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ABSTRACT

Title of Dissertation: The Role of Dopaminergic Neurons in the Regulation of Pituitary Beta-endorphin Secretion

John M. Farah, Jr., Doctor of Philosophy, 1985

Dissertation directed by: Gregory P. Mueller, Ph.D.
Associate Professor
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At the time this study was initiated, virtually nothing was known about neural mechanisms controlling pituitary secretion of the opiate peptide, beta-endorphin. The purpose of the present investigation was to determine the extent to which brain dopamine neurons regulate beta-endorphin secretion from the anterior and intermediate lobes of the pituitary gland.

Adult male rats or primary pituitary cell cultures were treated with dopaminergic agonists, antagonists, combinations of the two or appropriate vehicles. Total immunoreactive beta-endorphin and the major molecular forms of circulating immunoreactive beta-endorphin (beta-lipotropin- and beta-endorphin-sized immunoreactivity) were evaluated by radioimmunoassay in conjunction with gel filtration chromatography.

The results demonstrate that dopamine differentially controls beta-endorphin secretion from both the anterior and intermediate lobes by its effects on dopamine-1 and dopamine-2 receptor subtypes. Mixed dopaminergic agonists and selective dopamine-2, but not dopamine-1, agonists increased plasma levels of total immunoreactive beta-endorphin in a time- and dose-related fashion. These apparent dopamine-2-mediated increases were due to elevated beta-lipotropin-

sized immunoreactivity, material secreted exclusively by the anterior lobe. Conversely, beta-endorphin-sized immunoreactivity, which primarily reflects intermediate lobe secretion, was moderately reduced. The dopamine agonist-evoked release was prevented either by dopamine-2 receptor blockade or by glucocorticoid pretreatment which inhibits anterior but not intermediate lobe secretion of immunoreactive beta-endorphin. Since dopamine agonists had no direct effect on secretion of immunoreactive beta-endorphin from anterior lobe cultures, a dopamine-2 receptor mechanism within the brain probably enhances the release of hypothalamic corticotropin releasing factor. Unlike dopamine agonists, dopamine-2-specific antagonists increased only blood-borne beta-endorphin-sized immunoreactivity, whereas, mixed dopaminergic antagonists additionally increased beta-lipotropin-sized immunoreactivity and the latter effect was attenuated by pretreatment with glucocorticoid or a dopamine-1 agonist. Therefore, in contrast to inhibition of intermediate lobe secretion by a dopamine-2 receptor mechanism, anterior lobe release of immunoreactive beta-endorphin appears to be under the reciprocal control of dopamine receptor subtypes. Based on the present findings, a model is proposed for dopaminergic control of pituitary beta-endorphin secretion. Brain dopamine neurons mediate inhibition and stimulation of hypothalamic corticotropin releasing factor through actions on dopamine-1 and dopamine-2 receptors, respectively. In contrast, intermediate lobe secretion is inhibited by actions of dopamine on dopamine-2 receptors.

THE ROLE OF DOPAMINERGIC NEURONS IN THE REGULATION OF PITUITARY BETA-ENDORPHIN SECRETION

by

John Mitchell Farah, Jr.

Dissertation submitted to the Faculty
of the Department of Physiology
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dedicated to the ones that I love,

Sharon, John & Betty, Michael, Kathleen, Janine, Tina & Gregory

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I am always grateful to Doctor Gregory Mueller for his friendship, guidance and patience. I owe a great deal to many thoughtful individuals at the Uniformed Services University who have contributed unselfishly to my professional development and with whom I have enjoyed the coming of age of this fine university.

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ABBREVIATIONS

Abbreviation	Name
Ac-	acetyl-
ACTH	adrenocorticotropin, corticotropin
AL	anterior lobe (pituitary), pars distalis
AVP	arginine vasopressin, antidiuretic hormone
B-END	beta-endorphin, (beta-lipotropin 61-91)
iB-END	immunoreactive beta-endorphin
B-LPH	beta-lipotropin
C	celsius, centigrade
C-	carboxy-(terminus)
cm	centimeter(s)
CNS	central nervous system
CRF	corticotropin releasing factor
CSF	cerebrospinal fluid
D1	dopamine-1 (receptor subtype)
D2	dopamine-2 (receptor subtype)
DA	dopamine, 3-hydroxytyramine
DEX	dexamethasone
DNA	deoxyribonucleic acid
L-DOPA	L-dihydroxyphenylalanine
g	gram(s)
h	hour(s)
HAL	haloperidol
icv	intracerebroventricular, intraventricular
IL	intermediate lobe (pituitary), pars intermedia
kg	kilogram(s)
Kd	relative mobility (chromatography)
l	liter(s)
LY141865	trans-4,4a,5,6,7,8a,9-octahydro-5-propyl-2H-pyrazolo[3,4-g]quinolone
M	molar, moles per liter
mCi	millicurie(s)
mcg	microgram(s)
METYR	metyrapone
mg	milligram(s)
min	minute(s)
ml	milliliter(s)
mm	millimeter(s)
mRNA	messenger ribonucleic acid
MSH	alpha-melanocyte stimulating hormone, alpha-melanotropin
N-	amino-(terminus)
ng	nanogram(s)
NIL	neurointermediate lobe (pituitary), pars nervosa plus pars intermedia
6-OHDA	6-hydroxydopamine
pg	picogram(s)
POMC	pro-opiomelanocortin
PRL	prolactin

Abbreviation	Name
sc	subcutaneously
SE	Standard Error
SKF	SKF 38393
SULP	sulpiride
TFA	triflouroacetic acid
vs	versus
Ve	elution volume (chromatography)
VEH	vehicle
Vo	void volume (chromatography)
Vs	salt (total) volume (chromatography)

Chapter 1

INTRODUCTION

The field of neuroendocrinology has enjoyed an expansion of research efforts since the late 1970's, owing partially to the discovery of the pituitary peptide, beta-endorphin (B-endorphin). B-endorphin was first isolated as a peptide with remarkable opiate potency and was soon shown to be most highly concentrated in the adenohypophysis and present, but in lesser amounts, in a variety of other tissues. At the time, the analgesic actions of alkaloid opiates like morphine were thought to represent actions that could normally be mediated by the endogenous pituitary pool of B-endorphin. This notion has been tempered to reflect more recent discoveries that pituitary B-endorphin is synthesized in many forms, some of them having little activity as opiate compounds. Furthermore, B-endorphin represents just one among three families of endogenous opiate peptides including the enkephalins and dynorphins. Although all of these morphomimetic (opioid) peptides appear to be involved in endogenous opiate functions throughout the body, interest in the study of B-endorphin continues to increase as endocrine and neural functions of

opioids appear to be uniquely served by B-endorphin.

Enthusiasm for studies of B-endorphin increased when it was learned that B-endorphin is synthesized from a large protein precursor molecule together with two well-known pituitary hormones, adrenocorticotropin and alpha-melanocyte-stimulating hormone. This important revelation and subsequent investigation of the genetic origin and differential processing of the B-endorphin precursor between the anterior and intermediate lobes of the adenohypophysis continues to provide principles applicable to the study of polypeptide biosynthesis in general. B-endorphin's possible biologic actions and biosynthesis engendered numerous investigations into the mechanisms that govern the two pituitary stores of B-endorphin peptides. Among the many brain neurotransmitter systems that might regulate pituitary B-endorphin, dopamine neurons appeared well-suited to control both anterior and intermediate lobe secretion. This chapter first outlines what is currently known about B-endorphin's synthesis, tissue distribution and biologic actions, particularly as a pituitary hormone. Then, the neuroendocrine mechanisms involved with anterior and intermediate lobe release of B-endorphin are reviewed to establish a background for investigating dopaminergic influences on pituitary B-endorphin secretion.

1.0.0.1 Historical Overview

B-endorphin's history can be dated from the early 1970's when curiosity about the pharmacologic actions of morphine led to the description of opiate receptors. Using a radioisotopically-labeled opiate antagonist, [3H]-naloxone, Pert and colleagues (Pert et al, 1973; Pert and Snyder, 1973) were able to demonstrate specific binding sites for opiates in the central nervous system (CNS) and in other nervous tissues in the body (Pert et al, 1973; Pert and Snyder, 1973). They and others reasoned that these receptors probably existed for specific interactions with endogenous ligands. In 1975, Hughes and Kosterlitz and colleagues were the first to report the isolation and identification of two pentapeptides from porcine CNS which resembled potent alkaloid opiates both in radioreceptor assays and in opiate bioassays (Hughes et al, 1975). The pentapeptides they found were methionine- and leucine-enkephalin which are distinguished from one another only by their carboxy-terminal amino acid. Hughes and others recognized that methionine-enkephalin corresponded to amino acid residues 61 through 65 of beta-lipotropic hormone (B-lipotropin or B-LPH), a 91 residue peptide isolated a decade earlier from sheep pituitary by C H Li (Li, 1964). Thereafter, enzymatically-generated segments of B-LPH were found to exhibit varying degrees of opiate activity (Lazarus et al, 1976; Ling and Guillemin, 1976) but the most potent opioid constituted the last thirty-one amino acids in the B-LPH sequence (B-LPH 61-65) which

became known as B-endorphin (Bradbury et al, 1976a; Chretien et al, 1976; Cox et al, 1976; Goldstein, 1976; Li and Chung, 1976; Loh et al, 1976). Once B-endorphin had been identified as a potent analgesic, intense investigations were initiated to examine the synthesis, distribution and the regulation of this endogenous opioid.

1.1 BIOSYNTHESIS OF BETA-ENDORPHIN AND RELATED PEPTIDES

B-endorphin has been found to be biosynthetically-related to other peptide hormones in the pituitary gland through a common pro-hormone (Eipper and Mains, 1980; Krieger et al, 1980; Rosa et al, 1980). The same precursor molecule is synthesized in both anterior lobe (AL) corticotrophs and intermediate lobe (IL) melanotrophs for subsequent processing into adrenocorticotropic hormone (ACTH) and alpha-melanocyte-stimulating hormone (alpha-MSH), respectively, together with several molecular forms of B-endorphin (Mains and Eipper, 1979). Since endorphins, melanotropins and corticotropins are all derived from the same precursor, this pro-hormone has been named pro-opiomelanocortin (POMC) (Chretien et al, 1979). Although both lobes of the adenohypohysis produce B-endorphin, corticotrophs of the AL secrete only the potent opioid, B-endorphin 1-31. Melanotrophs, on the other hand, secrete mostly modified forms of B-endorphin 1-31

that have limited or no opioid activity (Deakin et al, 1980). Hence, dramatically different hormone products are secreted by AL corticotrophs and IL melanotrophs despite the fact that both cell types derive their constituent secretory products from the same pro-hormone, POMC. The distinct biological signals which emanate from corticotrophs and melanotrophs reveal the need for understanding differential processing and regulation of POMC in these two cell types. Since the present study examines pituitary release of B-endorphin, comparison of POMC processing between the AL and IL allows differences between blood-borne products to serve as markers for each of the two lobes.

Appreciation of differential POMC processing by the AL and IL has extended importance since several other tissues of the body also produce B-endorphin-related peptides. Depending on the sensitivity of the target tissue, alternative forms of B-endorphin peptides could determine whether the peptide serves hormonal, neuronal, paracrine or autocrine functions.

1.1.0.1 History of the B-endorphin Pro-hormone

The discovery of POMC actually preceded the discovery of B-endorphin and evolved from questions about the nature of larger molecular forms of ACTH (Eipper and Mains, 1980; Krieger et al, 1980). Biologically active ACTH

was known to be secreted as a polypeptide comprising 39 amino acids with an apparent molecular weight of about 4500 daltons. Nevertheless, immunoreactive ACTH had been found in forms as large as 31,000 daltons in the circulation as well as in the pituitary gland (Yalow and Berson, 1973; Yalow, 1974). Fortunately for those studying the biosynthesis of ACTH, these 'big' forms of ACTH are secreted in abundance by cloned mouse pituitary tumor cells (Orth et al, 1973; Canfield et al, 1970). Eipper and Mains used this pituitary cell line, AtT-20/D-16v, to demonstrate that the larger molecular weight forms of immunoreactive ACTH are actually the ACTH precursor and several biosynthetic intermediates for ACTH (Eipper and Mains, 1975; Mains and Eipper, 1976). The precursor found in pituitary tumor cells and, subsequently in the AL and in IL, is a glycoprotein with an apparent molecular weight of 31,000 daltons as determined by polyacrylamide gel electrophoresis (Mains and Eipper, 1976; Eipper et al, 1976). For this reason, the pro-hormone was previously referred to as 31 K from its molecular weight (Eipper et al, 1976). Carbohydrate moieties were found joined to one or two locations along the amino acid sequence of 31 K, one being in the ACTH 1-39 sequence. The conversion of 31 K to ACTH and glycosylated ACTH appeared to proceed via enzymatic cleavage of the precursor in steps similar to tryptic processing that had earlier been described for pro-insulin and pro-parathyroid hormone (Chan and Steiner,

1977; Habener and Potts, 1978a; 1978b; Eipper and Mains, 1980).

By the time that Eipper and Mains firmly established that ACTH is derived from 31 K, several lines of evidence suggested that this pro-hormone might also serve as the precursor for several other pituitary hormones. For example, the polypeptide, B-LPH, was known to be secreted by the AL (Li, 1964) and had been functionally and anatomically associated with ACTH as follows. Abe and colleagues reported that in human plasma, levels of beta-MSH, a peptide corresponding to amino acids 41-58 of B-LPH, covaried with ACTH (Abe et al, 1969). Although beta-MSH has since been found to be an extraction artifact generated from B-LPH (Scott and Lowry, 1975), this molecule nonetheless served as an anatomical marker for B-LPH. Immunohistochemistry revealed that ACTH and B-LPH are co-localized in AL corticotrophs as well as in IL melanotrophs (Moon et al, 1973; Phifer et al, 1974). In addition to the association of B-LPH and ACTH, Scott and colleagues (1974) provided evidence to support a biochemical relationship between ACTH and the IL peptide, alpha-MSH. They found that the amino acid sequence of alpha-MSH and of corticotropin-like intermediate lobe peptide (CLIP) correspond exactly to the ACTH sequences, ACTH 1-13 and ACTH 18-39, respectively (Scott et al, 1974). They concluded that ACTH itself serves as a biosynthetic intermediate to production of alpha-MSH and CLIP in the IL,

whereas, ACTH itself remains the ultimate product in AL corticotrophs. Interestingly, the newly described opioids, methionine-enkephalin and B-endorphin, were observed to be contained within the sequence of B-LPH (Hughes et al, 1975; Li and Chung, 1976) and could be extracted from the pituitaries of several mammalian species (Bradbury et al, 1976b; Chretien et al, 1976; Li and Chung, 1976; Li et al, 1976). This suggested that B-LPH might be the precursor to B-endorphin and methionine-enkephalin (Bradbury et al, 1976b; Lazarus et al, 1976). Considering the evidence for the co-existence of B-LPH with ACTH in the AL and with MSH in IL, the probability of a biosynthetic relationship between the opioid peptides, B-LPH and pro-ACTH became well worth pursuing. The possibility that methionine-enkephalin and B-endorphin might be derived from corticotropic precursors was enhanced by the report that a subclone of the AtT20/D-16v corticotrophic tumor secretes a variety of opiate peptides in addition to ACTH (Giagnoni et al, 1977). Appraised of these findings, several groups of investigators independently examined the biosynthetic processes which lead to pituitary production of B-endorphin, B-LPH and related peptides.

1.1.0.2 Pro-opiomelanocortin (POMC), the B-endorphin Precursor

The discovery that B-endorphin is synthesized along with ACTH from a common pro-hormone was first demonstrated

using AtT-20/ D-16v cells. Using anti-B-endorphin and anti-ACTH antisera, immunoreactive B-endorphin and ACTH were isolated from other polypeptide products of the AtT-20 cells and characterized according to molecular size and amino acid composition. Using this protocol, Mains, Eipper and Ling (1977) found that either ACTH or B-endorphin antisera immunoprecipitate the same 31 K glycoprotein from extracts of the mouse pituitary tumor. Similar results were obtained upon examination of the peptides producted in cell-free translation of messenger ribonucleic acid (mRNA) extracted from AtT-20 or bovine IL cells (Roberts and Herbert, 1977a; Nakanishi et al, 1977). In addition, it became clear that ACTH plus unprocessed biosynthetic intermediates equal the sum of B-endorphin and B-LPH (Eipper et al, 1976; Mains and Eipper, 1978; Roberts et al, 1978). Therefore, each mole of 31 K produced by corticotrophic cells yields one mole of ACTH-related peptides and one mole of B-endorphin-related peptides. Together, however, these two groups of hormones account for only about half of the molecular weight and amino acid content (approximately 260 residues) of the pro-ACTH precursor (Roberts and Herbert, 1977). The remaining fragment of the precursor could not be precipitated with anti-ACTH, B-endorphin or B-LPH antisera and became known as either the cryptic fragment of pro-ACTH or as 16 K (its molecular weight) (Roberts and Herbert, 1977a; Eipper and Mains, 1978; Keutmann et al, 1979).

The precursor-product relationship between pro-ACTH and B-endorphin-related peptides was determined through examination of the kinetics of their synthesis and later confirmed by sequencing the gene for POMC. The kinetics for biosysnthesis of B-endorphin and ACTH were determined by monitoring the fate of radioisotopically-labeled amino acid markers. Mains and Eipper (1978) briefly exposed AtT-20 cells to [3H]-labeled amino acids which were rapidly incorporated into the sequence of 31 K. This 'pulse' of radiolabel was followed by 'chase' periods of increasing duration in which unlabeled substrates were provided to the cells. Over time, the labeled intermediates and, finally, ACTH, B-LPH and B-endorphin were observed to gradually replace the labeled 31 K molecule (Mains et al, 1977). A similar succession of labeled products from the precursor was observed by others who translated mRNA and polysomes extracted from AtT-20 cells and bovine IL, respectively (Nakanishi et al, 1977; Roberts and Herbert, 1977a). Together, these findings proved that pro-ACTH serves as the biosynthetic precursor for MSH, B-LPH and B-endorphin-related peptides as well as ACTH. Furthermore, the temporal relationship between production of B-LPH and B-endorphin indicated that B-LPH is the immediate precursor to B-endorphin in the AL and IL just as ACTH gives rise to MSH in the IL. The multiple hormone potential of 31 K was reconfirmed in intact AL and IL (Crine et al, 1977; Eipper and Mains, 1978; Roberts et al, 1978; Seidah et al, 1978),

indicating that the precursor-product relationship between POMC and B-endorphin was not an anomaly of the tumor cells or of cell-free translation of POMC mRNA.

Interestingly, little methionine-enkephalin was found among the peptides isolated from B-endorphin-secreting cells (Mains and Eipper, 1978), despite the fact that methionine-enkephalin constitutes the amino (N)-terminal pentapeptide of B-endorphin (B-LPH 61-65). This indicated that although methionine-enkephalin is an integral part of B-endorphin's structure conferring B-endorphin with its opioid properties, the pentapeptide might not be biosynthetically derived from either B-endorphin or B-LPH. Thus, the mere presence of a peptide's sequence within the structure of a larger protein could not guarantee a precursor-product relationship between the two, a principle first derived from studies of B-endorphin's biosynthesis.

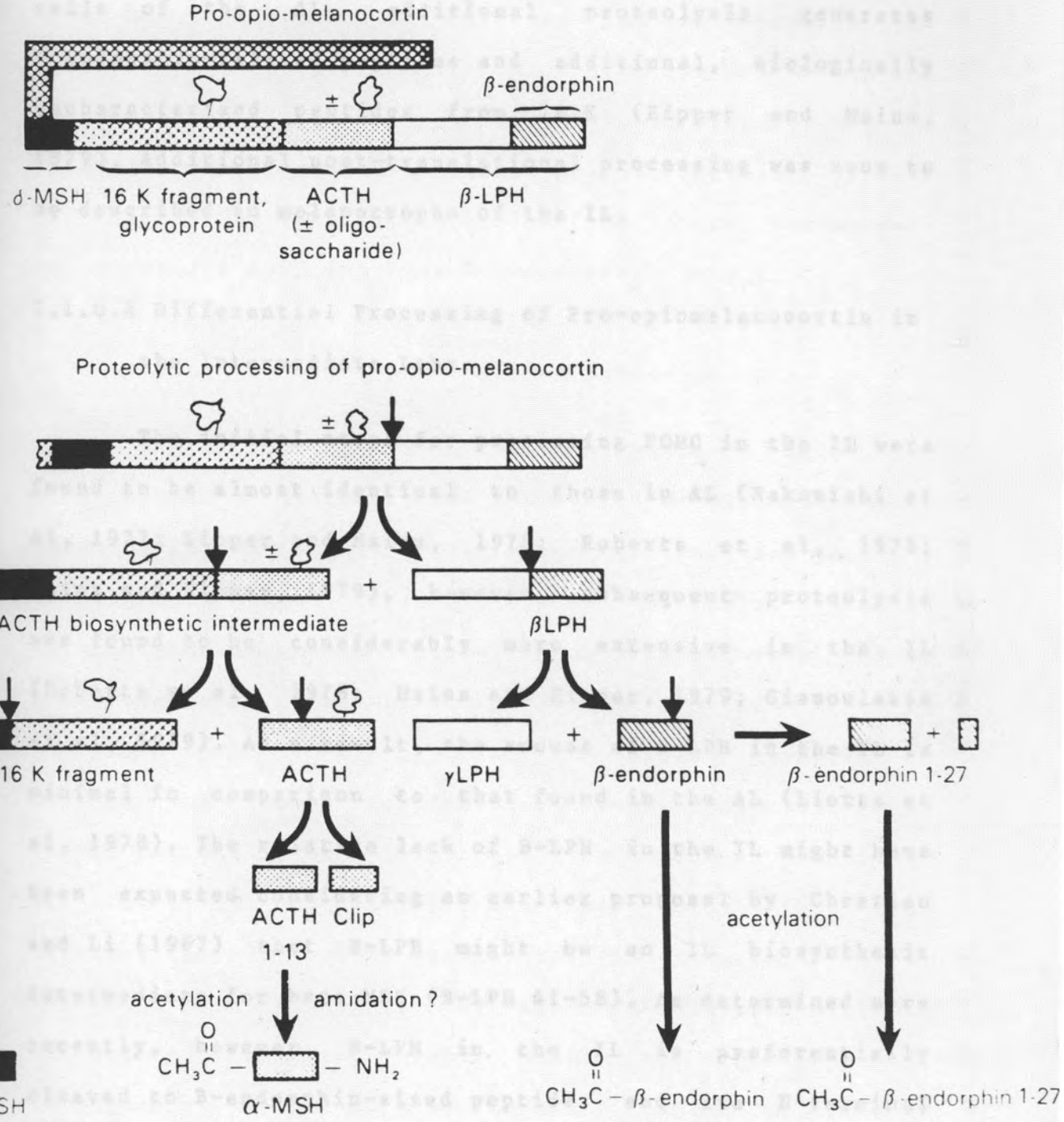
1.1.0.3 Structure and Processing of Pro-opiomelanocortin

The arrangement of peptides within POMC was first deduced by Roberts and Herbert (1977b). Using a polysome runoff procedure which allows cell-free translation of protein to proceed <u>in vitro</u>, these investigators found that incorporation of labeled amino acids was greatest into B-endorphin and B-LPH-sized peptides, less into ACTH-related peptides and least into the 16 K cryptic

fragment in extracts from AtT-20 cells. From these data, Roberts and Herbert surmised that B-endorphin and B-LPH are the last sequences translated from mRNA for the pro-hormone and, therefore, should occupy the carboxy (C)-terminus of pro-ACTH/endorphin preceded by ACTH. A more precise description of the structure and processing of POMC was formulated by Mains and Eipper (1979). Their model represented a condensation of results from studies of pro-ACTH/endorphin in AtT-20 and normal rodent AL cells (Mains and Eipper, 1976; Eipper et al, 1976; Roberts and Herbert, 1977a; 1977b; Nakanishi et al, 1977; Mains et al, 1977; Roberts et al, 1978; Eipper and Mains, 1978). Soon after translation of the protein precursor, carbohydrate moieties are attached to asparagine residues in the 16 K region and, sometimes, in the N-terminal region of the ACTH 1-39 sequence (Eipper et al, 1976). Thereafter, the glycoprotein is cleaved by proteolytic enzymes at sites which seemed likely to be demarcated by pairs of basic amino acids (Bradbury et al, 1976b; Lazarus et al, 1976) in a fashion similar to that described for pro-insulin and pro-parathyroid hormone (Habener and Potts, 1978a; 1978b; Docherty and Steiner, 1982). As shown in Figure 1, the first of these cleavages separates B-LPH from the remainder of POMC followed by a cleavage which frees ACTH or glycosylated ACTH from the 16 K fragment (Mains and Eipper, 1979). Thus, the initial enzymatic cleavage steps were found to yield three major segments of the precursor, i.e.,

Figure 1. Schematic illustration of pro-opiomelanocortin together with the enzymatic steps that result in distinct B-endorphin peptides in the anterior and intermediate lobes of the pituitary. The enzymatic events which yield gamma-MSH, alpha-MSH and acetylated and C-terminally shortened forms of B-endorphin occur only in the intermediate lobe (illustration courtesy of Dr. Thomas L. O'Donohue).

BIOSYNTHETIC PATHWAY OF αMSH, ACTH, γ-LPH, β-LPH AND β-ENDORPHIN



16 K from the N-terminus, ACTH from the mid-portion and B-LPH from the C-terminus (Figure 1). In normal and tumor cells of the AL, additional proteolysis generates B-endorphin-related peptides and additional, biologically uncharacterized peptides from 16 K (Eipper and Mains, 1979). Additional post-translational processing was soon to be described in melanotrophs of the IL.

1.1.0.4 Differential Processing of Pro-opiomelanocortin in the Intermediate Lobe

The initial steps for processing POMC in the IL were found to be almost identical to those in AL (Nakanishi et al, 1977; Eipper and Mains, 1978; Roberts et al, 1978; Mains and Eipper, 1979), however, subsequent proteolysis was found to be considerably more extensive in the IL (Roberts et al, 1978; Mains and Eipper, 1979; Gianoulakis et al, 1979). As a result, the amount of B-LPH in the IL is minimal in comparison to that found in the AL (Liotta et al, 1978). The relative lack of B-LPH in the IL might have been expected considering an earlier proposal by Chretien and Li (1967) that B-LPH might be an IL biosynthetic intermediate for beta-MSH (B-LPH 41-58). As determined more recently, however, B-LPH in the IL is preferentially cleaved to B-endorphin-sized peptides and its N-terminal fragment, gamma-LPH (B-LPH 1-58), which contains the B-MSH sequence (Chretien et al, 1976; Crine et al, 1978; Roberts et al, 1978; Eipper and Mains, 1978). In the IL, ACTH (like

B-LPH), appears to be a minor product which primarily serves as a biosynthetic intermediate for production of alpha-MSH (N-acetyl-ACTH 1-13 amide) and corticotropin-like intermediate lobe peptide (CLIP) (ACTH 18-39) (Mains and Eipper, 1979; Gianoulakis et al, 1979). In summary, the IL cleaves POMC to B-endorphin-sized peptides, gamma-LPH, CLIP and alpha-MSH, whereas, AL derivatives of the pro-hormone are B-LPH and ACTH and some B-endorphin.

1.1.0.5 Genetic Code for Pro-opiomelanocortin

Eipper and Mains (1980) proposed a model for biosynthesis of peptides from POMC which is similar to that shown in Figure 1. The details which confirmed and extended their model were established by molecular genetic techniques whereby the entire amino acid sequence of POMC was revealed. Nakanishi and colleagues took advantage of the IL's prolific synthesis of POMC-derived products to develop and clone complimentary deoxyribonucleic acid (cDNA) for the pro-hormone gene from bovine IL (Nakanishi et al, 1979). Sequencing and interpreting this cDNA revealed three important facts about the precursor. First, the amino acid sequences for known POMC-related peptides are encoded into translatable mRNA in the order presented in Figure 1. Second, each of the known peptide products of POMC is flanked by pairs of basic amino acids, i.e., two arginines, two lysines or combinations of the two, with no additional peptide leaders or fragments on each known

peptide. Third, a previously unidentified copy of an MSH fragment was found within the 16K cryptic region of POMC.

Dibasic amino acid residues in polypeptide sequences had previously been shown to be important in processing other proteins (Docherty and Steiner, 1982). Basic amino acids had been suspected to be recognition sites for post-translational cleavage of POMC but aren't included in the sequence of mature peptide products (Bradbury et al, 1976b; Eipper and Mains, 1979; Eipper and Mains, 1980). The most enlightening information provided by Nakanishi's gene code analysis was the amino acid sequence of the previously cryptic 16 K fragment at POMC's N-terminus (Nakanishi et al, 1979). Although Eipper and Mains (1979) had suggested that further processing of 16 K was possible, the cDNA method for sequencing POMC showed that additional peptide products could be derived from the cryptic segment of POMC (Nakanishi et al, 1979). Nakanishi and colleagues were able to identify these new peptides because their sequences were demarcated by pairs of basic amino acid residues. Striking sequence homology of one sequence with the ACTH 4-10 region of alpha- and beta-MSH led Nakanishi to name one peptide gamma-MSH (Nakanishi et al, 1979). Gamma-MSH has since been extracted from the pituitary of several species as a twenty-seven amino acid peptide which may be glycosylated like ACTH 1-39 (Benjannet et al, 1980; Shibasaki et al, 1980; Browne et al, 1981) and further C-terminally shortened to gamma 2- and gamma-3 forms. The gamma-MSH's

were found to be much less active at melanocyte dispersion than alpha-MSH (Ling et al, 1979), nonetheless, synthetic gamma-MSH, like the other melanotropins, has been shown to potentiate the steroidogenic actions of ACTH on mammalian adrenal cortex (Pederson and Brownie, 1980) and may have special natriuretic and cardiovascular roles (see Lymangrover et al, 1985). Thus, three species of melanotropic peptides, alpha-, beta- and gamma-MSH, are encoded in the POMC gene and they constitute sequences in all three major segments of the pro-hormone (see Figure 1). It is thought that since all three melanotropic peptides are encoded by uninterrupted expression regions of genomic DNA, called exons, POMC may have evolved through genetic recombinations of an ancient nucleotide sequence. Supporting this hypothesis is the homology found in nucleotide sequences for B-endorphin, ACTH and MSH between rodents, cattle and man (Drouin and Goodman, 1980; Nakanishi et al, 1981; Whitfeld et al, 1982). Species differences in polypeptide chain length and amino acid composition of 16 K and of the N-terminal region B-LPH suggest that these sequences of POMC are more recently evolved than biologically important regions of the multi-hormone precursor (Drouin and Goodman, 1980; Nakanishi et al, 1981; Whitfeld et al, 1982; Civelli et al, 1983; Notake et al, 1983).

1.1.0.6 Additional Enzymatic Modifications of

Pro-opiomelanocortin

Mechanisms must exist by which POMC is metabolized to distinctly different products within corticotrophs and melanotrophs (Rosa et al, 1980; Eipper and Mains, 1980). Glycolysis, probably the first post-translational modification of POMC, occurs in both the AL and IL and may stabilize the precursor during its packaging into secretory granules (Loh and Gainer, 1979). Cleavage of POMC to its constituent peptide products occurs in the granules (Glembotski, 1981). It is presently not known how more extensive proteolysis of B-endorphin and ACTH is directed in the IL than in the AL. Perhaps there are additional proteolytic enzymes in melanotrophs or else the enzymes common to both melanotrophs and corticotrophs exhibit different substrate specificity. Minor modifications of POMC such as phosphorylation or sulfation (Bennett et al, 1981; Hoshina et al, 1982) could alter the prohormone just enough to provide subtle differences in an otherwise identical amino acid sequence.

When Smyth and colleagues isolated multiple forms of B-endorphin from pig pituitary and brain (Smyth et al, 1978; Smyth et al, 1979; Zakarian and Smyth, 1979), the regional differences in POMC processing between corticotrophs and melanotrophs became all the more evident. In the IL but not in the AL, B-endorphin 1-31 was found to undergo additional C-terminal proteolysis directed

by lysine residues at positions 28 and 29 (Mains and Eipper, 1981). As compared to B-endorphin 1-31, C-terminally shortened forms of B-endorphin exhibit an opioid potency which is from 10- to 500-times less than that of B-endorphin (1-31) (Geisow et al, 1977; Li et al, 1978; Akil et al, 1981).

The IL modification of B-endorphin which has more profound effects on opioid potency is acetylation of the peptide's amino(N)-terminus. Although N-acetylation confers MSH with its biological potency (Waller and Dixon, 1960; Guttmann and Biossonas, 1961), N-acetyl forms of B-endorphin are totally devoid of opioid activity (Smyth et al, 1978; Deakin et al, 1980; Akil et al, 1981). The acetylase appears to have nearly as high an affinity for unacetylated forms of B-endorphin as for unacetylated and monoacetyl MSH (Rudman et al, 1979; Chappell et al, 1982; Glembotski, 1982c).

Since N-acetylation of B-endorphin procedes at a higher rate than C-terminal proteolysis, most B-endorphin in the IL is converted to N-acetyl B-endorphin prior to modification of the C-terminus (Eipper and Mains, 1981; Glembotski, 1982b). The forms of B-endorphin which are produced in the IL as a consequence of these modifications are N-acetyl B-endorphin 1-31, B-endorphin 1-27, N-acetyl B-endorphin 1-27 and some N-acetyl B-endorphin 1-26 in addition to unacetylated B-endorphin 1-31 (Smyth and

Zakarian, 1980; Mains and Eipper, 1981; Eipper and Mains, 1981; Liotta et al, 1981). Less than 10% of the total B-endorphin products of the IL is accounted for by the potent opioid, B-endorphin 1-31. The most abundant of the modified forms appears to be N-acetyl B-endorphin(1-27)(Smyth and Zakarian, 1980; Mains and Eipper, 1981; Eipper and Mains, 1981; Liotta et al, 1981).

Hence, B-endorphin-related products of the IL differ markedly from those of the AL where only B-endorphin and its immediate precursor, B-LPH, are produced (Liotta et al, 1978). Interestingly, the N-acetylation and C-terminal proteolysis, so consistently observed in mammalian IL, are modifications selectively exploited in lower vertebrates. For example, in the reptile, Anolis, B-endorphin in the IL is subjected only to C-terminal proteolysis (Dores and Suprenant, 1983; Dores, 1983). In contrast, B-endorphin produced by the IL in teleost fishes is principally N-acetylated without C-terminal shortening (Kawauchi and Muramoto, 1979). These unique molecular features of B-endorphin and related peptides are being used in order to ascertain the physiology and regulation of these peptides throughout the animal kingdom (Krieger, 1983). It will be important in the future to determine if differential regulation of AL and IL secretions, i.e., releasing factor stimulation versus inhibition, also controls the differences in biosynthetic products between the two lobes.

1.1.0.7 Chemistry of B-endorphin: Structure and Function

The opiate activity of B-endorphin, like that of dynorphin peptides, depends, in part, upon by the N-terminal enkephalin sequence which both peptides possess. Accordingly, modification of the N-terminus by acetylation destroys the morphomimetic potency of B-endorphin (Li et al, 1978; Deakin et al, 1980; Akil et al, 1981). The C-terminal region of B-endorphin is also important for opiate activity of the molecule, defining B-endorphin's potency and, perhaps, its specific affinity for the mu and epsilon subtypes of opiate receptor (see Cox, 1982; Snyder, 1984). Like N-acetylation, C-terminal shortening dramatically diminishes the potency of B-endorphin in both opiate receptor binding and biologic assays (Li et al, 1978; Deakin et al, 1980; Akil et al, 1981). These modifications are also likely to influence non-opioid actions of B-endorphin peptides that have been proposed for the immune system (Hazum et al, 1979; Schweigerer et al, 1982; Gilman et al, 1982; McCain et al, 1982; Simpkins et al, 1985).

There are features of B-endorphin's biological activity which may be accounted for, in part, by tertiary structure. The arrangement of hydrophobic and hydrophilic amino acid residues in the C-terminal region of B-endorphin has been postulated to provide B-endorphin with the ability to form an alpha or pi helix at lipid-water interfaces like

that presumed to occur at the cell membrane (Kaizer and Kezdy, 1984). This plasticity of B-endorphin may allow the peptide to form long-lasting associations with appropriate target cell receptors (Akil et al, 1980; Snyder, 1984). The stability of B-endorphin in blood is another physiologically important characteristic of this opioid which could be attributed to B-endorphin's tertiary structure. B-endorphin has an estimated circulating half-life of from 5-50 min (Pezalla et al, 1978; Foley et al, 1979; Houghten et al, 1980; Aronin et al, 1981). By contrast, the enkephalins are inactived by blood-borne peptidases at such a high rate that even in extra-corporeal serum, their estimated half-life is no greater than twelve minutes (Burbach et al, 1979). B-endorphin's resistance to N-terminal tyrosine cleavage which inactivates the enkephalins (Hambrook et al, 1976) may be due to folding of the B-endorphin molecule. Nicholas and colleagues (1981) found that, in aqueous solution, amino acid residues in the C-terminus of B-endorphin appear to non-covalently associate with residues in the N-terminus. It is likely that such potential tertiary conformation shields the N-terminus of the peptide from enzymatic attack and thereby prolongs the effective half-life of B-endorphin in blood.

B-endorphin's structure has, in addition, also availed investigators the antigenic and chemical features necessary to distinguish B-endorphin from other functionally and biosynthetically-related peptides.

Antisera directed towards the C-terminal region of B-endorphin are unable to detect the enkephalins or dynorphins yet recognize all B-endorphin peptides and precursors containing the same antigenic sequences of amino acids. The substantial molecular weight differences between the B-endorphin-sized peptides and their precursors, B-LPH and POMC, permit separation of these forms by gel filtration chromatography, whereas, similarly sized B-endorphin peptides (e.g., B-endorphin 1-31 vs N-Acetyl-B-endorphin 1-31) themselves are best separated on the basis of charge or hydrophobicity using ion exchange chromatography or high performance liquid chromatography, respectively. With cation exhange techniques, for example, N-acetylated and C-terminally shortened forms of B-endorphin are distinguishable from the opioid form, B-endorphin (1-31) (Zakarian and Smyth, 1979). Using these methods, it is possible to characterize and quantify specific forms of B-endorphin peptides and, thereby, attain a better understanding of factors which govern synthesis, regulation and biological activity of B-endorphin peptides.

1.2 DISTRIBUTION OF BETA-ENDORPHIN AND RELATED PEPTIDES

B-endorphin was first found in the pituitaries of camels (Li and Chung, 1976). Within months, B-endorphin had been

isolated from pituitary glands of several animals including pigs (Bradbury et al, 1976b), sheep (Chretien et al, 1976), and man (Li et al, 1976; Chretien et al, 1976) and has since been found in rodents, cows, horses and other mammals (Rubinstein et al, 1977; Liotta et al, 1978; Li et al, 1981). Subsequent studies have shown that B-endorphin or related peptides are produced in a variety of mammalian tissues including the brain, placenta and gonads (Rossier et al, 1977a; 1977b; Matsukura et al, 1978; Nakai et al, 1978; Odagiri et al, 1979; Liotta and Krieger, 1980; Sharp et al, 1980; Lim et al, 1983; Margioris et al, 1983; Pintar et al, 1984) as well as in associated fluids: cerebrospinal, amnionic, follicular and seminal, respectively (Jeffcoate et al, 1978; Gautray et al, 1977; Sharp and Pekary, 1981; Lim et al, 1983). Others have characterized the presence of B-endorphin peptides in the pancreas and in the gastrointestinal mucosa (Bruni et al, 1979; Watkins et al, 1980; Feurle et al, 1980; Orwoll and Kendall, 1980) where paracrine or autocrine functions have been postulated for B-endorphin (Feldman et al, 1983; Krieger, 1983). There is limited evidence to suggest that B-endorphin may also be used as a transmitter by motor neurons in neonatal rats (Haynes et al, 1982). Among all mammalian tissues, however, the pituitary synthesizes and secretes more B-endorphin and related peptides than any other tissue in the body (Voulteenaho et al, 1980). The AL and the IL each contribute substantially to basal levels of

circulating B-endorphin-related peptides (Hollt et al, 1978a; Akil et al, 1979; Wardlaw and Frantz, 1979; Mueller, 1980; Przewlocki et al, 1982), however, the AL secretions are generally more sensitive to a variety of stimuli. Consequently, circulating levels of B-endorphin-related peptides usually coincide with release evoked from AL corticotrophs. This is particularly true in man where the IL is poorly developed (Visser and Swaab, 1979).

1.2.1 Localization of B-endorphin

1.2.1.1 Pituitary B-endorphin

Just as Moon and coworkers had observed for the pituitary distribution of B-LPH (Moon et al, 1973), several groups found that B-endorphin can be immunohistochemically localized to corticotropic cells of the rat and human AL and in every cell of the rat IL (Bloom et al, 1977; Facer et al, 1977; Mendelsohn et al, 1979). As the biosynthetic relationship of B-endorphin to other POMC derivatives was unfolding, the presence of B-endorphin within secretory granules of corticotrophs and melanotrophs was inferred and later demonstrated using immunoelectron microscopy (Pelletier et al, 1977; Weber et al, 1978; Martin et al; 1979). Within these granules, the pro-hormone is processed to the peptides characteristic of either the AL or the IL (Glembotski, 1981; 1982a; 1982b). Humans and the great apes transiently develop an IL during fetal life (Silman et al,

1976; Visser and Swaab, 1979) which largely involutes after birth (Visser and Swaab, 1979). Recent evidence suggests that the enzymatic capabilities of the homonid IL resemble those of melanotrophs of other mammals only insofar as proteolysis is concerned; the enzyme which catalyzes N-acetylation seems to be lacking in the human fetal IL (Tilders et al, 1981; Ackland et al, 1983). The rat is one of the many mammalian species in which the IL is developed and appears to be functional in the adult animal. The concentration of B-endorphin and related POMC products in the rat IL exceeds that in the AL by ten-fold (Rossier et al, 1977b; Liotta et al, 1978; Mueller, 1980). This relationship holds up at the transcriptional level where Herbert and co-workers have found that there is twenty times more POMC messenger RNA in the IL than in the AL (Civelli et al, 1983).

1.2.1.2 Ontogeny of Pituitary Corticotrophs and Melanotrophs

As the distribution of POMC-secreting cell types is considered, the importance of pituitary sources of B-endorphin and related peptides is highlighted by the precocious embryological development of corticotrophs and melanotrophs relative to other pituitary cell types. Chatelain and colleagues found that in rat fetuses, B-endorphin and other POMC derivatives are being synthesized by well-differentiated cells of the anterior

and intermediate lobes several days prior to the appearance of the first detectable growth hormone- or prolactin-secreting cells (Chatelain et al, 1979). Although immunoreactive B-endorphin and related peptides don't appear in the AL until day 15 or 16 of rat fetal life, the cells appear to be committed by day 12 (Begeot et al, 1982). Similarly, corticotrophs are the first of the adenohypophyseal cells to become functional in humans (see Daughaday, 1981). Once the hypophyseal germ cells for corticotrophs and melanotrophs appear, they are able to differentiate independently from hypothalamic influences, i.e., even in human and rat anenchephalic fetuses (Begeot et al, 1978; Chatelain et al, 1979). Both lobes of the adenohypophysis are embryologically derived from the same enlage of ectodermal tissue, Rathke's pouch (Wingstrand, 1966a; 1966b). Recent discoveries that many of the same biogenic amines and peptides are ubiquitously present in neural and endocrine tissues emphasizes an embryologic kinship between secretory cells throughout the body (Pearse and Takor, 1976; Pearse and Polak, 1978). Thus Pearse and colleagues have proposed that cells of the adenohypophysis belong to a diffuse neuroendocrine system. Widespread distribution of B-endorphin and other POMC peptides in secretory and neural cells support their hypothesis.

1.2.1.3 Brain B-endorphin

The discovery of B-endorphin and related peptides in

tissues other than the pituitary, particularly in neurons of the brain, closely followed the isolation and description of B-endorphin in the pituitary. Because of the initial astonishment at B-endorphin's analgetic potency and its presumed biosynthetic relationship to the enkephalins (initially isolated from brain), the central nervous system (CNS) was the first and most thoroughly examined extra-pituitary region of the body for localization of B-endorphin and related peptides. It was soon evident that B-endorphin could be found in CNS neurons but its distribution was clearly distinct from that of the enkephalins. Numerous groups of enkephalinergic cell bodies with intranuclear or short internuclear projections have been demonstrated throughout the rat CNS (Elde et al, 1976; Simantov et al, 1977; Bloom et al, 1978). In contrast, B-endorphin was found in a well-defined band of perikarya along the basolateral border of the arcuate and adjacent periventricular hypothalamus (Watson et al, 1978; Sofroniew, 1979). Fibers from these neurons innervate other nuclei within the hypothalamus and terminate also in the median eminence. Endorphinergic neurons arch dorsally out of the hypothalamus to innervate the septum, the amygdala, portions of the thalamus and cortex, the central grey of the brainstem and the locus coeruleus (Rossier et al, 1977b; Bloch et al, 1978; Bloom et al, 1978; Watson et al, 1978). The rather restricted nuclear origin and CNS-wide distribution of brain B-endorphin contrasts markedly with

the extensive nuclear distribution of local enkephalinergic neurons (Elde et al, 1976; Bloom et al, 1978). This distinction is similarly evident from differential regional concentrations of the two opioids (Hughes et al, 1977; Rossier et al, 1977b; Gramsch et al, 1979). The difference in CNS distribution of B-endorphin and the enkephalins was part of the evidence which made it increasingly obvious that these two endogenous opioids are biosynthetically unrelated. Current literature supports an equally diverse distribution of the dynorphin opioids in the CNS which more closely resembles the distribution of the enkephalin neurons than of CNS B-endorphin neurons (Khachaturian et al, 1982).

Several lines of evidence indicate that CNS B-endorphin is distinct from pituitary B-endorphin in synthesis as well as in secretion and function. Krieger and coworkers (1979) found that lesioning the arcuate nucleus [which comprises no more than 5% of the total hypothalamus (Palkovits, 1977) with monosodium glutamate significantly reduces CNS levels of B-endorphin and ACTH without changing pituitary content of either peptide (Krieger et al, 1979). Conversely, hypophysectomy fails to substantially alter brain content of B-endorphin-related peptides (Rossier et al, 1977b; O'Donohue et al, 1979). Furthermore, pulse-chase studies have shown that B-endorphin and related peptides are synthesized <u>de novo</u> in hypothalami from adult and neonate animals (Liotta at el,

1979; 1980). B-endorphin-related peptides have been found within hypothalamic granules (Barnea et al, 1981) from which calcium-dependent release has been demonstrated (Fukata et al,1980; Vermes et al, 1981). An important physiological distinction of CNS from pituitary B-endorphin is that forms which are produced in the brain, though similar to forms synthesized by melanotrophs, are nonetheless unique to the CNS (Gramsch et al, 1980; Weber et al, 1981; Evans et al 1982; Zakarian and Smyth, 1982). Additional evidence which distinguishes brain from pituitary B-endorphin is that fetal development of B-endorphin neurons precedes that of pituitary B-endorphin (Schwartzenberg and Nakane, 1982). The first sign of B-endorphin immunoreactivity in the cerebral anlage emerges on the twelfth day of rat fetal life in neurons of the developing hypothalamus, whereas, AL corticotropic cells don't appear until fetal day 16 with the IL melanotroph precursors appearing twenty-four hours later (Chatelain et al, 1979; Schwartzenberg and Nakane, 1982). Although recent experiments have shown that brain B-endorphin may be functionally influenced by pituitary-related endocrine functions (Gambert et al, 1980; Barden et al, 1981; Wardlaw et al, 1982a; 1982b; Wardlaw and Frantz, 1983) it is nonetheless clear that B-endorphin producing cells of the CNS and pituitary function independently.

1.2.1.4 Phylogenetic Distribution of B-endorphin

As anti-B-endorphin antisera became available, exploration for B-endorphin broadened to organisms other than mammals. Immunohistochemical techniques have demonstrated iB-endorphin in organisms as diverse as the protozoan, Tetrahymena pyriformis (LeRoith et al, 1982), earthworms (Alumets et al, 1979), bony fishes (Kawauchi et al, 1979; Van Eys and Van den Oetelaar, 1981), amphibians (Loh, 1979), reptiles (Dores, 1982) and birds (Naude et al, 1981). Similar to observations in the mammalian kingdom, B-endorphin was found in secretory or neural tissues. In Drosophila, for instance, complimentary DNA probes for the POMC gene have been recently used to locate B-endorphin- and ACTH-producing cells in the fly's reproductive organs as well as in nervous tissues (see Krieger, 1983), findings which parallel the presence of B-endorphin in mammalian gonads and CNS.

1.3 PHYSIOLOGY OF BETA-ENDORPHIN SECRETION

There are numerous putative roles for B-endorphin in mammals. As indicated above, the broadest description of B-endorphin peptides is that they are intercellular messengers. Pituitary B-endorphin peptides most likely exert hormonal actions in the periphery. Like other pituitary hormones, B-endorphin peptides are synthesized in endocrine cells where they are processed and stored in

secretory granules. Upon depolarization, B-endorphin peptides are released via calcium-dependent mechanisms from both the AL and IL (Simantov, 1978; Przewlocki et al, 1978a; Vermes et al, 1980a). The most convincing evidence that the pituitary is the primary source of circulating B-endorphin peptides is that both basal and stimulated levels in blood are generally found to be undetectable after hypophysectomy (Guillemin et al, 1977; Akil et al, 1979; Mueller, 1980). It should be noted, however, that blood-borne B-endorphin may arise from lymphocytes under experimental conditions of viral infection (Smith and Blalock, 1981). Both the AL and IL appear to be involved in basal and stimulated release of B-endorphin peptides. This is evident, for instance, by reductions in blood levels of B-endorphin-related peptides after either selective anterior- or intermediate-lobectomy (Przewlocki et al, 1982). A final consideration regarding B-endorphin's hormone status is that the peptide has been shown to be relatively stable in blood. Its circulating half life is comparable to that of other pituitary hormones (see Chemistry of B-endorphin, p 19).

Demonstrating that pituitary B-endorphin peptides are hormone-like in their origin, secretion and presence in the circulation has been an easier task than determining their biologic actions. An extensive body of literature on biological actions of opiate alkaloids together with an especially versatile antagonist of opiate receptors,

naloxone, has facilitated the search for physiological actions of the opioid forms of B-endorphin, particularly B-endorphin 1-31. These may include, but are not limited to, functions in analgesia, neuroendocrine regulation, immune function, thermoregulation, cardiovascular control, respiration and gastrointestinal motility and secretion. It should be emphasized that none of these actions can specifically be ascribed to B-endorphin since two other families of endogenous opioids, the enkephalins and dynorphins, are also lkely to serve at least some of the biological functions listed above. An additional factor which has complicated efforts to define B-endorphin's hormonal actions relates to the multiple forms of B-endorphin secreted by the pituitary gland, forms for which physiologic functions are only now being elucidated.

Consideration of the endocrine functions of pituitary B-endorphins is also confounded by limited information regarding access of these peptides to an important target organ, the brain. There, numerous actions mediated by opiate receptors are described and additional non-opioid effects are possible. Whether or not pituitary B-endorphin is able to reach CNS structures remains an unsettled issue which is addressed below.

1.3.0.1 Physiological Release and Possible Functions of Pituitary B-endorphin Peptides

One approach leading to a better understanding of the physiologic functions of pituitary B-endorphin stems from appreciation of conditions under which AL and IL forms of the peptide are normally secreted. Studies of this sort have relied almost exclusively on radioimmunoassay as the tool for measuring changes in pituitary B-endorphin release. As reviewed previously, precursors, as well as modified forms of B-endorphin 1-31, are detected equally well by most antisera used for radioimmunoassays. Without additional chromatographic procedures that separate AL and IL forms of B-endorphin from one another and from B-endorphin's immediate precursor, B-LPH, there can be no certainty as to the true chemical identity of the immunoreactive B-endorphin in blood. For this reason, uncharacterized, radioimmunoassayable forms of B-endorphin are collectively referred to as immunoreactive B-endorphin (iB-endorphin).

1.3.1 Stress-Induced Release of Pituitary B-endorphin

If a single condition is to be named which characteristically evokes pituitary secretion of B-endorphin, that condition is stress. Selye (1936) defined stress as any internal or external situation capable of activating the pituitary-adrenocortical axis. Pituitary release of iB-endorphin now shares the distinction, along with ACTH, of being a definitive pituitary response to stress. A wide variety of stressors

stimulate pituitary release of iB-endorpin: trauma (Guillemin et al, 1977), hypovolemia (Knepel et al, 1982a), septic shock (Bone et al, 1981; Carr et al, 1982), surgery (Dubois et al, 1981; Mueller, 1981), fetal distress (Gautray et al, 1977), parturition and birth (Csontos et al, 1979 ; Fletcher et al, 1980), strenuous physical exertion (Colt et al, 1981; Carr et al, 1981) and noxious experimental stimuli like inescapable electrical footshock (Rossier et al, 1977a; Hollt et al, 1978a; Akil et al, 1979; Millan et al, 1981), cold water swimming (Wardlaw and Frantz, 1980; Lim and Funder, 1983), immobilization (Mueller, 1980) and ether (Mueller, 1981).

Unusual stressful stimuli such as flashing light or loud noise may preferentially enhance IL secretion (Smelik, 1960; Moriarty et al, 1975; Moriarty and Moriarty, 1975; Miahle and Briaud, 1977). In most forms of stress, however, AL release dominates the increase in plasma levels of iB-endorphin. Several observations support this view. Concomitant (apparently equimolar) amounts of iB-endorphin and ACTH are secreted in response to stress in rats (Guillemin et al, 1977, Rossier et al, 1977a). When the forms of stress-induced plasma levels of iB-endorphin have been characterized by chromatography, stress stimuli were found to elevate not only B-endorphin-sized immunoreactivity but also B-LPH, a marker for AL secretion of iB-endorphin (Millan et al, 1981; Carr et al, 1981; Carr et al, 1982; Lim and Funder, 1983). Furthermore,

glucocorticoid treatment, which specifically reduces AL release of ACTH, has been repeatedly shown to depress stress-induced release of iB-endorphin (Guillemin et al, 1977; Rossier et al, 1977a; Akil et al, 1979; Mueller, 1980; Lim and Funder, 1983).

AL concentrations of iB-endorphin tend to remain stable during short-term secretory changes, however, some investigators have reported that acute stimulation of IL release temporarily depletes glandular content of MSH and B-endorphin peptides (Kastin et al, 1969; Moriarty and Moriarty, 1975; Moriarty et al, 1975; Millan et al, 1981; Lim and Funder, 1983). Shortly after inescapable footshock, for example, IL content of total iB-endorphin declines by approximately 25% (Millan et al, 1981; Lim et al, 1982a). Within an hour or two, however, peptide levels in the IL are replenished indicating that synthesis of POMC peptides in melanotrophs is stimulated by stress to keep pace with glandular secretion (Rossier et al, 1977a; Millan et al, 1981; Lim and Funder, 1983).

Considering the numerous forms of B-endorphin peptides secreted by the AL and IL, the responsiveness of the two lobes to stress indicates that B-endorphin peptides may be involved in a wide spectrum of crisis-related biological needs. The sensitivity of AL and IL B-endorphin release underscores the need to discern how secretion from corticotrophs and melanotrophs are regulated by the central

nervous system.

1.3.1.1 Pituitary B-endorphin and Stress-Induced Analgesia

Pituitary B-endorphin has been considered an essential element in certain mechanisms of analgesia. Like morphine, B-endorphin 1-31 has analgesic actions which are readily reversed by the opiate antagonist, naloxone (see Cox, 1982). Direct admininstration of B-endorphin 1-31 into the cerebrospinal fluid produces profound and long-lasting analgesia in animals and man (Feldberg and Smyth, 1976; Loh et al, 1976; Oyama et al, 1980). Furthermore, like morphine, B-endorphin is physically addicting (Wei and Loh, 1976). Since the pituitary is the body's largest resevoir of B-endorphin 1-31, pituitary B-endorphin was assumed to be an mediator of autoanalgesic mechanisms. Much of the evidence supporting this hypothesis has been circumstantial, correlating stress-induced increases in circulating iB-endorphin with nociception.

Stress, the principle physiologic stimulus for release of pituitary B-endorphin, is often associated with a state of analgesia (Basbaum and Fields, 1978). This stress-induced analgesia is evoked by many of the forms of stress that elevate circulating iB-endorphin, e.g., footshock, cold water swimming, immobilization and centrifugal acceleration (Akil et al, 1978; Bodnar et al, 1978; Hayes et al, 1978; see Millan, 1981). In addition,

the amount of iB-endorphin which is released in response to stress appears dependent upon the intensity of the stressful experience (Mueller, 1981), a relationship which parallels the correlation between stress intensity and the degree of analgesia induced by stress (Hayes et al, 1978). As an example, during the stress of parturition, pituitary release of B-endorphin is elevated and thought to diminish the pain of delivery (Csontos et al, 1979; Fletcher et al, 1980; Akil et al, 1978). This hypothesis is consistent with the finding that during the course of gestation, pain thresholds increase, especially just prior to delivery (Ginzler, 1980).

Additional evidence for involvement of pituitary B-endorphin in stress-induced analgesia comes from studies of hypophysectomized animals or animals whose AL secretion of POMC peptides is experimentally increased or decreased before testing levels of nociception. Ablation of the pituitary has been shown to attenuate or abolish stress-induced analgesia (Amir and Amit, 1979; Bodnar et al, 1979; Lewis et al, 1981; Millan et al, 1980). Evidence which further implicates AL B-endorphin in stress-induced analgesia is that long-term enhancement of B-endorphin secretion by adrenalectomy potentiates footshock-induced analgesia (Mareck et al, 1982; Mareck et al, 1983). Conversely, selective inhibition of corticotroph secretions with glucocorticoids attenuates stress-induced analgesia (Bodnar et al, 1979; Cheng et al, 1979; Lewis et al, 1980;

Marek et al, 1982; Marek et al, 1983; Gaiardi et al, 1983).

Nevertheless, the role of pituitary B-endorphin in analgesia is controversial. In contrast to the evidence cited above, Millan and coworkers found that glucocorticoids had no effect on footshock-induced analgesia suggesting that AL B-endorphin plays no essential part in mechanisms of stress-induced analgesia (Millan et al, 1980; Millan et al, 1981). In accord with Millan's conclusions, Lim and colleagues found that although footshock-induced analgesia is accompanied by increased secretion of iB-endorphin and partial depletion of both AL and IL content, there is no positive correlation between these hormonal changes and the time-course of stress-induced analgesia (Lim et al, 1982b).

The principle contention against pituitary B-endorphin's involvement in analgesic mechanisms is the issue of the hormone's access to the brain. CNS structures which are believed to mediate analgesia, like the periaqueductal grey area, may be insulated from circulating peptides by the blood-brain barrier (Rapoport, 1976). Although a growing body of evidence indicates that B-endorphin could mediate analgesia through peripheral antinociceptive mechanisms (Bentley et al, 1981; Rios and Jacob, 1983; Brodin et al, 1983; Randich and Maixner, 1984), the ability of circulating B-endorphin peptides to influence perception of pain as well as numerous other

physiologic functions may depend on the entry of blood-borne B-endorphin into brain target sites.

1.3.1.2 Access of Pituitary B-endorphin to the CNS

For the brain to be considered a target organ for pituitary B-endorphins, the peptides must be able to penetrate the blood-brain barrier. B-endorphin and a radiolabeled analog of B-endorphin were found to enter the cerebrospinal fluid (CSF) within minutes after systemic infusion (Pezalla et al, 1978; Rapoport et al, 1980). Similarly, B-endorphin in human CSF rapidly equilibrates to a maximum of 20% of plasma levels after intravenous infusion of the opioid (Gerner et al, 1982). Other investigators, however, have contested the possibility that B-endorphin rapidly enters the CNS even after enhanced pituitary release of the magnitude achieved during stress (see Meisenberg and Simmons, 1983). Houghten and coworkers reported that although radiolabeled B-endorphin accumulates in CSF after peripheral administration, the intact peptide was undetectable in the brain parenchyma (Houghten et al, 1980). In contrast, Merin and colleagues found radiolabeled B-endorphin in the hypothalamus as well as in the CSF after systemic administration of the peptide (Merin et al, 1980). The abilty of blood-borne B-endorphin to rapidly accumulate in the ventricles and B-endorphin's stability in the CSF (Pezalla et al, 1978) are important considerations in support of pituitary B-endorphin's influence on analgetic

mechanisms. The CNS structure best known for mediation of analgesia, the periaqueductal grey area, abutts the cerebral aqueduct. Therefore, the CSF bathes the periaqueductal grey matter with opioid concentrations of B-endorphin which reflect ongoing pituitary release conditions.

Although the ability of B-endorphin to penetrate the blood-brain barrier may be limited, certain structures of the brain do not possess this exclusionary interface with the circulation. Circumventricular areas of the CNS, like the medio-basal hypothalamus and the region around the fourth ventricle, permit free exchange of neuronal and sytemic substances. In addition to access through the circumventricular areas, there is another route through which pituitary B-endorphin peptides could gain access to the brain to influence CNS-mediated functions.

Direct routing of pituitary peptides to the brain is possible by retrograde transport which involves flow through unique vascular connections that have been demonstrated from the posterior and superior aspects of the pituitary to the medio-basal hypothalamus (Bergland and Page, 1978; Mezey and Palkovits, 1982). The concept of retrograde transport originated from the observation that pituitary hormones are more highly concentrated in effluent portal blood from the hypothalamus than in the peripheral circulation (Oliver et al, 1977). More recently, it has

been shown that the very high levels of iB-endorphin in portal blood are dramatically reduced after hypophysectomy (Lissitsky et al, 1980) indicating that most iB-endorphin in the portal circulation normally originates in the pituitary.

Stimuli which are known to provoke AL secretion of ACTH have also been shown to rapidly elevate intraventricular concentrations of ACTH (Bergland et al, 1980), but these data do not address the possibility that CSF peptides may not represent what has access to neural structures. Other studies on the types of B-endorphin in brain suggests that the N-acetylated forms of B-endorphin which are found in the CNS (Zakarian and Smyth, 1982) may actually originate in the IL (Weber et al, 1981; Evans et al, 1982), a finding which also supports retrograde flow of pituitary B-endorphin. Additional research is needed to settle the issue about circulating B-endorphin's access to the brain before pituitary B-endorphin can be an accepted participant in CNS-mediated analgetic mechanisms such as those brought on by stress.

1.3.1.3 Effects on Pituitary Secretions

Recent evidence suggests that the release of several pituitary hormones that occurs in response to stress may, in fact, be mediated by B-endorphin. The most convincing evidence for neuroendocrine actions of B-endorphin is in

control of stress-induced prolactin release. Increased prolactin secretion is as reliable a hormonal index of stress as release of ACTH and adrenal glucocorticoids and catecholamines (Nicholl et al, 1960). Interestingly, stress-induced secretion of prolactin is blocked by naloxone pretreatment (Van Vugt et al, 1978), a finding which is consistent with the ability of opiates to stimulate prolactin release (Bruni et al, 1977). Although B-endorphin has been shown to increase circulating prolactin *in vivo* (Rivier et al, 1977; Foley et al, 1979), neither this nor other opioids directly stimulate prolactin secretion *in vitro* (Grandison and Guidotti, 1977). Consequently, the involvement of opioid peptides in stress-induced release of prolactin is likely to be mediated by actions within the CNS.

One of the earliest reports indicating that corticotroph secretions might modulate prolactin secretion was that of Harms and coworkers (1975) who found that the synthetic glucocorticoid, dexamethasone, inhibits secretion of prolactin due to ether stress. They also noted that adrenalectomy potentiates stress-induced prolactin secretion (Harms et al, 1975). More recent findings by Rossier and colleagues (1980) specifically linked AL B-endorphin secretion with stress-induced prolactin secretion and indicated that Harms results reflect direct opioid effects on prolactin release. Stronger support for physiological involvement of B-endorphin in the regulation

of prolactin was obtained from the observation that anti-B-endorphin anti-sera decreased basal and stress-induced release of prolactin in rats (Ragavan and Frantz, 1981). Since B-endorphin is unable to directly influence AL release of prolactin, it is generally accepted that B-endorphin inhibits the secretion of dopamine at the median eminence (MacLeod, 1976). In fact, this hypothesis offers one of the best-supported roles for pituitary B-endorphin to date. Through a naloxone-reversible mechanism, B-endorphin decreases the activity of dopamine neurons in the basal hypothalamus (Deyo et al, 1979; Van Loon et al, 1980). As a consequence dopamine release from the tuberoinfundibular nerve terminals is reduced (Wilkes and Yen, 1980) lowering the concentration of dopamine in portal blood (Gudelsky and Porter, 1979) thereby removing tonic inhibitory control of prolactin secretion. Therefore, stress-stimulated B-endorphin release appears to disinhibit prolactin secretion through its actions on hypothalamic dopaminergic mechanisms. Consistent with this hypothesis is the observation that lesioning brain dopaminergic neurons prevents B-endorphin from increasing circulating levels of prolactin (Okajima et al, 1980). It is likely that B-endorphin influences the secretion of other AL hormones through analogous mechanisms, whereas, B-endorphin appears to affect vasopressin secretion through direct actions on the neural lobe of the pituitary.

Vasopressin release is characteristically unchanged

or lowered by stress. By contrast, animals or human subjects pretreated with naloxone respond to stress with dramatically elevated circulating levels of vasopressin (Lightman and Forsling, 1980; Knepel et al, 1982b) suggesting that vasopressin release is normally suppressed through an opiate receptor mechanism. Opiates have been shown to inhibit vasopressin release *in vitro* (Iversen et al, 1980;Lightman et al, 1982) and *in vivo* (van Wimersma Greidanus et al, 1979). Although the neurohypophysis is innervated by both enkephalin and dynorphin neurons, the following considerations indicate that pituitary B-endorphin is the endogenous opioid involved in inhibitory control of vasopressin release. As in the case of naloxone, dexamethasone-pretreatment allows stress to evoke the release of vasopressin (Knepel et al, 1982b). A common element linking these two findings is the ability of dexamethasone and naloxone to block the secretion and actions, respectively, of B-endorphin from the AL. Furthermore, dexamethasone has no effect on either methionine-enkephalin or dynorphin content in the neural lobe (Hollt et al, 1981). In addition, animals bearing lesions of brain endorphinergic neurons, like normal animals, fail to release vasopressin in response to stress (Knepel et al, 1982c), indicating that pituitary rather than brain B-endorphin is important in suppressing vasopressin release during exposure to stress.

Similar to its putative inhibitory control of

vasopressin release, pituitary B-endorphin may inhibit other pituitary hormones during stress. Consistent with the known inhibitory effects of opiates on pituitary and gonadal reproductive hormones (Cicero et al, 1976; Bruni et al, 1977), naloxone has been shown to elevate luteinizing hormone (LH) secretion in man (Quigley and Yen, 1980). As in the case of prolactin, opioids do not directly influence gonadotropin secretion indicating that B-endorphin acts through the CNS to inhibit gonadotropin releasing hormone (GnRH) (Cicero et al, 1979). Considering opioid inhibition of LH secretion, the enhanced release of pituitary iB-endorphin during strenuous physical exertion, and circulating B-endorphin's easy access to GnRH terminals in the median eminence, Carr and colleagues proposed that pituitary B-endorphin may underly the amenorrhea which is often experienced by highly conditioned female athletes (Carr et al, 1981).

The other glycoprotein hormone that could be physiologically influenced by pituitary B-endorphin is thyroid stimulating hormone (TSH). The stress-induced fall in TSH has been shown to be inhibited by naloxone (Judd and Hedge, 1982) indicating that endogenous opioids are probably involved in control of the pituitary-thyroid axis. Since intrahypothalamic injections of morphine or B-endorphin have been shown to depress TSH secretion in rats (Judd and Hedge, 1982), there may be a role for stress-induced release of pituitary B-endorphin in the

regulation of thyrotropin releasing hormone in much the same way as that for its postulated actions on prolactin and vasopressin secretion during stress.

Interestingly, B-endorphin has been shown to activate the adrenocortical axis together with its own release *in vivo* (Hollt et al, 1978b; Haracz et al, 1981). This is consistent with the report that morphine and opioid peptides stimulate CRF release from the hypothalamus both *in vitro* and *in vivo* (Buckingham, 1982). B-endorphin may also influence IL secretions. A limited number of reports provide mostly conflicting views of B-endorphin's acute effects on MSH release (van Wimersma Greidanus et al, 1979; Celis, 1980). One line of evidence, however, clearly supports an inhibitory role of opioids in IL physiology since chronic morphine treatment inhibits synthesis and secretion of IL POMC products (Przewlocki et al, 1979a; Hollt et al, 1981a; Gianoulakis et al, 1981a; Gianoulakis, 1981b). Whether this results from opioid-mediated control of IL stimulatory factors or from autoinhibition of melanotrophs by POMC opioids is matter of speculation at present.

In summary, pituitary B-endorphin secretion has the potential for influencing the global hypophyseal response to stress. These effects, in turn, could modify hormonal conditions throughout the body. The evidence for B-endorphin's effects in stress are most convincing for

stimulation of prolactin release and concomitant suppression of vasopressin secretion. Further investigations are likely to reveal whether opioid influences on pituitary responses to stress are modulated by AL and/or IL B-endorphin.

1.3.1.4 Effects on Adrenal Function

Trophic and stimulatory actions of ACTH on steroid synthesis in the adrenal cortex are well-established (see Daughaday, 1981). Although other POMC peptides from both the AL and IL may participate in regulating glucocorticoid and mineralocorticoid production (see Farah et al, in press), the effects of B-endorphin on adrenocortical physiology are quite unclear. Low to moderate (10 picomolar to 10 micromolar) concentrations of B-endorphin were shown in one study to inhibit corticosterone production in the zona fasciculata and block ACTH-stimulated steroidogenesis in both the fasciculata and the glomerulosa, whereas, extremely high doses of B-endorphin stimulated steroidogenesis in the adrenal cortex (Shanker and Sharma, 1979; Szalay and Stark, 1981). Others have found, however, no ability of B-endorphin to stimulate either corticosterone or aldosterone production, except when working solutions of peptides were found to be contaminated with ACTH (Matsuoka et al, 1981; Vinson et al, 1981a). By contrast, nanomolar doses of B-endorphin's immediate precursor, B-LPH, have been shown to stimulate

mineralocorticoid production by the adrenal cortex *in vitro* (Matsuoka et al, 1980; Matsuoka et al, 1981). New interest for this putative role of B-LPH emerged with a recent case report in which infusion of the peptide to simulate blood concentrations that occur during stress was found to elevate circulating aldosterone in man (Wiesen et al, 1983). Therefore, *in vitro* and *in vivo* evidence supports a stimulatory role of B-LPH in mineralocorticoid secretion. The B-MSH sequence contained within B-LPH may account for the steroidogenic activity of B-LPH. A growing body of evidence points to MSH as an additional POMC hormone that stimulates aldosterone secretion, especially in conditions of sodium restriction (Vinson et al, 1980; 1981a; 1981b; Szalay and Stark, 1982). Recent evidence that indirectly supports this possibility is the responsiveness of the IL to chronic mineralocorticoid treatment reported by Lim and colleagues (Lim et al, 1982a). In summary, though B-endorphin itself has no clear role in either the maintenance or secretory activity of the adrenal cortex, its immediate precursor, B-LPH, as well as MSH and ACTH collectively appear to contribute to the normal functioning of the adrenal gland.

1.3.1.5 Effects on Gonadal Function

As mentioned in relation to stress-induced release of B-endorphin, reproductive hormones are likely to be influenced by pituitary B-endorphin. The most profound

effects of endogenous opioids on reproductive hormones are evident in immature female rats. During a rather short period of prepubertal development, blockade of opioid receptors with naloxone increases luteinizing hormone (LH) secretion ten-fold (Blank et al, 1979). This rise in serum LH can be induced by treatment with anti-B-endorphin antisera (Schulz et al, 1981). This suggests that endogenous B-endorphin may normally exert an inhibitory influence over the developing hypothalamic-pituitary-ovarian axis (Schulz et al, 1981). It is not presently clear whether neuronal or pituitary B-endorphin normally inhibits LH secretion <u>in vivo</u>. Because the sensitivity of LH secretion to naloxone is blocked by estradiol (Blank et al, 1979), a treatment which acutely mobilizes IL secretion of iB-endorphin, supports a role for the pituitary opioid in endogenous opioid inhibition of LH.

1.3.1.6 Effects on Pancreatic Function

Hypoglycemia induced by insulin has been shown to increase circulating levels of iB-endorphin in humans and experimental animals (Krieger et al, 1977; Hollt et al, 1978). This response is of interest for the possible effects of B-endorphin on pancreatic function. Pancreatic B-endorphin (Bruni et al, 1979; Watkins et al, 1980) is likely to behave as a paracrine substance modulating the secretion of other islet cell hormones (Krieger, 1983).

There is some evidence to suggest, however, that circulating B-endorphin may also influence pancreatic physiology. Infusions of B-endorphin into human subjects produces hyperglycemia preceded by elevated serum glucagon and, to a lesser extent, insulin (Reid and Yen, 1981; Feldman et al, 1983). Some believe that these effects are mediated by opioid inhibition of somatostatin secretion in the pancreas (Ipp et al, 1978). By contrast, others have shown that the ability of B-endorphin to inhibit glucose-stimulated insulin secretion is a non-opiate action of the peptide which nonetheless requires both intact N- and C-termini of B-END 1-31 for full activity (Rudman et al, 1984). In another animal model, Feldman and colleagues (1983) had evidence to suggest that B-endorphin could synergistically produce hyperglycemia with glucocorticoids and adrenaline (Feldman et al, 1983). Others believe, however, that the B-endorphin-induced hyperglycemia may be mediated by the opioid's ability to enhance general sympathetic tone (Van Loon and Appel, 1981).

1.3.1.7 Effects on Fat Mobilization and Renal Function

Another putative role of B-endorphin released by stress which may, more appropriately, be considered a physiologic function of its immediate precursor, B-LPH, is in the mobilization of body fat stores. B-lipotropin was so named because of its lipolytic action on rabbit adipocytes (Lohmar and Li, 1968). B-endorphin was also

found to stimulate glycerol production by adipocytes *in vitro* and this activity was mediated independent of B-endorphin's interaction with opiate receptors (Schwandt et al, 1979; Jean-Baptiste and Rizack, 1980). Of the two peptides, B-LPH exhibits the most potent lipolytic activity *in vitro* indicating that sequences which enhance lipolysis are contained in B-endorphin's structure (Schwandt et al, 1981; Richter et al, 1984).

Other potential functions of circulating B-endorphin which have been noted but for which there is limited research are its actions on the kidney, an organ which is known to be responsive to MSH sequences from POMC in its handling of sodium excretion (Orias and McCann, 1976; Lymangrover et al, 1985). Soon after its discovery, B-endorphin like morphine, was shown to stimulate renal ornithine decarboxylase activity in rats, effects that were blocked by naloxone pretreatment (Haddox and Russell, 1979). Since ornithine decarboxylase is involved in polyamine synthesis necessary for normal growth and repair processes of cells, the authors postulated that circulating B-endorphin levels, especially those elevated by stress, could be functionally important to maintenance of renal function (Haddox and Russell, 1979).

1.3.1.8 Effects on Gut Motility and Neuromuscular Functions

The inhibition of gut motility by opiates is so

well-characterized that the guinea pig ileum is used in a classic bioassay for opioids. These actions are not mediated directly on gut smooth muscle, but on the myenteric plexi that modulate contractility of intestinal smooth muscle. Although parasympathetic enkephalinergic neurons are plentiful throughout the gut (Hughes et al, 1977), circulating B-endorphin may also influence gastrointestinal motility through its actions on myenteric plexi (Williams and North, 1979).

As noted previously, iB-endorphin has been found in developing ventral horn motoneurons of immature rats (Haynes et al, 1982). Here, the function of the opioid peptide might be to enhance neuromuscular transmission since B-endorphin was found to potentiate contractile responses of skeletal muscles to acetylcholine (Haynes, 1980). This effect is probably due to inhibition of the motor endplate form of acetylcholinesterase by B-endorphin (Haynes and Smith, 1982). Although naloxone weakly reverses B-endorphin's ability to inhibit acetylcholinesterase, the most important internal sequence for B-endorphin's effect appears to be the midportion of the peptide. Whereas methionine enkephalin failed to inhibit the enzyme, nanomolar doses of both B-LPE and C-terminally shortened IL forms of B-endorphin retain inhibitory potency (Haynes and Smith, 1982). It is an intriguing possibility that IL forms of B-endorphin as well as the AL products, B-LPE and B-endorphin 1-31, may be involved in neuromuscular function

through actions at the motor endplate.

1.3.1.9 Effects on Immunity

Stress is known to influence the body's ability to resist infection and tumor growth (Solomon and Amkraut, 1981). Previously, endocrine effects of stress on immunity focused on the role of adrenal corticosteroids but, recently, pituitary B-endorphin peptides have become candidates as regulators of immune function. One reason for the association of B-endorphin peptides and immunity was the recent discovery that interferon-alpha contains an opioid sequence which, like B-endorphin, has been found to enhance the cytotoxicity of lymphocytes (Blalock and Smith, 1981; Smith and Blalock, 1981; Mathews et al, 1983). Depending on the test of immune function, dose and type of opioid treatment administered, a wide range of effects have been described.

The types of stress which evoke naloxone-reversible analgesia have been shown to depress both the cytotoxicity and the proliferation of T-lymphocytes in vivo (Laudenslager et al, 1983; Shavit et al, 1984). Opiate antagonists have been shown to retard or enhance tumor growth in rodents depending on the dose and duration of opiate receptor blockade (Zagon and McLaughlin, 1983). This bimodal type of reaction to opiate manipulations is also characteristic of the immune system's responses to

glucocorticoids (see Healy et al, 1983) and appears repeatedly in B-endorphin's effects on tests of cellular immunity. For example, micromolar doses of B-endorphin inhibit T-cell dependent antibody production (Johnson et al, 1982). By contrast, lower doses of B-endorphin enhance the cytotoxic activity of T-lymphocytes in vitro (Mathews et al, 1983). Whereas B-endorphin enhances neutrophil chemotaxis, its N-acetylated forms inhibit motility of the white cells towards chemoattractants (Van Epps et al, 1983; Simpkins et al, 1984; Simpkins et al, 1985). These seemingly conflicting findings might be explained by diversity of opioid receptor mechanisms and by non-opioid actions of B-endorphin. For example, met-enkephalin (a delta agonist) and morphine (a mu agonist) have opposite effects in vitro on rosette formation by T-cells (Wybran et al, 1979). Another explanation for disparate immune actions of B-endorphin is that, in addition to opiate binding (Lopkor et al, 1980; McDonough et al, 1980), non-opiate binding of B-endorphin has been demonstrated on white blood cells (Hazum et al, 1979). It is, perhaps, through non-opioid receptors that B-endorphin influences lymphocyte proliferation (Gilman et al, 1982; McCain et al, 1982). Likewise, in complexes of serum complement and in neutrophil chemotaxis, non-opioid forms of B-endorphin have been found either to be equipotent or exert opposite actions from B-endorphin 1-31 (Schweigerer et al, 1982; Simpkins et al, 1985). Although studies of B-endorphin's

effects on cellular and humoral immunity are preliminary, the evidence outlined above indicates that both opioid and non-opioid forms of pituitary B-endorphin may be importantly involved in mammalian immune competence, particularly under conditions of stress.

1.3.1.10 Effects on Thermoregulation

One physiological response to stress which is naloxone-reversible is increased core body temperature (Blasig et al, 1978). Administration of subanalgesic doses of morphine or B-endorphin into the cerebroventricles induces hyperthermia, whereas, analgesic doses produce hypothermia (Huidibro-Toro and Way, 1979; Bloom and Tseng, 1981; Millan, 1981). Treatment of heat-exposed rats with naloxone induces hyperthermia, an effect which is blocked by prior hypophysectomy (Holaday et al, 1978a; Holaday et al, 1978b). These results indicate that pituitary B-endorphin may participate in thermoregulatory adjustments to stress and high ambient temperatures. Recently, however, Millan and colleagues reported that although stress-induced hyperthermia is probably mediated by B-endorphin, the increased thermogenesis in rats is probably due to CNS rather than pituitary B-endorphin (Millan et al, 1981).

1.3.2 Circadian and Age-Related Variation in Pituitary B-endorphin

Plasma and pituitary levels of iB-endorphin have been shown to covary with ACTH and MSH under a variety of conditions other than stress. Basal synthesis and secretion of pituitary POMC peptides appear sensitive to endocrine status, photoperiod, consumptive behaviors and age. These conditional changes in the physiology of pituitary B-endorphin may suggest functions which involve actions of circulating B-endorphin peptides.

1.3.2.1 Circadian Changes in Pituitary B-endorphin Secretion

As in the case of ACTH, diurnal variations of plasma and AL concentrations of iB-endorphin have been observed that are quite similar to the corresponding fluctuations in circulating corticosterone (Guillemin et al, 1959; Gibson et al, 1983; Lim and Funder, 1983). At the beginning of the activity cycle (corresponding to the onset of darkness for rats), the iB-endorphin content in both the AL and IL reaches or begins to approach its daily maximum (Gibson et al, 1983; Lim and Funder, 1983; Millington et al, personal communication). Circulating levels of iB-endorphin follow a similar course. After the peak of the activity cycle, blood and pituitary concentrations of iB-endorphin decline towards a daily nadir at a time which is reported to occur between 0400 and 1000 in the rat. Interestingly, Gibson and coworkers (1983) found greater diurnal changes occuring

in IL content of iB-endorphin than in the AL. This is curious because the amplitude of circadian changes in pituitary and blood-borne MSH is not noted to be as dramatic as that for ACTH and adrenal glucocorticoids. In addition, basal MSH secretion had not previously been shown to be as closely entrained with the day-night cycle as what Gibson and colleagues reported for IL iB-endorphin (Tilders, 1973; Usategui et al, 1976; Gibson et al, 1983). Whatever the differences, various studies concur on the observation that spontaneous changes do occur in pituitary content of most POMC derivatives and these changes appear to be circadian in nature. Preliminary evidence indicates that changes in pituitary content are reliable measures of secretion and POMC synthesis in the IL (Millington et al, personal communication).

1.3.2.2 Correlation of Pituitary B-endorphin and Feeding

Diurnal variations in pituitary and plasma content of B-endorphin peptides may signify their importance to other physiological events that are synchronized to the activity cycle in animals. Endogenous opioid peptides have been associated with waxing and waning sensitivities to pain (McGivern et al, 1979) and diurnal variations in food intake (Lowy et al, 1980). Sensitivity of rats to noxious stimuli is highest during the awake cycle and lowest during the sleep cycle (Frederickson et al, 1977) roughly corresponding to the peak and trough, respectively, of

pituitary B-endorphin levels and secretion. This cycle of nociceptive threshold may actually be entrained to feeding activity rather than to the photoperiod or activity cycle per se. Rats that are permitted unrestricted access to food are nocturnal feeders whose enhanced nightime sensitivity to painful stimuli seems to be associated with their nightime consumptive behavior. Evidence for this was obtained by restricting access to food. By the end of the period of food restriction, irrespective of the time of day, rats are significantly less responsive to noxious stimuli. Furthermore, this state of relative analgesia can be reversed with feeding or naloxone (McGivern and Berntson, 1980). Others have recently found that during spontaneous or provoked feeding, plasma levels of iB-endorphin are elevated (Davis et al, 1983). Unlike lean littermates, genetically obese rats (fa/fa) and mice (ob/ob) lack periodicity in their food intake and have been shown to have elevated pituitary, but not plasma, concentrations of iB-endorphin (Garthwaite et al, 1980; Gibson et al, 1981). Since food consumption can be provoked with opioid administration (Grandison and Guidotti, 1977) or inhibited by opiate antagonists (Brands et al, 1979; Lowy et al, 1980), it is possible that pituitary B-endorphin is causally-related to food consumption and circadian variations in nociception threshold.

1.3.2.3 Age-Related Changes in Pituitary B-endorphin

Secretion

Aging has also been associated with changes in secretion and, in the case of the IL, content of iB-endorphin. In general, circulating levels of iB-endorphin and IL concentrations of the peptides are elevated in older animals (Forman et al, 1981; Forman et al, 1983; Missale et al, 1983). Although AL content of iB-endorphin is no different in young and old rats, changes in the secretory activity of the AL could have contributed to increased plasma levels of iB-endorphin in the aged animals, especially considering that the AL normally contributes the larger amount of iB-endorphin under basal conditions (Przewlocki et al, 1982). In contrast to the changes seen with aging in pituitary and plasma iB-endorphin, brain levels of the peptide tend to decline with age (Gambert, 1981; Missale et al, 1983). Whether these changes reflect decreased utilization of IL stores and increased utilization of brain B-endorphin is not clear. However, since plasma levels of iB-endorphin increase with age in rats, the secretory capacity of the combined AL and IL do not appear impaired by senescence (Forman et al, 1981; Forman et al, 1983).

It is evident that there may be both peripheral and CNS roles for B-endorphin and related peptides. Some of these functions would best be mediated by hormonal B-endorphin, for instance actions of the peptide on the

immune system. Other actions are best served by endorphinergic neuronal systems in the brain. In some cases, such as in neuroendocrine control of prolactin or in mechanisms of analgesia, B-endorphin peptides from neuronal and pituitary sources are likely to act cooperatively.

1.4 REGULATION OF PITUITARY BETA-ENDORPHIN SECRETION

1.4.0.1 General Principles of Pituitary Regulation

Pituitary release of B-endorphin is subject to both central nervous system (CNS) and systemic hormonal control mechanisms. The most important source of regulation is the CNS which controls the synthesis and secretion of anterior (AL) and intermediate lobe (IL) B-endorphin through hypothalamic hormones and neurotransmitters (Szentagothai et al, 1972). As illustrated in Figure 2, these substances reach the AL and IL via neurovascular and neuronal routes, respectively. The special anatomical and functional relationships between the basal hypothalamus and the two lobes of the adenohypophysis are responsible for differential CNS control of B-endorphin synthesis and secretion by corticotrophs and melanotrophs.

The research described here addresses the role of dopamine neurons in regulating pituitary B-endorphin secretion. Background directly related to this research is

found in section 1.5. The following discussion is a more general consideration of the physiological factors known to control AL and IL B-endorphin secretion.

1.4.1 Regulation of the Anterior Pituitary

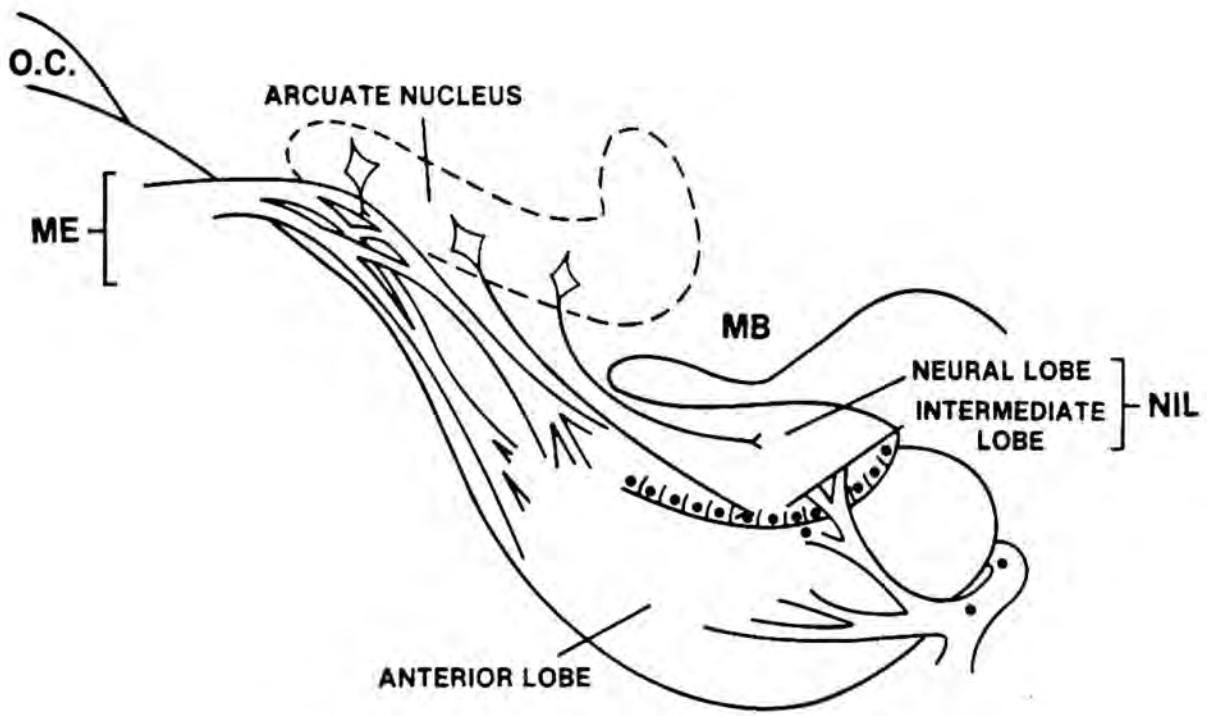
1.4.1.1 Brain Control of Anterior Lobe B-endorphin Secretion

The anatomical relations between the hypothalamus and AL provide for the management of a diverse grouping of hypophyseal cell types of which corticotrophs constitute only 3-10% (Baker et al, 1970; Moriarty, 1973). Although the AL lacks direct innervation (except vasomotor) (Rasmussen, 1938), the gland is invested with sinusoidal capillaries which are the secondary plexus of a hypothalamic-hypophyseal portal vasculature (Wislocki and King, 1936). Through this portal circulation, hypothalamic hormones and nutrients reach the gland and the AL's secretions are, in turn, transported to the systemic circulation by way of the cavernous sinuses that drain the entire pituitary (Harris, 1955). As shown in schematic in Figure 2, the primary plexus of the portal vasculature is an extensive system of fenestrated capillaries that mingle with nerve terminals at the base of the third ventricle in an area called the median eminence. By volume, these capillaries have been estimated to occupy about 30% of the median eminence (Rinne, 1966; Knigge and Scott, 1970).

Although recent studies support the possibility of retrograde blood flow in the hypophyseal-portal circulation (Oliver et al, 1977; Bergland and Page, 1978; Bergland et al, 1980; Flerko, 1980; Mezey and Palkovits, 1982), the ability of the median eminence to act as the final common pathway for regulation of the AL relies on largely unidirectional blood flow from the hypophyseal arteries through the primary portal plexus prior to entering the AL (Harris, 1955). This portal system delivers a variety of hypothalamic hormones directly to the AL but corticotrophs are responsive to only a limited number of these CNS factors. Therefore, the portal circulation provides the vascular link for neurohumoral control of AL B-endorphin secretion by the hypothalamus and, hence, by the entire CNS.

The importance of the hypothalamic-hypophyseal portal connection in the regulation of AL B-endorphin and related POMC hormones is most clearly demonstrated when the vascular connection between the gland and the basal hypothalamus is disrupted. Under these conditions, corticotrophs (and most other cell types of the gland) fail to thrive and there is an attendent loss of corticotropin activity (Harris and Jacobsohn, 1952) and immunoreactive B-endorphin (Panerai et al, 1980). These findings emphasize the trophic and stimulatory influence which hypothalamic factors exert over corticotrophs.

Figure 2. Schematic illustration of sagittal section through the rat pituitary and medio-basal hypothalamus. Hypothalamic hormones released at the median eminence (ME) are carried in the hypophyseal-portal vessels to the anterior lobe (AL) where corticotroph secretions are humorally regulated. In contrast, melanotrophs of the intermediate lobe (IL) are innervated by dopamine neurons from the arcuate hypothalamus. POMC-derived peptides secreted by the AL and IL enter the general circulation through the cavernous sinuses which drain the pituitary.


O.C.
ARCUATE NUCLEUS
ME
MB
NEURAL LOBE
INTERMEDIATE LOBE
NIL
ANTERIOR LOBE

1.4.1.2 Corticotropin Releasing Factor is the Principle Regulator of Anterior Lobe B-endorphin Secretion

Hypothalamic hormones which regulate corticotrophs are produced by neurons that terminate in the external layer of the median eminence near the fenestrated capillaries of the primary portal plexus (Hokfelt et al, 1978). The most important hypothalamic hormone controlling AL release of B-endorphin and related POMC derivatives is corticotropin releasing factor (CRF)(Buckingham, 1980). CRF was recently isolated by Vale and colleagues from brain extracts in which other hypothalamic hormones had previously been identified (Vale et al, 1981). CRF is uniquely larger than other known hypothalamic hormones, comprised of forty-one amino acids rather than the customary few to ten which are the size range of other peptidergic releasing factors. Hypothalamic CRF originates predominantly in parvocellular neurons of the paraventricular nucleus (Olschowka et al, 1982a; 1982b; Swanson et al, 1983). Although CRF neuronal systems are distributed elsewhere in the rat brain, the highest concentrations of bioactive and immunoreactive CRF is present in the median eminence (Lang et al, 1976; Fishman and Moldow, 1982; Palkovits et al, 1983). Interestingly, CRF neurons are not detectable during embryogenesis of the rat hypothalamus until several days after ACTH and B-endorphin-producing corticotrophs are first evident in

the fetal pituitary (Bugnon et al, 1982; Schwartzenberg and Nakane, 1982). Nonetheless, evidence that CRF is the principle hypothalamic hormone regulating corticotrophs is overwhelming.

Like other hypothalamic hormones, CRF is found in neurosecretory terminals of the median eminence (Pelletier et al, 1982) and measurable in portal blood at concentrations that are known to stimulate secretion of B-endorphin and related peptides from the AL (Gibbs and Vale, 1982). Corticotrophs have been found to bind and internalize [125 I]-CRF within minutes after its systemic administration (Leroux and Pelletier, 1984). Picomolar concentrations of synthetic CRF directly stimulate AL secretion of B-endorphin and ACTH with maximal stimulation (6- to 10-fold over basal release) occurring at concentrations no greater than 5 nanomoles per liter (Vale et al, 1981; Vale et al, 1983). Similarly, CRF is a potent secretogogue of AL B-endorphin and ACTH secretion in vivo (Rivier et al, 1982). The maximal stimulatory activity of synthetic CRF equals the activity previously achieved using purified hypothalamic or median eminence preparations (Vale et al, 1978; Vale et al, 1979; Gillies and Lowry, 1978). Not only does CRF have an acute stimulatory influence on corticotrophs, but prolonged CRF treatment in vitro or in vivo increases transcription of the POMC gene, POMC messenger RNA levels and the total releasable content of B-endorphin-related peptides (Vale et al, 1983; Bruhn et

al, 1984).

With the ability to quantify CRF by radioimmunoassay, experimental *in vivo* manipulations such as adrenalectomy and hemorrhage have been found to alter median eminence and portal blood content of CRF in directions expected for the major hypothalamic regulator of corticotroph secretions (Suda et al, 1983; Plotsky and Vale, 1984). Furthermore, Conte-Devolx and colleagues (1983) found that administration of anti-CRF antisera to intact and adrenalectomized rats significantly lowered immunoreactive levels of both ACTH and B-endorphin in plasma. Another piece of evidence supporting the role of CRF as the principle physiologic regulator of AL B-endorphin secretion *in vivo* was provided by lesioning the paraventricular nucleus of the hypothalmus. This treatment resulted in lower blood levels and higher AL concentrations of immunoreactive B-endorphin indicating that release had been curtailed as a consequence of damage to the CRF perikarya (Millan et al, 1984).

Corticotrophs are not soley responsive to hypothalamic CRF. It has long been appreciated that corticotroph function is an integrated response to a variety of substances (see Buckingham, 1980). Many of the factors other than CRF which directly affect B-endorphin secretion from corticotrophs are listed in Figure 3. These include other hypothalamic hormones as well as circulating

Figure 3. Schematic illustration of pituitary including list of substances that are presently thought to directly influence secretion of POMC-derived peptides from the anterior (AL) and intermediate lobes (IL) of the pituitary. Stimulatory effects are marked by (+), inhibitory effects, by (-) and putative direct effects, by (?). Abbreviations and appropriate references are included in the list below.

AII = angiotensin II (Gaillard et al, 1981; Anhut et al, 1982)
AVP = arginine vasopressin (Vale et al, 1983)
B = corticosterone, glucocorticoids (Vale et al, 1979)
CRF = corticotropin releasing factor (Vale et al, 1983)
DA = dopamine (Bower et al, 1974; Vale et al, 1979)
DOC = deoxycorticosterone, mineralocorticoids (see Lim et al, 1982a)
E2 = estrogens (see Lim & Funder, 1984)
EPI = epinephrine (Vale et al, 1979; Cote et al, 1982)
GABA = gamma-amino butyric acid (Taraskevich & Douglas, 1982)
5-HT = serotonin (Randle et al, 1983)
NE = norepinephrine (Vale et al, 1979; Cote et al, 1982)
PGE = prostaglandin E2 alpha (Vale et al, 1979)
SP = substance P (Matsumura et al, 1982)
SRIF = somatostatin (Heisler et al, 1982; Correa & Saavedra, 1983)
TSN = thymosin [fraction 5] (Healy et al, 1983)
VIP = vasoactive intestinal polypeptide (Reisine et al, 1982; Westundorf et al, 1983)

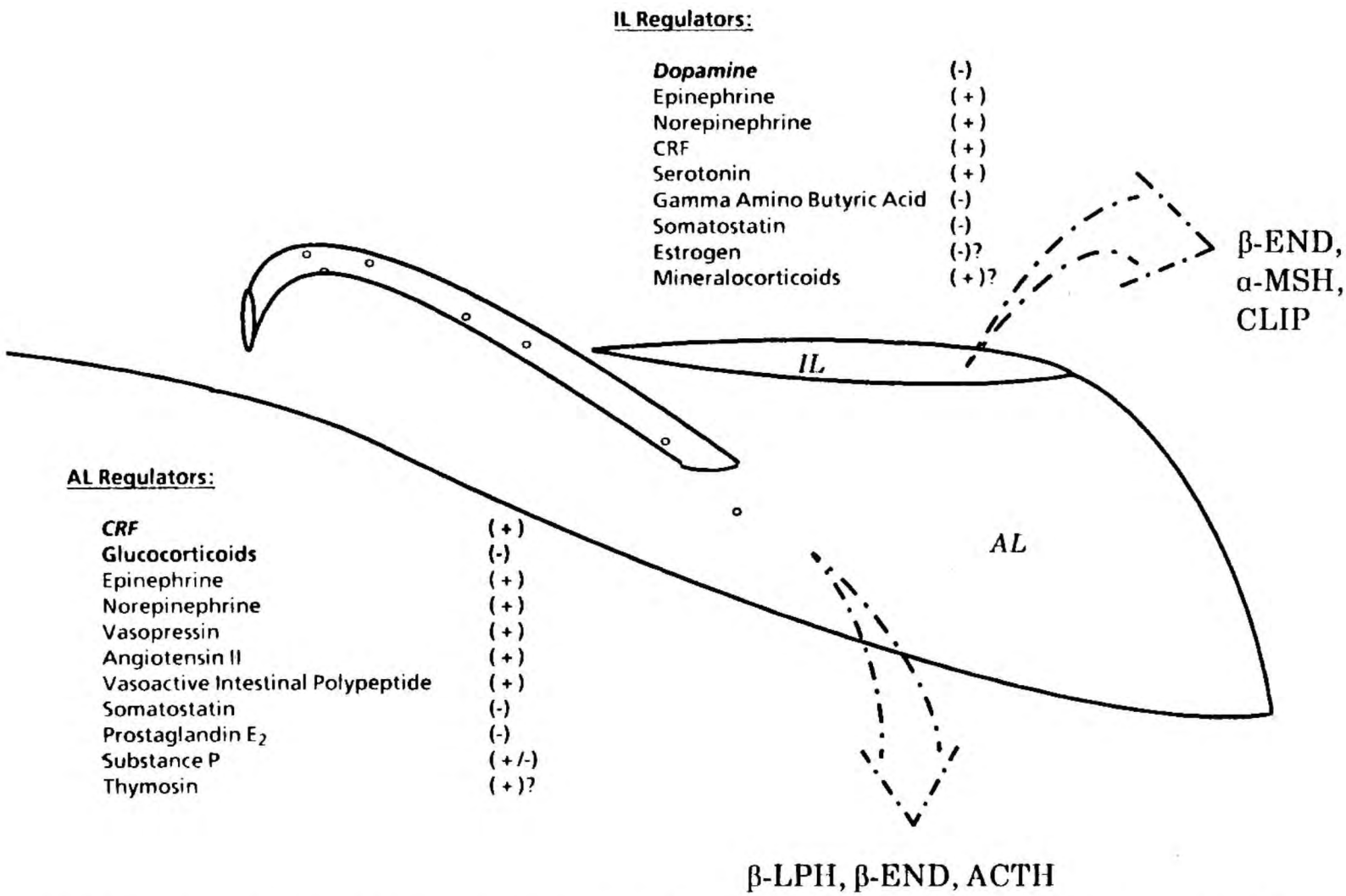


FIGURE 3. Schematic illustration of pituitary with lists of substances that directly influence secretion of pro-opiomelanocortin-derived peptides from the AL and IL.

catecholamines and adrenal glucocorticoids. Also presented are the agents to be discussed later which directly influence IL B-endorphin secretion. Non-CRF secretagogues exhibit a fraction of the effects of CRF *in vitro* (Vale et al, 1979; Gillies et al, 1982; Vale et al, 1983). Nonetheless, their ability to stimulate B-endorphin release after *in vivo* treatment, in some instances, exceeds their direct effects on corticotrophs (Knepel and Meyer, 1980; Anhut et al, 1981; 1982; Berkenbosch et al, 1981a; 1981b; Beuers et al, 1982). For example, treatment of conscious rats with vasopressin stimulates ACTH secretion far greater than effects of the peptide on corticotrophs *in vitro*. Therefore, vasopressin must act *in vivo* either by enhancing CRF release or by potentiating CRF's actions on the AL. Support for these possibilities is available from several studies. Vasopressin-induced ACTH release is blocked by pretreatment of rats with anti-CRF antiserum indicating that vasopressin acts by enhancing release of hypothalamic CRF (Rivier and Vale, 1983). Additionally, several laboratories have shown that vasopressin greatly potentiates CRF's direct actions on AL release of B-endorphin or ACTH *in vitro* (Yates et al, 1971; Portanova and Sayers, 1973; Gillies et al, 1982; Vale et al, 1983). Similar synergistic actions of angiotensin II or alpha-adrenoceptor agonists with CRF *in vitro* have also been reported (Vale et al, 1983). Whether alone or in combination with other regulatory agents, CRF is the most

physiologically important regulator of AL B-endorphin secretion. Thus, the regulation of hypothalamic CRF neurons serves as the final common pathway for CNS control of AL corticotrophs.

1.4.1.3 Hypothalamic Regulation of Corticotropin Releasing Factor

In addition to interaction of hypothalamic hormones directly at the level of the AL, several CNS neurotransmitters are known to participate in the regulation of corticotroph secretions through influences on CRF release (Buckingham, 1980). Acetylcholine is a well-documented stimulator of CRF release from hypothalamic preparations in vitro (Jones et al, 1976; Buckingham and Hodges, 1977; Jones and Hillhouse, 1977). This is consistent with diminished stress-induced secretion of corticosterone observed after cholinergic blockade in animals (Hedge and Smelik, 1968) and with enhanced adrenocortical, ACTH and B-endorphin release following cholinergic stimulation in rats and man, respectively (Abe and Hiroshige, 1974; Risch et al, 1980; 1981a, 1981b). Similar actions of serotonin and angiotensin II on CRF have been reported (Jones et al, 1976; Buckingham and Hodges, 1977) and the actions of serotonin will be described further below. Other CNS transmitters are known for their inhibitory effects on CRF release in vitro and attenuation of adrenocortical activation in animals due to stress.

These include norepinephrine and gama-aminobutyric acid (GABA) (Jones and Hillhouse, 1977; Buckingham, 1980). The actions of norepinephrine, however, are a matter of controversy as discussed below. In summary, the ability of neurotransmitters to modulate CRF synthesis and release may account for the ability of many substances that lack direct actions on pituitary B-endorphin release to influence circulating B-endorphin and adrenocortical function in vivo. For good reason, the growing consensus is that CRF as the primary mediator of CNS influence over corticotroph function (Buckingham, 1980).

1.4.1.4 Extra-Hypothalamic Regulation of Corticotropin Releasing Factor

There are several areas of the brain other than the hypothalamus which are thought to indirectly participate in controlling CRF and, hence, AL secretion of B-endorphin. Information about the relationship of non-hypothalamic brain structures to control of AL B-endorphin secretion has been gleaned from studies on CNS control of glucocorticoids and ACTH. Some of these extra-hypothalamic structures include the hippocampus (Knigge and Hayes, 1963; Mandell et al, 1963), the amygdala (Mandell et al, 1963; Allen and Allen, 1974) and the brainstem (Taylor, 1969). The neurotransmitters involved in extra-hypothalamic control of corticotrophs are not well-established. There is some evidence to suggest that the amininergic neurotransmitters,

serotonin and norepinephrine participate in the regulation of CRF by extra-hypothalamic brain structures. Serotonin neurons which originate in the dorsal raphe nuclei and terminate in the hypothalamus have been implicated in both stimulatory and inhibitory actions on corticotroph secretions (Vernikos-Danellis et al, 1977). Recent pharmacologic evidence suggests that serotonin's principle effect is to enhance CRF release under basal and stress conditions (Fuller, 1981; Sapun et al, 1981; Bruni et al, 1982; Sapun-Malcolm et al, 1983). A stimulatory role of serotonergic neurons in corticotroph function is supported by the observation that serotonin increases the synthesis and release of CRF from basal hypothalamus in vitro (Jones et al, 1976; Buckingham and Hodges, 1977). Furthermore, serotonin is one of the few transmitters in the hypothalamus known to be responsive to the glucocorticoids products of the corticotroph target organ, the adrenal cortex (Vermis et al, 1976).

Evidence for noradrenergic participation in control of corticotroph function is abundant but conflicting, perhaps due to the differences in direct versus indirect actions of norepinephrine on corticotrophs. The earlier view (established by in vivo pharmacologic manipulations of endogenous adrenergic activity) was that brain noradrenergic neurons inhibit the secretion of ACTH (and consequently B-endorphin)(Ganong, 1972; Van Loon, 1973; Eisenburg, 1975). This conclusion was supported by the

ability of norepinephrine to inhibit spontaneous and evoked CRF release from isolated hypothalamic preparations *in vitro* through an alpha- adrenoceptor mechanism (Jones et al, 1976; Buckingham and Hodges, 1977). In recent support for noradrenergic inhibition of CRF, Millan and colleagues have shown that lesioning brainstem noradrenergic neurons which innervate the hypothalamus increases basal and stress-induced release of AL B-endorphin in rats (Millan et al, 1982a; 1982c). By contrast, several recent studies show that alpha- and beta-adrenergic mechanisms are involved in stimulatory control of AL B-endorphin release. In these studies specific adrenergic receptor agonists and antagonists were administered ***in vitro*** and ***in vivo*** (Pettibone and Mueller, 1981a; 1981b; 1982a; 1982b; Berkenbosch et al, 1981a; 1981b; Knepel et al, 1981). The results of these studies show that direct stimulation of AL (as well as IL) release occurs through beta-adrenoceptor mechanisms and that an alpha-adrenoceptor-stimulated release is also possible via CNS mechanisms. Furthermore, Smythe and colleagues recently reported a strong positive correlation between the activity of noradrenergic neurons in rat hypothalamus and the secretion of ACTH over a wide range of endocrine conditions (Smythe et al, 1983). Their findings reaffirmed results by others who had observed that adrenalectomy increased hypothalamic noradrenergic activity in association with increased ACTH release (Hedge et al, 1976).

1.4.1.5 Dopaminergic Participation in Brain Control of Corticotropin Releasing Factor

Brain dopamine neurons may be uniquely suited to influence AL B-endorphin secretion. As outlined in later sections, a small group of hypothalamic dopamine neurons are known to control secretion of MSH from the IL and presumably exert comparable control of B-endorphin secretion from melanotrophs. Although dopamine has no direct influence on AL corticotrophs (see Figure 3), considerations reviewed in section 1.5 indicate that brain dopamine neurons might govern AL as well as IL secretion of B-endorphin through actions on CRF.

1.4.1.6 Hormonal Control of Anterior Lobe B-Endorphin Secretion

Of the circulating hormonal factors that routinely modulate AL secretion of iB-endorphin, the most important regulators are adrenal glucocorticoids. Corticosterone is the principle glucocorticoid in the rat and has long been suspected of inhibiting the secretion of ACTH (Sayers and Sayers, 1947). Yates first demonstrated that corticosterone mediates negative feedback control of adrenocortical secretion at some level higher than the adrenal cortex itself and suggested that the pituitary might be its target (Yates et al, 1961). Corticosterone and other

glucocorticoids have recently been shown to suppress AL release of B-endorphin a well as ACTH *in vitro* (Vale et al, 1978; Vale et al, 1979; Simantov, 1979; Vale et al, 1983).

Treatments which acutely or chronically reduce circulating levels of glucocorticoids enhance the synthesis, storage and secretion of AL POMC peptides. Metyrapone, which acutely inhibits adrenocortical synthesis of glucocorticoids (Chart et al, 1958), has been shown to elevate blood-borne iB-endorphin commensurate with the documented increase of circulating ACTH (Hollt et al, 1978a; Wardlaw and Frantz, 1979; Pettibone and Mueller, 1984). Chronic loss of glucocorticoid negative feedback following adrenalectomy also increases circulating levels of both iB-endorphin and ACTH, stimulating the synthesis and increasing AL content of POMC peptides (Guillemin et al, 1977; Rossier et al, 1979; Rosa et al, 1980; Hollt et al, 1981b; DeSouza and Van Loon, 1983; Lim and Funder, 1983).

Although glucocorticoids are known to directly inhibit corticotroph synthesis and secretion of POMC peptides beginning at the level of gene transcription (Roberts et al, 1982; Sachter et al, 1982; Civelli et al, 1983), corticosterone is likely to exert inhibitory control through actions on the CNS as well (Buckingham, 1979; McEwen, 1977). Limbic structures mentioned earlier which are known to be anatomically associated with the endocrine

hypothalamus (Raisman and Field, 1971) have been shown to accumulate [3H]-corticosterone more effectively than the AL itself (Stumpf, 1971; deKloet et al, 1975; Warembourg, 1975a). There is some evidence that these same CNS structures may be importantly involved in feedback control of corticotrophs under conditions of stress (Feldman et al, 1976; Feldman and Conforti, 1980; Feldman et al, 1982). The most widely accepted CNS site for feedback actions of glucocorticoids is the hypothalamus (Smelik, 1977). Although hypothalamic binding of glucocorticoids is weaker than that observed in other brain structures (Warembourg, 1975a; McEwen, 1977), functional inhibition of hypothalamic CRF activity has been demonstrated repeatedly using either corticosterone or dexamethasone (Jones and Hillhouse, 1977; Smelik, 1977; Buckingham, 1980). Furthermore, the interaction of glucocorticoids with both serotonergic and noradrenergic neurons terminating in the hypothalamus are linked to feedback control of the CNS-pituitary-adrenal axis (Vermis et al, 1976; Stith and Person, 1982). Thus, the ability of glucocorticoids to regulate AL secretion of B-endorphin and other corticotroph products is likely mediated at the level of hypothalamic CRF as well as directly at the pituitary.

As indicated in Figure 3, numerous neuronal and hormonal signals are liable to be direct physiological regulators of AL B-endorphin secretion via their common route of access to corticotrophs, the portal vasculature.

Evidence from early studies of adrenocortical activity as well as recent investigation of corticotroph secretions have implicated adrenal medullary catecholamines, primarily epinephrine, as stimulatory factors in pituitary release of B-endorphin-related hormones (Sayers and Sayers, 1947; Tilders et al, 1980; Berkenbosch et al, 1981a; 1981b; Pettibone and Mueller, 1981b; 1982a; 1982b). Similarly, another systemically produced hormone, angiotensin II, has also been implicated in the control of B-endorphin secretion from the AL by evidence from *in vivo* and *in vitro* studies (Knepel and Meyer, 1980; Gaillard et al, 1981; Beuers et al, 1982; Anhut et al, 1982; Vale et al, 1983). Thyroid hormone may also be important to maintenance of pituitary concentrations of B-endorphin (Gambert et al, 1980) but neither the extent to which thryoid hormones affect normal release nor the level at which their effects are mediated are presently known.

1.4.2 IL Regulation

1.4.2.1 CNS Control of IL B-endorphin Secretion

In light of the differences that exist between the biosynthetic products of POMC in the IL and AL, it is not surprising that regulation of IL B-endorphin is distinct from that of the AL. Even though both tissues share a common embryonic origin (Wingstrand, 1966a; 1966b), in most adult mammals the IL adheres to the neural lobe and is

physically separated from the AL and its vasculature by a cleft (Wingstrand, 1966b; Howe, 1973). Together, the IL and neural lobe each contribute about half of the total mass of the pituitary structure commonly referred to as the neurointermediate lobe (NIL) (Gosbee et al, 1970). In the rat, the NIL is about one-tenth the size of the AL yet the IL's concentration of B-endorphin and related peptides exceeds that of the AL by ten-fold (Rossier et al, 1977b; Liotta et al, 1978; Mueller, 1980). Part of the reason for this difference in total content is that, in contrast to the mixture of cell types comprising the AL, the IL is a homogeneous population of melanotrophs (Howe, 1973).

In primary culture, melanotrophs exhibit a high level of spontaneous depolarizations which corresponds to their predisposition to elevated secretory activity (Douglas and Taraskevich, 1978). In vitro, melanotrophs secrete from 7% to 16% of their hormonal content each day (Mains and Eipper, 1979; Eipper and Mains, 1981). This propensity for high level secretory activity in vitro only becomes evident in vivo when the IL looses its functional connections with the central nervous system (CNS). This indicates that the CNS normally inhibits IL secretion of POMC peptides. Tilders and Smelik (1978) found, for instance, that shortly after lesioning the basal hypothalamus, the MSH content of the IL decreases. This loss of hormonal content results from unreplenished release of POMC peptides. Similarly, ILs autotransplanted to the

kidney capsule become acutely depleted of MSH stores but gradually recover hormonal content while maintaining elevated secretory activity (Kastin and Ross, 1964). Therefore, in contrast to primary stimulatory control and trophism of ACTH and B-endorphin secretion by AL corticotrophs, IL secretion of POMC peptides is tonically suppressed by the CNS. As outlined below, there are anatomical and biochemical basis for this differential control of the IL.

With respect to neuroendocrine regulation, the most prominant anatomical difference between the AL and IL is that the IL of rodents is "virtually avascular" yet is directly innervated by the hypothalamus (Wingstrand, 1966; Howe, 1973). The only capillary beds in the proximity of melanotrophs are those supplying the neurohypophysis. These neural lobe capillaries appear capable of sustaining the IL since the NIL survives vascular lesions of the AL (Daniel and Prichard, 1956). Although short portal vessels connect the neural lobe plexus with that of the AL, there is no direct vascular route accessing the hypothalamic hormones of the median eminence to the IL. Not only does this limit neurohumoral control of the IL by the hypothalamus, but the lack of parenchymal blood flow to melanotrophs might seem incompatible with an endocrine role for the IL. This, however, is not the case since IL peptides are measureable in systemic blood and their levels vary in normal and experimental conditions like stress

(Kastin et al, 1969; Usategui et al, 1976; Van Wimersma Greidanus et al, 1979). Bulk exchange of nutrients and IL secretory products may occur by way of channels in the IL that resemble bile canaliculi of the liver. These channels are intercalated among the clusters of melanotrophs and lead to the capillary network at the IL's border with the neural lobe (Howe and Maxwell, 1968; Howe, 1973; de Bold et al, 1980).

After isolation and synthesis of CRF, this hypothalamic factor was found to be a direct secretagogue for melanotrophs in vitro (Vale et al, 1983) and in vivo (Proulx-Ferland et al, 1982), however, the potency of CRF on cultured melanotrophs is far less than that of epinephrine (Vale et al, 1983). Since the vascular access for CRF to the IL is limited at best and because it is not clear that CRF neurons innervate the IL, it is unlikely that CRF normally governs IL secretion of POMC peptides in rats. This view is supported by the finding that passive immunization of rats with anti-CRF antiserum suppresses ACTH secretion in vivo without causing similar reductions in circulating MSH (Conte-Devolx et al, 1983). Therefore, although the IL may be sensitive to pharmacologic application of CRF, this hypothalamic hormone is probably not normally involved in the regulation of melanotroph secretions.

Since the anatomy of the IL doesn't readily provide

for neurovascular control of melanotroph function, how does the brain regulate secretion from the IL? As will be discussed in the next section, the IL is richly innervated by hypothalamic neurons which, in most mammals, are predominantly dopaminergic (see Howe, 1973). Dopamine neurons regulate melanotroph secretions through tonic inhibition and have been shown to be the principal controllers of IL function.

Other neurotransmitters also have been detected in nerve terminals of the IL in addition to dopamine. They are listed in Figure 3 and include gamma-amino butyric acid (GABA) (Oertel et al, 1982; Vincent et al, 1982), serotonin (Westlund and Childs, 1982; Leranth et al, 1983) and somatostatin (Saavedra et al, 1983). Recent evidence suggests inhibitory roles of both GABA and somatostatin versus stimulatory actions of serotonin (Fisher and Moriarty, 1977; Taraskavich and Douglas, 1982; Correa and Savaadra, 1983; Randle et al, 1983). Whether any of these neurotransmitters is as potent a regulator of melanotrophs as dopamine remains to be seen. Judging from extensive evidence for potent dopaminergic inhibition of alpha-MSH secretion, it is unlikely that other neural systems will serve as pivotal a role as tuberohypophyseal dopamine neurons in controlling the IL (Tilders and Smelik, 1977; 1978; 1979).

1.4.2.2 Hormonal Control of IL B-endorphin Secretion

It is presently thought that similar to corticotrophs, melanotrophs release B-endorphin and related IL peptides upon stimulation by circulating catecholamines (Tilders et al, 1980; Berkenbosch et al, 1981b; Pettibone and Mueller, 1982b; 1984). Melanotrophs are stimulated by adrenergic agonists and this effect is mediated by beta-adrenoceptors in the IL (Cote et al, 1980). Beta-adrenergic stimulation of the IL increases the activity of adenylate cyclase (Munemura et al, 1980; Cote et al, 1980) along with an associated release of MSH and B-endorphin (Cote et al, 1980; Vermes et al, 1980b; Pettibone and Mueller, 1982a; 1982b). After dilution in systemic blood, hypothalamic hormones (e.g., CRF) might not reach the IL in high enough concentrations to influence melanotroph secretion. In contrast, circulating catecholamines appear capable of stimulating IL release of B-endorphin and MSH in the rat (Tilders et al, 1980; Berkenbosch et al, 1981b; Berkenbosch et al, 1983).

Unlike corticotrophs, melanotrophs are not inhibited by physiological doses of glucocorticoids (see Roberts et al, 1982). The relative lack of glucocorticoid binding in melanotrophs (Warembourg, 1975b; Rees et al, 1977) is consistent with the well-documented unresponsiveness of the IL to circulating glucocorticoids (Kastin et al, 1969; Dunn et al, 1972a; Usategui et al, 1976). Accordingly, IL content of immunoreactive B-endorphin is unaffected by

chronic glucocorticoid treatment or adrenalectomy (Rossier et al, 1979; Hollt et al, 1981b; Lim et al, 1982; Lim and Funder, 1983). Interestingly, Lim and colleagues have found that iB-endorphin in the NIL is increased by mineralocorticoid treatment, however, this response of melanotrophs is not nearly as sensitive as the AL response to glucocorticoids (Lim et al, 1982; Lim and Funder, 1983).

Ovarian steroids appear to influence IL but their net influence on POMC secretion from this lobe remains an unsettled issue. Consistent with reports which show that estrous is accompanied by decreased pituitary MSH content (Taleisnik and Tomatis, 1969; Norman et al, 1972), others have found that just prior to estrous, circulating levels of MSH increase (Celis, 1975; Thody et al, 1981). Synthesis and release of IL B-endorphin appear to be similarly associated with cycles in levels of ovarian steroids. During the afternoon of proestrous, both plasma and IL content of iB-endorphin increase but fall again before diestrous (Ishizuka et al, 1982). These reciprocal changes in content and release of POMC peptides are thought to occur in response to high levels of estrogen since acute estrogen treatment reportedly elevates plasma levels of MSH in ovariectomized rats (Celis, 1977; Thody et al, 1981).

Curiously, there are several reports which indicate that chronic exposure to ovarian steroids inhibit the synthesis and secretion of POMC peptides from the IL,

rather than stimulating IL production of B-endorphin. To begin with, the concentration of iB-endorphin in the IL of female rats is lower than that in males (Mueller, 1980). That estrogens are involved has been shown by the ability of chronic estrogen treatment to reduce IL content and plasma levels of iB-endorphin in both male and ovariectomized female rats (Mueller, 1980; Lim and Funder, 1984). What is particularly interesting about inhibitory effects of ovarian steroids on pituitary B-endorphin is that these actions may amount to feedback loops in the pituitary-gonadal axis since opiates are known to reduce circulating levels of pituitary and gonadal reproductive hormones (Cicero et al, 1976; Bruni et al, 1977). The differences between spontaneous changes of IL POMC peptides during the estrous cycle and the long term effects of ovarian steroids on the IL require further investigation.

Experimental evidence indicates that the synthesis and secretion of IL B-endorphin is sensitive to opiates (Przewlocki et al, 1979a; Hollt et al, 1981a; Gianoulakis et al, 1981a, 1981b; Lim and Funder, 1983) perhaps indicating a regulatory role of an endogenous opioid system in IL function. Opiates acutely elevate plasma levels of iB-endorphin (Hollt et al, 1978b; Haracz et al, 1981) but chronic opiate administration is associated with diminished synthesis of IL B-endorphin and reduced secretory responsiveness of the AL as well as the IL to stimulation (Przewlocki et al, 1979a; Hollt et al, 1981b; Gianoulakis

et al, 1981a; 1981b). The long term effects of opiates on AL B-endorphin secretion are consistent with well-known ACTH and adrenocortical responses to morphine dependence. An involvement of opioids in the regulation of IL secretions is further supported by the finding that depletion of IL iB-endorphin due to cold water swimming can be prevented by naloxone pretreatment (Lim and Funder, 1983). It is not presently known whether the inhibitory actions of estrogens and opiates result from direct or indirect actions of these compounds on melanotrophs.

1.5 THE ROLE OF DOPAMINE NEURONS IN REGULATING PITUITARY BETA-ENDORPHIN SECRETION

Regulation of circulating B-endorphin involves control of secretion from the IL and AL, two anatomically and physiologically distinct tissues of the adenohypophysis. Most is known about the physiologic role of dopamine neurons in regulating melanotroph secretions.

1.5.0.1 Dopamine Neurons and Intermediate Lobe B-endorphin Secretion

Unlike the AL, the IL is known to be innervated by hypothalamic neurons which are predominantly inhibitory to the secretion of melanotrophs in mammals (Howe, 1973). In the rat, most of these hypothalamic neurons are

dopaminergic (Bjorklund et al, 1973) with perikarya in the arcuate and adjacent periventricular hypothalamic nuclei. These so-called tuberohypophyseal dopamine neurons project to the IL by way of the neural lobe (Bjorklund et al, 1973; Moore and Bloom, 1978) and the nerve terminals make synaptic-like contacts with melanotrophs (Baumgarten et al, 1972). Conditions which enhance neuronal depolarization have been shown to increase efflux of dopamine from the NIL in vitro (Sharman et al, 1982). Depolarization-induced dopamine release probably accounts for the ability of high potassium to inhibit rather than stimulate the release of B-endorphin related peptides from freshly excised rat NIL (Vermes et al, 1980a; 1980b).

Dopaminergic agonists dramatically slow the frequency of spontaneous action potentials recorded from these cells (Douglas and Taraskevich, 1978; 1982) and similar doses of dopamine have been shown to inhibit basal and stimulated release of B-endorphin and other POMC peptides from freshly dissociated IL (Bower et al, 1974; Munemura et al, 1980; Cote et al, 1982) and from primary cultures of the IL (Vale et al, 1979). It is likely, therefore, that dopamine controls melanotrophs partly by inhibiting stimulus-secretion coupling (Douglas and Taraskevich, 1978; 1980). As discussed below, more recent evidence points to the inhibitory influence of dopamine on the enzyme, adenylate cyclase, and its relationship to IL secretion (Cote et al, 1982).

The IL contains dopaminergic receptors (Cronin et al, 1978; Sibley and Creese, 1980) that have recently been classified as the dopamine-2 (D2) subtype (Stefanini et al, 1980; Cote et al, 1982). According to Kebabian and Calne (1979), dopamine is able to exert actions on target tissues through pharmacologically and biochemically distinct receptors that can be broadly classified as either dopamine-1 (D1) or dopamine-2 (D2) receptor subtypes. D2 receptor subtypes display a high affinity for dopamine agonists and modulate the physiological activity of target tissues without interacting with or via inhibition of adenylate cyclase (Kebabian and Calne, 1979). Dopamine receptors on melanotrophs are now used as model D2 receptors, but cyclic adenosine monophosphate production and related AL secretion of prolactin or the synaptic release of acetylcholine in the neostriatum have also been shown to be inhibited by D2 receptor activation (Kebabian and Calne, 1979; Wong et al, 1983). In contrast, D1 receptor subtypes, which have not been found in the IL, have a much lower affinity for dopaminergic agents and mediate biological responses through positive interactions with the enzyme, adenylate cyclase. For example, stimulation of D1 receptors in the bovine parathyroid gland results in increased adenylate cyclase activity with associated production of the cyclic adenosine monophosphate second messenger and elevated secretion of parathyroid hormone (Brown et al, 1980). Although additional types of

dopamine receptors have been proposed (see Costall and Naylor, 1981), the present consensus is that the D1 and D2 classifications represent the most durable model for representing biologically meaningful interpretations of dopaminergic mechanisms.

Using a variety of dopaminergic agonists and antagonists, Kebabian and colleagues have found that the rat IL contains about 15 femtomoles of high affinity dopaminergic binding sites (Frey et al, 1982). Through interaction with these presumed D2 sites, dopaminergic agonists inhibit MSH secretion and adenylate cyclase activity in a correlated fashion (Cote et al, 1982; Frey et al, 1982; Meunier and Labrie, 1982). The ability of D2 specific antagonists to reverse dopaminergic inhibition of electrical activity (Douglas and Taraskevich, 1978), hormone secretion (Vermes et al, 1980b; Tilders et al, 1981) and adenylate cyclase activity (Cote at al, 1982) are each taken as evidence to support the view that tuberohypophyseal inhibition of IL B-endorphin release is physiologically mediated by D2 receptors on melanotrophs (Cote et al, 1982). The importance of cAMP as an intracellular regulator of melanotrophs is becoming increasingly evident as recent evidence indicates that circulating catecholamines may stimulate IL secretion of POMC peptides through a beta-adrenoceptor mechanism which acts through increased intracellular production of cAMP (Tilders et al, 1980).

1.5.0.2 Dopamine Neurons and AL B-Endorphin Secretion

Although dopamine has no direct effect on corticotrophs, considerations reviewed here indicate that brain dopamine neurons might indirectly govern AL as well as IL secretion of B-endorphin and related POMC peptides.

1.5.0.3 Neuroendocrine Importance of Brain Dopamine Neurons

Intra- and extrahypothalamic dopaminergic pathways are known to be anatomically and functionally linked to hormone secretion from the pituitary gland. Hypothalamic dopamine neurons which are most clearly associated with neuroendocrine control originate from cell bodies in the arcuate and adjacent periventricular nuclei and project to the neural and intermediate lobes of the pituitary and to the median eminence (Dahlstrom and Fuxe, 1964; Ungerstedt, 1971; Bjorklund et al, 1973; Moore and Bloom, 1978). Dopamine neurons originating in the rostral pole of the arcuate nucleus innervate the IL and a slightly more caudal group innervates the neural lobe (Bjorklund et al, 1973). These neurons are collectively refered to as tuberohypophyseal dopamine neurons. Virtually all of the dopamine in the IL is accounted for by the tuberohypophyseal innervation of the lobe (Smith and Fink, 1972; Alper et al, 1980). Consequently, dopaminergic inhibition of B-endorphin and MSH release from melanotrophs

is attributed entirely to this discrete dopaminergic system.

Most of the intrahypothalamic dopamine neurons are situated from the mid- to caudal arcuate nucleus and project to the median eminence. These are tuberoinfundibular dopamine neurons (Ungerstedt, 1971; Lindvall and Bjorklund, 1978; Moore and Bloom, 1978) which are known to be the primary inhibitory regulators of prolactin secretion from the AL (MacLeod, 1976). In addition to inhibiting prolactin release, these neurons are also believed to govern gonadotropin secretion by inhibiting secretion of gonadotropin releasing hormone (GnRH) at the median eminence (Lofstrom et al, 1976; MacLeod, 1976; Wiesel et al, 1978; Sladek et al, 1978). In a similar manner, the tuberoinfundibular system could influence AL B-endorphin secretion by acting on CRF terminals in the median eminence.

Although most of the dopamine in the median eminence and basal hypothalamus originates in tuberoinfundibular dopamine neurons, a significant amount cannot be accounted for by the arcuate neurons alone. Incertohypothalamic, periventricular and mesencephalic dopamine neurons have been described (Lindvall and Bjorklund, 1978; Moore and Bloom, 1978) which innervate the hypothalamus and may also influence CRF.

Incertohypothalamic dopamine neurons most closely

resemble the intrahypothalamic dopaminergic system in that both originate within the diencephalon and appear to innervate structures within the vicinity of their nucleus of origin. The incertohypothalamic cell bodies are located in the caudal hypothalamus and zona incerta (Bjorklund et al, 1975). Also included in this system are dopamine neurons situated around the third ventricle in the anterior hypothalamus/preoptic area (Bjorklund et al, 1975; Lindvall and Bjorklund, 1978). Little is known about the physiology of incertohypothalamic dopamine neurons, however, their innervation of regions like the septum, medial preoptic area and, particularly, the paraventricular nuclei where CRF neurons originate suggests their involvement with control of AL B-endorphin secretion (Bjorklund et al, 1975; Palkovits et al, 1977a; Moore and Bloom, 1978).

The periventricular system is a diffuse network of dopaminergic (and noradrenergic) neurons along the ventricular system of the diencephalon and brainstem which shares overlapping innervations with the incertohypothalamic system in the rostral hypothalamus. The ventral group of the periventricular system is predominantly dopaminergic and innervates the paraventricular nucleus as well as septal nuclei and the bed nucleus of the stria terminalis (Lindvall and Bjorklund, 1978). Each of these structures has been associated with regulation of paraventricular neurons and/or AL corticotrophs (Willoughby and Martin, 1978;

Ellendorff and Parvisi, 1980; Millan et al, 1984).

Another extrahypothalamic dopaminergic system which may participate in controlling AL B-endorphin secretion is located in the ventral tegmental area of the brainstem. In addition to major projections of these neurons to limbic structures like the septum and the amygdala (Lindvall and Bjorklund, 1978; Moore and Bloom, 1978), the mesencephalic dopamine system also innervates the hypothalamus, contributing up to 40% of the dopamine concentrated there (Kizer et al, 1976; Palkovits et al, 1977b).

The non-hypothalamic dopaminergic pathways described above offer multiple anatomical pathways for conveying diverse physiological signals to the hypothalamus where CNS control of AL B-endorphin release is expressed through CRF release. There is an additional innervation of the frontal cortex by mesencephalic dopamine neurons which could also be involved with dopaminergic control of AL B-endorphin release despite its lack of direct anatomical connections with the hypothalamus. In summary, brain dopaminergic systems decribed here offer several neural substrates through which CRF neurons can be appraised of CNS stimuli that ultimately govern AL as well as IL B-endorphin secretion.

1.5.0.4 Functional Associations of Dopamine Neurons with CRF

By and large, the evidence which functionally associates dopamine with AL POMC secretions is rather mixed. Some evidence supports a stimulatory role but most indicates that dopamine neurons should have little effect or else act as inhibitors of CRF release. The earliest indication that any of the catecholamines neurotransmitters might stimulate the pituitary-adrenocortical axis came from experiments in which amphetamine was found to diminish the adrenal content of ascorbic acid, an indirect measure of stimulated steroid synthesis and secretion (Ohler and Sevy, 1956). Amphetamine enhances release and reduces inactivation of catecholamines (Glowinski and Axelrod, 1965), therefore, its ability to increase circulating glucocorticoid metabolites (Naumenko, 1967) and corticosterone (Knych and Eisenberg, 1979) was taken as evidence for aminergic stimulation of the pituitary-adrenal axis. More recent findings that circulating ACTH and B-endorphin are elevated in amphetamine-treated subjects is more direct evidence consistent with the possibility that catecholamines stimulate CRF secretion (Brown et al, 1978; Cohen et al, 1981). Since the amphetamine-induced secretion of adrenal steroids is prevented by pretreatment with phenoxybenzamine, a well-known adrenoceptor antagonist (Ohler and Sevy, 1956; Knych and Eisenberg, 1979), the effects of amphetamine were interpreted as evidence for adrenergic stimulation of ACTH secretion. Two recent findings, however, suggest that dopamine is just as likely

as norepinephrine or epinephrine to stimulate the pituitary-adrenal axis. First, amphetamine stimulates dopaminergic more potently than adrenergic neurotransmission (Holmes and Rutledge, 1976). Second, phenoxybenzamine has recently been shown to be an irreversible antagonist of dopaminergic as well as adrenergic receptors (Lehmann and Langer, 1981). Therefore, phenoxybenzamine-inhibited activation of the pituitary-adrenal axis by amphetamine may also support dopaminergic stimulation of ACTH and B-endorphin release.

Results from direct administration of dopamine favors the possibility of a stimulatory dopaminergic role in ACTH and AL B-endorphin secretion. Peripheral or central injections of dopamine elevate blood levels of corticosteroids in rats (King, 1969; Abe and Hiroshige, 1974). These hormonal effects of dopamine occur at doses lower than those shown to exert cardiovascular effects (King, 1969) indicating that the effects can be attributed to dopamine rather than to its conversion to other catecholamines in the sympathetic nervous system. Consistent with these results is the finding that peripherally-administered L-dihyroxyphenylalanine (L-DOPA), the precursor for dopamine synthesis, increases circulating levels of glucocorticoids in man (Wilcox et al, 1975; Lightman, 1981). Similar effects of L-DOPA on iB-endorphin release have likewise been observed (Cohen et al, 1981). The additional ability of L-DOPA to potentiate

metyrapone-induced release of ACTH (Hsu et al, 1976) suggests that brain dopamine neurons might participate in glucocorticoid feedback control of the pituitary-adrenal axis. Together these results indicate that dopamine neurons stimulate CRF release, thereby increasing AL secretion of iB-endorphin.

Consistent with a stimulatory action of dopamine on CRF release are data which indicate that, like B-endorphin and ACTH release, the activity of certain hypothalamic and extra-hypothalamic dopamine neurons parallels release of corticotroph hormones during stress (Palkovits et al, 1975; Hedge et al, 1976; Roth et al, 1982; Saavedra, 1982; Bannon and Roth, 1983; Smythe et al, 1983) and throughout the circadian cycle (Simon and George, 1975; Owasoyo et al, 1979). Additionally, stress-induced activation of mesocortical dopamine neurons is sensitive, like secretion of AL POMC peptides, to glucocorticoid inhibition (Dunn et al, 1981; Delanoy et al, 1982). Dopaminergic activity in the arcuate hypothalamus increases soon after adrenalectomy (Versteeg et al, 1984), at a time when ACTH and B-endorphin release is dramatically increased in response to loss of negative feedback control by circulating glucocorticoids (DeSouza and Van Loon, 1983). These results are consistent with previous findings that adrenalectomy increases tyrosine activity in the median eminence, a response that is blocked by glucocorticoid treatment (Kizer et al, 1974). Perhaps, as suggested by the L-DOPA-metyrapone experiments

(Hsu et al, 1976), tuberoinfundibular dopamine neurons are involved in feedback control of CRF release. The findings outlined above allow for the possibility that dopamine neurons may normally stimulate corticotrophs, presumably through CRF.

Until the present study, a popular assumption had been that dopamine exerts no significant influence on corticotroph function (Fuxe et al, 1970; Van Loon, 1973; Ganong et al, 1976). Consistent with this view, Smythe and colleagues (1983) report that although the activity of hypothalamic dopamine neurons parallels ACTH secretion in one or two isolated circumstances, overall, there is no correlation between dopaminergic activity in the hypothalamus and patterns of ACTH secretion in the rat (Smythe et al, 1983). Buckingham's 1980 review of the literature on control of CRF similarly denied dopamine any influence on CRF release. These conclusions may have been premature, however, since CRF has only recently been identified and sequenced (Vale et al, 1981). Hence, accurate investigations of how specific neurochemicals like dopamine affect CRF secretion are only now possible.

Contrary to the evidence for no effect or a stimulatory role of dopamine neurons in AL B-endorphin release, there are a number of studies whose results suggest that dopamine acts as an inhibitory regulator of CRF. Dopamine reportedly attenuates stimulated but not

basal CRF release from hypothalamic preparations (Edwardson and Bennet, 1974; Hillhouse et al, 1975). Consistent with these *in vitro* findings, destruction of catecholaminergic neurons with the neurotoxin, 6-hydroxydopamine (6-OHDA), has been found to elevate basal secretion of adrenal glucocorticoids (Cuello et al, 1974; Smith et al, 1982). Although the diurnal rhythm of circulating corticosterone remains intact in 6-OHDA-lesioned animals (Ulrich and Yuwiler, 1973; Abe and Hiroshige, 1979), the lesion-induced increase in plasma glucocorticoid levels has been interpreted to indicate that noradrenergic neurons tonically inhibit basal secretion of AL corticotroph (Van Loon, 1973; Ganong et al, 1976). Since 6-OHDA also destroys dopaminergic neurons (Kostrzewa and Jacobowitz, 1974), these findings could also be interpreted to indicate that dopamine neurons tonically inhibit CRF release.

Additional evidence for an inhibitory role of brain dopamine neurons in the regulation of corticotroph function are a few reports which contradict the evidence for stimulatory actions of either amphetamines or L-DOPA on pituitary-adrenal activation (Marantz et al, 1976). Furthermore, the turnover of hypothalamic dopamine has been shown by some to be decreased rather than increased in response to stress (Lidbrink et al, 1972; Fuxe et al, 1983).

1.6 SPECIFIC AIMS

At the outset of this research project, virtually nothing was known about the role of dopamine neurons in the physiologic regulation of pituitary B-endorphin secretion. This was due to the very newness of B-endorphin's discovery. Based upon the evidence that brain dopamine neurons importantly control the secretion of several anterior pituitary hormones in addition to alpha-melanotropin from the intermediate lobe, the overall goal of the research described here was to determine the physiologic importance of dopamine in governing B-endorphin secretion from these two pituitary sources. As the project evolved, the research was directed towards the specific aims of determining:

1. The effects of general and receptor-specific dopamine agonists on secretion of anterior and intermediate lobe B-endorphin peptides *in vivo* and *in vitro*
2. The effects of general and receptor-specific dopamine antagonists on anterior and intermediate lobe secretion of B-endorphin *in vivo* and *in vitro*
3. The role of brain dopamine neurons in mediating physiologically-evoked release of anterior and

intermediate lobe B-endorphin peptides *in vivo*.

Chapter 2

METHODS

2.0.1 Animals

Adult male albino rats of the Sprague-Dawley strain (Taconic Farms, Germantown, NY; Hilltop Laboratory Animals, Scottdale, PA) weighing 150-300 grams were housed four per cage in a controlled environment (23 C, ~70% relative humidity) with 12 hours of light daily (0600-1800) and free access to food (Rat, Mouse & Hamster Formula, Charles River, Syracuse, NY) and tap water. In order to minimize effects of non-specific stress (Hodges and Mitchley, 1970) on pituitary release of immunoreactive B-endorphin (iB-endorphin), all animals were briefly handled daily for 3 to 5 days prior to an experiment and, on the afternoon preceding an experiment, were transfered to the experimental room to acclimate to the new environment overnight (Fortier, 1958). Rats were numbered with indelible tail marks, weighed and assigned to treatment groups following a randomized block design that included 6-8 animals per group. In each experiment, animals were

Table 1. Directory of pharmacologic agents for examining involvement of dopamine in controlling release of iB-endorphin and prolactin from the pituitary gland *in vivo*

Compound	Description	Vehicle
	D1 agonist	
SKF 38393		water
	D2 agonists	
Bromocriptine *		0.03 M tartaric acid
LY141865		0.9% NaCl
	D2 antagonists	
Domperidone *		0.9 M acetic acid
Sulpiride *		0.03 M tartaric acid **
	D1, D2 agonists	
Apomorphine		0.1% metabisulfite
Pergolide		0.03 M tartaric acid
Piribedil		0.1% metabisulfite
	D1, D2 antagonist	
Haloperidol		0.03 M tartaric acid
Pimozide		0.03 M tartaric acid
	Miscellaneous	
Dexamethasone	glucocorticoid	water
Metyrapone	glucocorticoid synthesis blocker	0.9% NaCl suspension
Nomfensine	catecholamine reuptake inhibitor	0.03 M tartaric acid

* poor or delayed entry into the brain after systemic administration
** except for intracerebroventricular injection where sulpiride was dissolved in a small volume of glacial acetic acid then diluted to an injectable form using sodium bicarbonate and water

treated and killed between 0800 and 1300.

2.0.2 Treatments

2.0.2.1 Drugs

Compounds listed in Table 1 were prepared in concentrations such that intraperitoneal (ip) or subcutaneous (sc) injection volumes were 0.1 or 0.2 cc per 100 gram body weight (see RESULTS for specifications). For every drug treatment, the corresponding vehicle (Table 1) was administered to control animals.

2.0.2.2 Behavioral Observations

Classic dopaminergic drugs like haloperidal and apomorphine are known to have behavioral effects on rodents which are easily distinguished from normal activity patterns. Since several novel dopaminergic compounds were used in the present study, overt, stereotypic behaviors which were unmistakably different from normal (i.e., vehicle-treated control) rat activity were noted as time permitted. Drug-induced catalepsy was defined as a state in which the animal would retain an unusual posture (hind paw crossed over ipsilateral front paw) for at least 15 seconds.

2.0.2.3 Stress

Animals were subjected to physical immobilization as an experimental method of evoking stress (Dunn et al, 1972b). Physical immobilization was administered by strapping rats to test tube racks with tape and placing the animals on their backs for 20-30 minutes. This procedure reproducibly provokes maximal pituitary secretion of iB-endorphin without physically injuring the animals (Mueller, 1980; Mueller, 1981).

2.0.3 Sample Collection

2.0.3.1 Plasma

Rats were decapitated within 15 seconds after removal from their cages and trunk blood was collected into plastic tubes containing ~ 70 mg of ethylenediamine tetra-acetic acid (EDTA, Sigma Chemical Company, St. Louis, MO) as anticoagulant and 25 micrograms (mcg) of bacitracin (Sigma) as peptidase inhibitor dissolved in 0.5-0.6 ml of 0.05 M sodium phosphate buffer (pH 7.4). Blood tubes were immediately chilled in an ice bath. Plasma was separated by centrifugation (7000 rpm for 20 min), decanted into 12 x 75 mm polysterene tubes, covered with Parafilm and stored at -70 C (Harris LoTemp Products, North Billerica, MA).

2.0.3.2 Pituitary

Within 2 min after decapitation, the brain was

removed from the skull and, with the aid of a dissecting microscope and fine forceps, the neurointermediate lobe (NIL = neural plus intermediate lobes) was freed from the anterior lobe (AL) in situ. Pituitary samples were placed into labeled polypropylene microcentrifuge tubes containing 1.0 ml of ice-cold 1 M acetic acid, homogenized by sonication (Heat Systems-Ultrasonics, Plainview, NY) and frozen at -70 C.

2.0.4 Pituitary Cell Cultures

Primary cultures of AL and IL cells were established by modification of a method described by Vale and colleagues (1978). Pituitaries were harvested from 40-50 male Sprague-Dawley rats weighing 100-150 g. After dissection of the pituitary as described above, the AL and NIL samples were separately pooled into ice-chilled solutions of Dulbecco's Modified Eagles Medium containing 4500 g/l glucose (DMEM) and 2% horse serum (Grand Island Biological Company [GIBCO], Grand Island, NY). Pituitary samples were minced with a razor blade and enzymatically dispersed as follows: pituitary fragments were incubated for 45 min at 37 C in calcium- and magnesium-free Hanks Balanced Salt Solution (HBSS) (GIBCO) containing 0.35% collagenase (CLS IV, 150-170 U/mg, Worthington Enzymes, Cooper-Biomedical, Malvern, PA) and 0.1% hyaluronidase (Type III, Sigma) plus 3% bovine serum albumin (BSA, Sigma); a second incubation in HBSS containing 0.25%

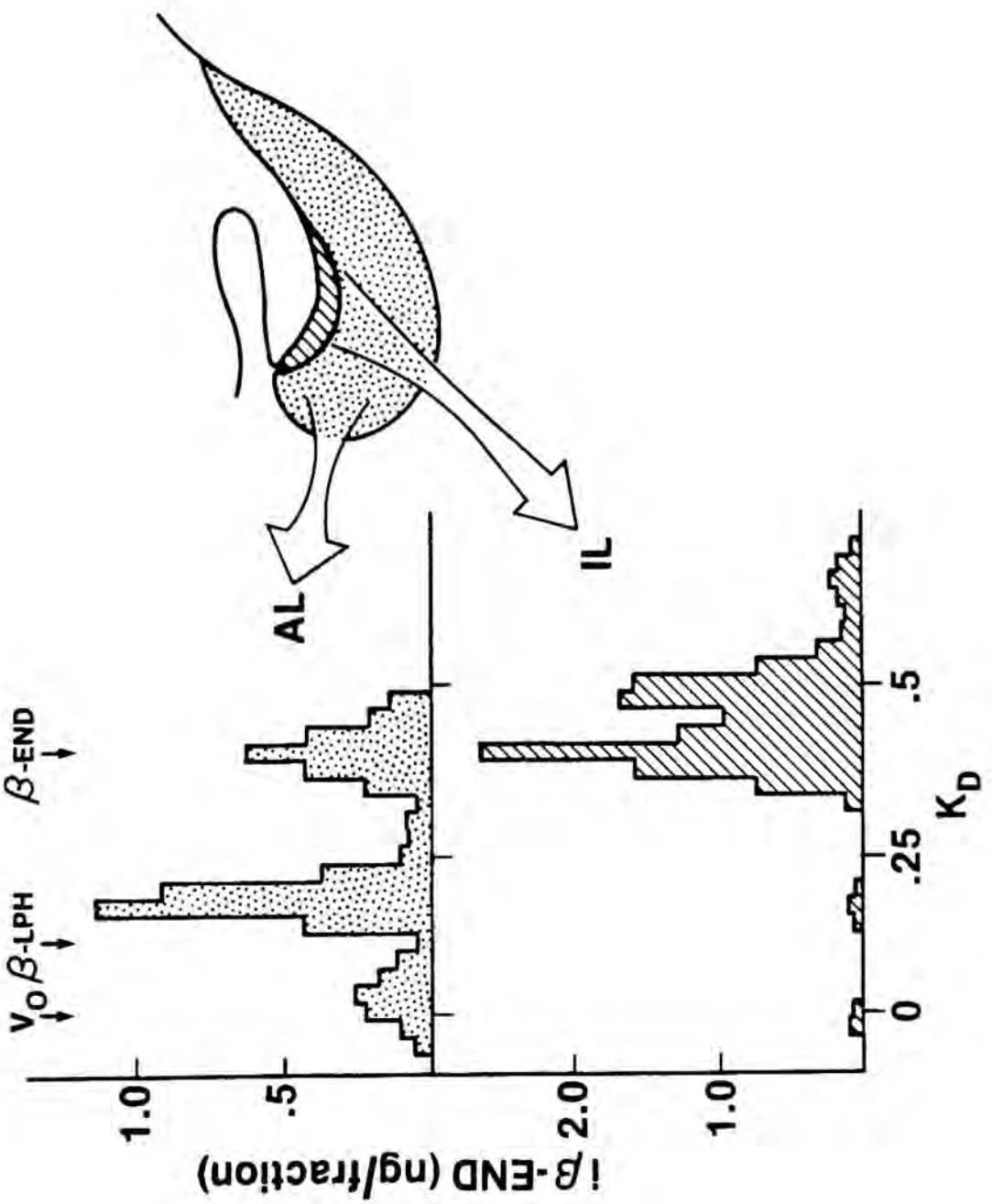
pancreatin (Grade III, Sigma) and 3% BSA (15-30 min at 37 C) completed the enzymatic dissociation. Approximately 70,000 AL cells and 30,000 NIL cells (estimated with a hemocytometer and trypan blue) were plated into 10 x 16 mm and 15 x 35 mm culture dishes, respectively. Cells were grown at 37 C (Hotpack, Philadelphia, PA) in humidified, filtered room air containing 5% carbon dioxide in sterile DMEM culture medium containing 10% horse serum, 2.5% fetal calf serum (GIBCO), 0.1% glutamine and 1% non-essential amino acids (GIBCO). Using this protocol, AL and IL cells (approximately 80 and 40 plates, respectively) were established on a substrate of fibroblasts within 5-7 days (Pettibone and Mueller, 1981b; Pettibone and Mueller, 1982). Cell viability was determined initially by trypan blue exclusion (Pettibone and Mueller, 1981b) and the condition of the cultures was routinely examined using a phase-contrast microscope (Leitz Wetzler, Germany). Release experiments were conducted after repeatedly (x4) rinsing the cultures with 2 ml of DMEM containing 2% horse serum. Thereafter, cells were incubated for 2 h with 2.0 ml (AL) or 1.5 ml (IL) of release medium (DMEM containing 2% horse serum, 0.6 mg/ml ascorbic acid (Sigma) and 30 mcg/ml bacitracin). Release media were removed without cells and stored at -70 C.

2.0.5 Biochemical Techniques

2.0.5.1 Gel Filtration Chromatography

The major plasma and cell culture components of iB-endorphin (B-endorphin- and B-lipotropin-sized peptides) were separated by gel filtration chromatography according to molecular weight. Plasma or release media from single treatment groups were pooled (5-8 ml), a small aliquot of the pool was saved for estimating recovery and the pool was layered on a column (2.5 x 80 cm) of dextran gel resin (Sephadex G-50 fine, Pharmacia Fine Chemicals, Piscataway, NJ). Columns were equilibrated at 4 C and eluted at a flow rate of ~ 4.0 ml/min with a mobile phase of 0.1 M acetic acid (pH ~3) containing 0.05% BSA, 0.02% sodium azide and 5 mg% bacitracin. Fractions (7.0-7.5 ml) were collected with a Microfractionator (Gilson Medical Electronics, Middleton, WI) into 13 x 100 mm borosilicate glass tubes. Three to 6 ml aliquots of fractions were lyophilized (Unitrap, Vitris, Gardiner, NY) and reconstituted in a 0.05 M sodium phosphate buffer (pH 7.4) for determination of iB-endorphin by radioimmunoassay as described below. Columns were equilibrated using blue dextran (Sigma) as the void volume marker (Vo) and sodium [125]-iodide (Amersham Corporation, Arlington Heights, IL) as the salt volume marker (Vs). Characteristic elution volumes (Ve) for B-endorphin- and B-lipotropin-sized peptides were determined using camel B-endorphin 1-31 (Peninsula Laboratories, San Carlos, CA) and purified human B-lipotropin (A. Parlow, NIADDK, Baltimore, MD), respectively. As shown in Figure 4,

Figure 4. Gel filtration chromatography of immunoreactive beta-endorphin (iB-END) released *in vitro* by primary cultures of anterior (AL) and intermediate lobe (IL) cells. Control release medium from 7 day cultures of AL or IL cells were eluted on a column of Sephadex G-50 fine and fractions were assayed for iB-END. Recovery of total immunoreactivity was 69% for the AL and 72% for the IL samples. Arrows indicate the elution positions of calibration standards (Vo = blue dextran, B-LPH = purified human B-lipotropin and B-END = camel B-endorphin 1-31).


AL
IL
V_O β-LPH
β-END
1.0
.5
2.0
1.0
0
.25
.5
K_D
i β-END (ng/fraction)

chromatography of control release medium from primary cultures of AL and IL clearly resolves the major molecular forms of iB-endorphin. The amount of iB-endorphin in each fraction (y-axis) is graphed with respect to a measure of relative mobility, Kd (x-axis), which is calculated as (Ve-Vo)/(Vs-Vo). As indicated by the pointers from the schematic rat pituitary gland, AL and IL cultures release markedly different molecular forms of iB-endorphin. Three major forms of iB-endorphin are secreted by the AL which elute, respectively, near Vo, slightly later than the human B-lipotropin standard and at the position of the camel B-endorphin 1-31 standard. In contrast, nearly all the iB-endorphin released by the IL corresponds to B-endorphin-sized material (lower chromatogram, Figure 4). The IL peptides elute as two poorly resolved peaks with the second peak corresponding to C-terminally shortened forms of B-endorphin, i.e., free and N-acetylated B-endorphin 1-27 and 1-26 (not shown). The chromatographic differences between B-lipotropin- and B-endorphin-sized immunoreactivity shown here were exploited throughout the in vivo studies to distinguish AL from IL release since only the AL secretes appreciable amounts of B-lipotropin. In a representative group of chromatographic runs, recovery of iB-endorphin was 82 +/- 8% (mean +/- SE, n=10). Importantly, the proportion of molecular forms of iB-endorphin detected in plasma from similar treatment groups were unaffected by variations in recovery from run

to run. Accordingly, all subsequent chromatographic profiles are corrected to 100% recovery.

2.0.5.2 Radioimmunoassays

Radioimmunoassays for B-endorphin peptides and alpha-melanotropin (MSH) were established using rabbit antisera (C-55 and H-50, respectively) that were developed against camel B-endorphin 1-31 or alpha-MSH (Peninsula Labs) conjugated to bovine thyroglobulin (Mueller, 1980; Mueller, 1981; Pettibone and Mueller, 1984; see Appendix for details). Anti-prolactin antiserum and other reagents for the prolactin radioimmunoassay were provided through the National Hormone Distribution Program of the National Institute on Arthritis, Digestive Disorders and Kidney. All radioimmunoassays were performed at 4 C in 10 or 12 x 75 mm borosilicate glass tubes in 0.05 M sodium phosphate assay buffer (pH 7.4) containing 0.05% BSA, 0.02% sodium azide and 5 mg% bacitracin. For details of the assays see Appendix. Briefly, iodinated tracers were prepared by the chemical oxidation method of Greenwood et al (1963). Two mcg of peptide and 1 mCi of sodium 125-iodide (carrier free, Amersham Corporation, Arlington Heights, IL) were reacted for 30 seconds with 10 mcg of the oxidant, chloramine-T (Sigma), in a phosphate buffered reaction volume of 0.06 ml. The reaction was quenched by addition of ~ 15 mcg of the reducing agent, sodium metabisulfite (Sigma). Radioisotopically-labeled B-endorphin or MSH were

purified by reverse-phase chromatography using commercially available C18 cartridges (Bennett et al, 1977). The iodination mixture was transferred to a Sep-Pak cartridge (Waters Associates, Milford, MA) in an aqueous solution of 0.05% triflouroacetic acid (TFA) and the purified tracer was eluted with step gradients of acetonitrile containing 0.05% TFA and stored at 4 C. 125 I-labeled prolactin was purified by gel filtration chromatography on a column of Sephadex G-50 medium (Pharmacia) using the sodium phosphate assay buffer for elution of the hormone.

All radioimmunoassays were incubated for a minimum of 60 hours at 4 C. The B-endorphin and MSH assays were terminated by charcoal separation of free from bound peptides, whereas, second antibody precipitation of bound hormones was used to complete the prolactin radioimmunoassay (see Appendix).

At a final dilution of 1/100,000, the anti-B-endorphin antiserum, C-55, bound 30-35% of 125 I-camel B-endorphin 1-31 in the absence of unlabeled peptide (see Appendix). Addition of 10 picograms (~2.9 femtomoles) of unlabeled B-endorphin standard displaced 10% of specifically bound 125 I-B-endorphin; 50% displacement occurred in the presence of approximately 85 pg (~20 femtomoles) of standard. This radioimmunoassay detected equimolar amounts of camel, rat or human B-endorphin 1-31, C- and N-terminally modified forms characteristic of the IL

as well as purified rat or human B-lipotropin. C-55 did not, however, cross-react with methionine-enkephalin (B-endorphin 1-5) or alpha-endorphin (B-endorphin 1-16) [indicating that the antigenic determinants for C-55 are located between amino acids 17-27 in the B-endorphin sequence] nor with many other hypothalamic and pituitary peptides such as adrenocorticotropin, melanotropin, somatostatin, luteinizing hormone and prolactin (Mueller, 1980; Mueller, 1981). Increasing doses of unextracted plasma, culture media, pituitary extracts or Sephadex G-50 column eluates displaced 125 I-B-endorphin in parallel with unlabeled standards.

At a final dilution of 1/200,000, the anti-MSH antiserum, H-50, specifically bound 30% of 125 I-labeled MSH. Thirty (30) picograms (~18 femtomoles) of unlabeled alpha-MSH standard produced 10% displacement. This assay detected equimolar amounts of des Ac-MSH, mono Ac-MSH and di Ac-MSH (ACTH 1-13 amide, N-Ac ACTH 1-13 amide and N, O-diAc ACTH 1-13 amide, respectively) but did not measure deamidated MSH or larger forms of ACTH (Pettibone and Mueller, 1984). The C-terminal amide moeity and amino acid sequence of MSH appear critical for detection by H-50.

The prolactin antiserum was diluted according to specifications in the NIADDK rat prolactin radioimmunoassay kit to achieve 30% specific maximum binding. Generally, the minimal detectable amount of prolactin was 60 pg per

tube. In the course of these studies, the National Hormone Distribution Program under the NIADDK changed the reference standard for rat prolactin from RP-1 to RP-2. This resulted in a downward shift in basal plasma levels of plasma prolactin (see Results, Table 3 vs Table 2), however, the magnitude of experimentally-induced changes was not noticeably affected.

2.0.5.3 Protein Determination

Protein was measured by a Coomassie brilliant blue dye binding assay (Bradford, 1976) using commercially supplied reagents and standards (Bio-Rad Laboratories, Richmond, CA).

2.0.6 Statistical Analysis

Statistical differences between treatments were determined by one- or two-way analysis of variance followed by Duncan's new multiple range test of group means (Winer, 1971). Statistical differences were accepted when the probability of error was less than 5% ($P < 0.05$).

Chapter 3

RESULTS

The studies described below were designed to examine the possible mechanisms by which dopamine may regulate the release of pituitary immunoreactive beta-endorphin (iB-endorphin) in rats. Results of these investigations are presented in three sections organized according to: 1) the effects of dopaminergic agonists, 2) effects of dopaminergic antagonists and 3) the actions of dopaminergic agonists and antagonists on physiologically stimulated release of iB-endorphin. Since it is well-established that the secretion of prolactin is regulated principally by dopaminergic inhibition, changes in plasma levels of prolactin were monitored to demonstrate the efficacy of dopaminergic drug treatments.

In overview, the results demonstrate that dopamine independently inhibits anterior lobe (AL) and intermediate lobe (IL) release of iB-endorphin through dopamine-1 (D1) and dopamine-2 (D2) receptor mechanisms, respectively. The most profound action of dopaminergic agonists, however, was stimulation of AL secretion of iB-endorphin through a D2 receptor mechanism. Together, these observations suggest

that dopamine neurons may be involved in regulating circulating levels of iB-endorphin through dopaminergic mechanisms which can influence both AL and IL release of iB-endorphin.

3.0.1 Dopaminergic Agonist Effects on Circulating iB-endorphin

The three parts of this section describe experiments which characterize the effects of dopaminergic agonists on plasma levels of iB-endorphin in rats. In the first section, time- and dose-related effects of dopaminergic agonists on circulating levels of total iB-endorphin and on the underlying molecular forms of iB-endorphin were examined. Rats received injections of classical dopaminergic agonists which are thought to be active at both D1 and D2 dopamine receptor subtypes (apomorphine and piribedil) or which preferentially stimulate either D1 receptors (SKF 38393) or D2 receptors (LY141865 and bromocriptine). The next section describes the effects of dopamine agonists on iB-endorphin released specifically from the AL. The actions of apomorphine and LY141865 on iB-endorphin release <u>in vivo</u> were challenged with exogenous glucocorticoid pretreatment (dexamethasone), a means by which AL (but not IL) secretion of iB-endorphin is inhibited. Furthermore, the possible direct actions of dopamine on spontaneous and evoked release of iB-endorphin from the AL was examined <u>in vitro</u> by exposing primary

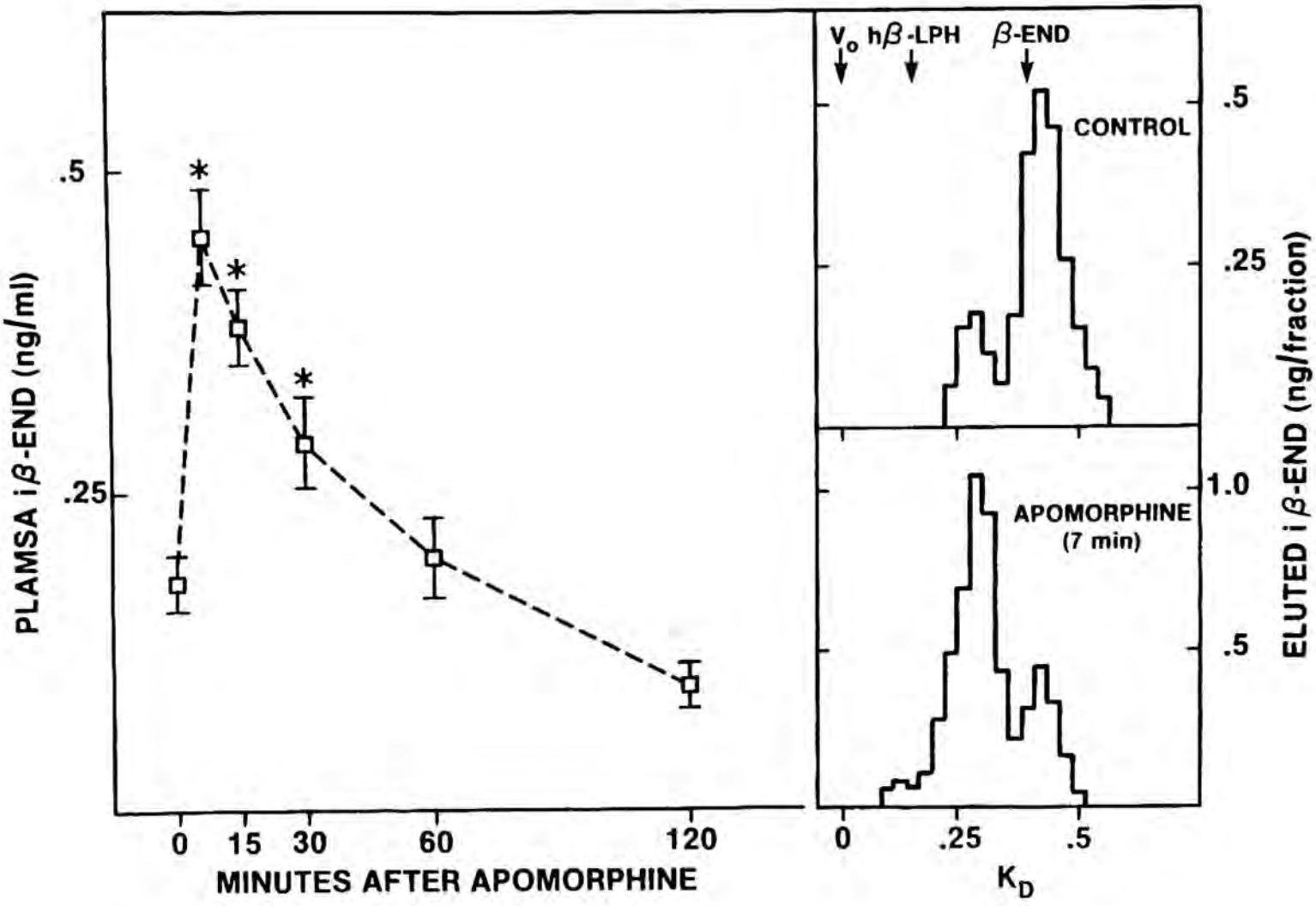
cultures of the rat AL to dopamine or the D2 agonists, LY141865 and bromocriptine. Experiments in the last section further define, pharmacologically, dopaminergic mechanisms regulating pituitary release of iB-endorphin *in vivo*. The actions of apomorphine and LY141865 were challenged by pretreating rats with antagonists of dopamine receptors which block both D1 and D2 receptor subtypes (haloperidol) or which preferentially inhibit only D2 receptors (sulpiride).

3.0.1.1 Time- and Dose-related Effects of Dopaminergic Agonists

Figures 5 and 6 illustrate the effects of two classical dopaminergic agonists, apomorphine and piribedil (mixed D1, D2 agonists), on circulating levels of total iB-endorphin and on the underlying molecular forms of that immunoreactivity. As compared to control values of 0.18 +/- 0.02 ng/ml (mean +/- SE), circulating levels of total iB-endorphin were elevated 2.5-fold by 7 min after a single injection of apomorphine (3 mg/kg, sc) and remained significantly elevated ($P<0.05$) up to 30 min after apomorphine administration; thereafter, plasma iB-endorphin tended towards control values (Figure 5, left). As shown in the right hand panels of Figure 5, gel filtration chromatography of plasma clearly resolved two forms of circulating iB-endorphin that resembled B-lipotropin (B-LPH) and B-endorphin standards in molecular size. The

Figure 5. Effects of apomorphine on circulating levels of immunoreactive beta-endorphin (iB-END) in rats. Composite illustration showing the time-course effects (left) and the gel chromatographic profiles (right) of apomorphine versus control treatments. Rats received sc injections of vehicle or apomorphine (3 mg/kg) at each of the times indicated below the abscissa (left) prior to decapitation. Points and vertical lines represent the group means +/- SE, N=6, in the time course. Pools of treatment group plasma (~7 ml) were filtered on a column of Sephadex G-50 resin and the resulting profiles were graphed (corrected to 100% recovery) with respect to the mobility coefficient, Kd. Pos itions of calibration peaks, i.e., blue dextran (Vo), human beta-lipotropin (B-LPH) and camel beta-endorphin (B-END) are shown (arrows) above the control profile.

* Significantly different ($P<0.05$) from zero time controls

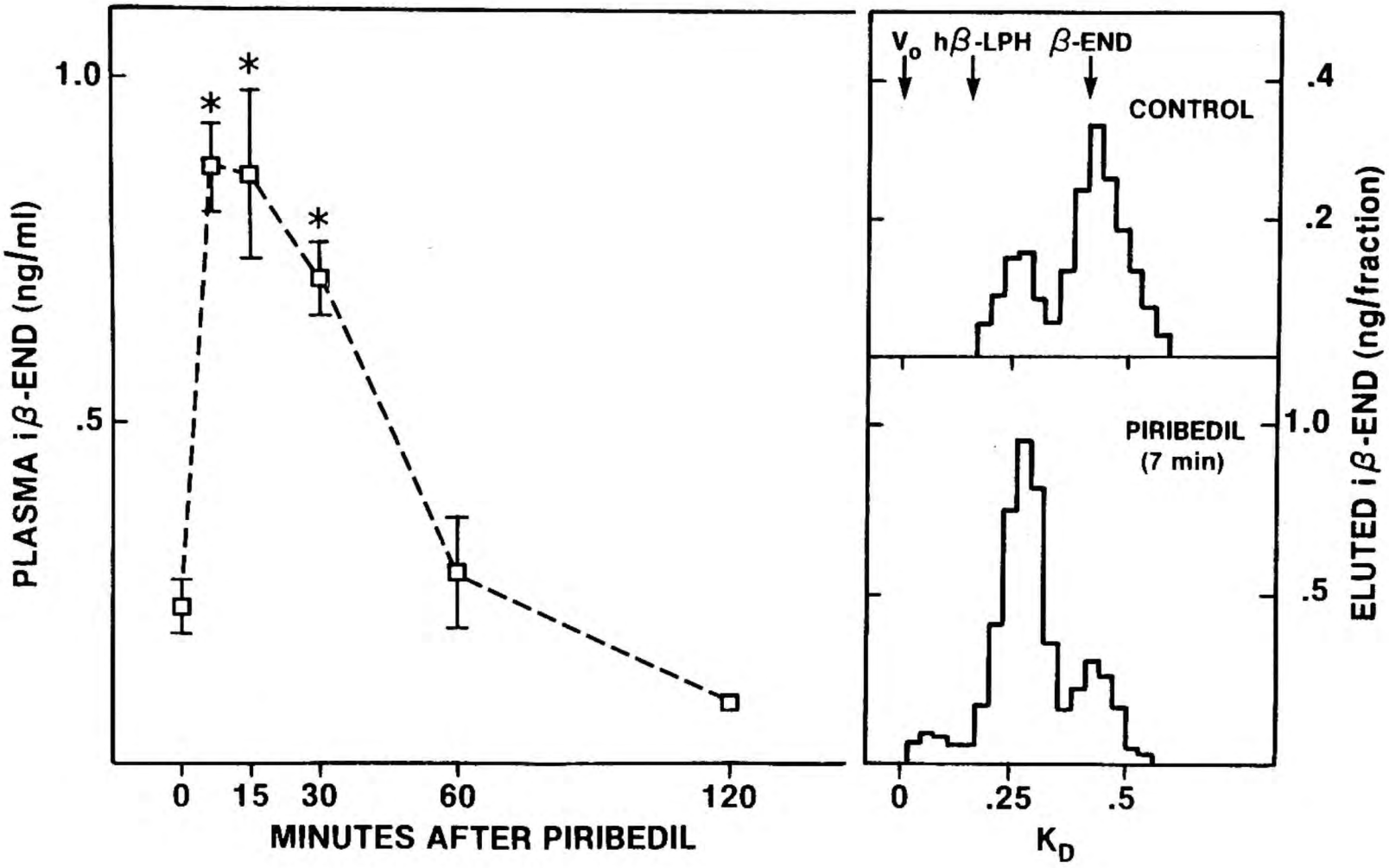

PLAMSA iβ-END (ng/ml)
.5
.25
0
15
30
60
120
MINUTES AFTER APOMORPHINE
Vo
hβ-LPH
β-END
CONTROL
APOMORPHINE
(7 min)
.5
.25
1.0
.5
0
.25
.5
KD
ELUTED iβ-END (ng/fraction)

higher molecular weight peak of immunoreactivity co-chromatographed with rat B-LPH and eluted just beyond purified human B-LPH which is significantly larger than rodent beta-lipotropin (Eipper & Mains, 1979). The second peak of plasma immunoreactivity coeluted with synthetic camel B-endorphin. Under basal conditions, B-endorphin-sized material was the principle molecular form representing 80% of total iB-endorphin (Figure 5, top right).

In contrast, the increase in total circulating iB-endorphin 7 min after apomorphine treatment was dominated by immunoreactivity resembling B-LPH in size (bottom right, Figure 5). The B-LPH peak comprised 72% of total iB-endorphin in plasma of apomorphine-treated rats and this represented an 8-fold increase in B-LPH relative to the amount in control rat plasma. Accompanying this rise in plasma B-LPH was a concomitant reduction in B-endorphin-sized immunoreactivity. The B-endorphin peak decreased by 34% relative to the corresponding peak in control plasma. Similar to apomorphine, piribedil (10 mg/kg, sc) also induced a brief increase (7-30 min) in circulating levels of total iB-endorphin that maximally exceeded control values (0.23 +/- 0.04 ng/ml) by 280% at 7 min after treatment (Figure 6, left). Further, the gel filtration elution profiles of plasma from control and piribedil-treated rats (right-hand panels, Figure 6) were

Figure 6. Effects of piribedil on circulating levels of immunoreactive beta-endorphin (iB-END). Composite illustration showing the time course effects (left) and gel chromatographic profiles (right) of piribedil versus control treatments. Rats received a sc injection of vehicle (10 min) or of piribedil (10 mg/kg) at each of the times indicated (left) prior to decapitation. Symbols and vertical lines represent the group mean +/- SE, N=5-7, in the time course. Pools of rat plasma (7 ml) were filtered on a column of Sephadex G-50, and the elution profile was graphed (corrected to 100% recovery) with respect to the mobility coefficient, Kd. Positions of calibration peaks, i.e., blue dextran (Vo), human beta-lipotropin (B-LPH) and camel B-endorphin (B-END), are shown (arrows) above the control profile (right).

* Significantly different ($P<0.05$) from the zero time controls


PLASMA iβ-END (ng/ml)
1.0
.5
0
15
30
60
120
MINUTES AFTER PIRIBEDIL
V_o
hβ-LPH
β-END
CONTROL
PIRIBEDIL
(7 min)
.4
.2
1.0
.5
0
.25
.5
K_D
ELUTED iβ-END (ng/fraction)

virtually the same as those observed in the apomorphine experiment. The B-LPH peak constituted only 27% of total iB-endorphin in the plasma of control animals yet represented greater than 70% in the plasma of piribedil-treated rats. These changes in plasma iB-endorphin of rats treated with piribedil reflected a 7-fold elevation of iB-endorphin resembling B-LPH together with a simultaneous 18% loss of the immunoreactivity in the B-endorphin peak. Since only AL corticotrophs produce appreciable amounts of B-LPH (Eipper and Mains, 1980), the dominance of B-LPH-sized iB-endorphin in profiles of plasma from apomorphine- and piribedil-treated animal suggests that these dopamine receptor agonists preferentially stimulate anterior lobe release of iB-endorphin in rats. Although B-endorphin-sized peptides are secreted by both the AL and IL, the observation that material resembling B-endorphin was selectively decreased by the agonists suggests that apomorphine and piribedil inhibit IL release of iB-endorphin <u>in vivo</u>. This interpretation received additional support from the observation that an apomorphine treatment (1 mg/kg, ip, 60 min) which failed to increase total levels of circulating iB-endorphin nonetheless shifted the major constituent of total immunoreactivity from the B-endorphin-sized form to the form resembling B-LPH in molecular size (not shown).

As expected, both apomorphine and piribedil significantly reduced circulating prolactin by 15 min after

Table 2. Time-course effects of apomorphine and piribedil on plasma levels of prolactin

Treatment	Minutes after treatment					
	0	7	15	30	60	120
Apomorphine	5.8 ± 1.6	5.2 ± 0.8	3.4 ± 0.3*	2.2 ± 0.3*	1.9 ± 0.1*	4.0 ± 1.0
Piribedil	13.0 ± 2.8	8.8 ± 3.9	2.7 ± 0.2*	2.2 ± 0.3*	1.9 ± 0.1*	1.7 ± 0.2

Rats received sc injections of vehicle, apomorphine (3 mg/kg) or piribedil (10 mg/kg) as described in the legends of Figures 5 and 6, respectively. Values are group means ± SE of plasma prolactin levels (ng/ml); N=5-7.

* Significantly different ($P<0.05$) from corresponding zero time controls

injection (Table 2). The duration of inhibition by piribedil persisted throughout the 2 h time-course, whereas, apomorphine's suppression of prolactin was evident only up to 60 min after injection. In addition to the endocrine effects described above, rats treated with apomorphine or piribedil exhibited stereotypic behavior which was markedly different from vehicle-treated animals. The stereotypy consisted of ptosis, repetitive gnawing and sniffing and, in the case of apomorphine, pawing as if to dig through the cage floor. Although these behavioral responses were not quantified, the apparent time-course of the stereotypic displays paralleled the endocrine responses to the mixed D1, D2 agonists (onset usually occuring within 5 min of administration and lasting for about one hour).

In order to determine if endogenous activity of dopamine neurons is involved in basal release of iB-endorphin from the pituitary <u>in vivo</u>, rats were treated with the indirect dopaminergic agonist, nomfensine. Nomfensine enhances dopaminergic transmission in the brain by its ability to preferentially inhibit reuptake of dopamine at the dopaminergic nerve terminal (Hunt et al, 1974). Accordingly, nomfensine prevents inactivation of dopamine at the synapse and, thereby, increases the duration of dopamine receptor activation. Its effects, however, require the integrity of active dopaminergic pathways. Rats were treated with a dose of nomfensine (3 mg/kg, sc) which has been found elsewhere to increase

Table 3. Time course effects of nomfensine on circulating immunoreactive β-endorphin and prolactin in rats

Treatment	N	Plasma Hormones (ng/ml) iβ-endorphin	prolactin
Controls	5	0.07 ± 0.01	2.7 ± 0.7
Nomfensine, 15 min	6	0.13 ± 0.02	1.4 ± 0.2 [a]
" 30 min	6	0.10 ± 0.02	1.2 ± 0.1 [a]
" 60 min	6	0.08 ± 0.02	1.3 ± 0.2 [a]
" 120 min	6	0.10 ± 0.03	1.0 ± 0.1 [a]

Rats received sc injections of vehicle or nomfensine (3 mg/kg) at each of the times indicated prior to sacrifice. Values are the group mean ± SE; number of samples is indicated under N.

[a] Significantly different ($P<0.05$) from vehicle-treated controls

levels of dopamine in the AL (Apud et al, 1980). Here, nomfensine-treated rats exhibited some of the stereotypy of the classical dopaminergic agonists, namely sniffing, but, relative to control animals, the striking behavioral feature of nomfensine-treated rats was repetitive, rearing and swaying. This behavior appeared most intense by 30 min after injection of nomfensine but was occasionally evident up to 2 h after the treatment. As shown in Table 3, nomfensine also lowered plasma prolactin during this time period and prolactin remained significantly depressed as compared to vehicle-treated controls up to 2 h after nomfensine administration. In this experiment, basal levels of prolactin are lower than in other experiments of this study. This may be due to the use of a different reference standard for prolactin (RP-2) than that most frequently used in other experiments (RP-1, see Methods). At no time after nomfensine were circulating levels of total iB-endorphin significantly different from control values. Gel chromatography (not shown) revealed that nomfensine treatment, like substimulatory apormorphine administration, increased the B-LPH form of iB-endorphin and slightly decreased B-endorphin-sized immunoreactivity. Despite these alterations in circulating molecular forms of iB-endorphin, no net change in plasma levels of total iB-endorphin were detectable.

Dose-related stimulation of pituitary iB-endorphin release by apomorphine is shown in Table 4. Even at a dose

Table 4. Dose-response effects of apomorphine on circulating levels of immunoreactive β-endorphin and prolactin in rats

Dose of Apomorphine (mg/kg)	N	Plasma Hormones (ng/ml) iβ-endorphin	prolactin
Vehicle	7	0.20 ± 0.03	13.0 ± 2.8
0.3	6	0.56 ± 0.10 [a]	14.4 ± 3.1
1.0	6	0.74 ± 0.12 [a]	8.0 ± 1.5
3.0	6	0.79 ± 0.03 [a]	8.1 ± 1.2
10.0	6	0.95 ± 0.08 [a]	11.4 ± 3.1

Rats received sc injections of vehicle or apomorphine (doses indicated) 10 min prior to decapitation. Values are the group mean ± SE; number of samples is indicated under N.

[a] Significantly different ($P<0.05$) from vehicle-treated controls

(0.3 mg/kg) which was ten times less than that used in the time-course experiment, apomorphine significantly increased circulating iB-endorphin 2.8-fold as compared to control values by 10 min after administration. Higher doses of apomorphine evoked progressively higher levels of plasma iB-endorphin with the highest dose, 10 mg/kg, increasing total circulating iB-endorphin ~4.8-fold relative to control values. Together, these findings suggest that pituitary release of iB-endorphin <u>in vivo</u> may be physiologically stimulated by dopamine receptor mechanism.

To better define the receptor which mediates the effects of dopaminergic drugs on pituitary secretion of iB-endorphin, the effects of more selectively active dopaminergic agonists were also examined in rats. The D1 receptor agonist, SKF 38393 (Setler et al, 1978), had little effect on either basal levels of total circulating iB-endorphin (Table 5) or on the chromatographic profile of iB-endorphin in plasma (not shown). Even though higher doses of SKF 38393 (3 and 10 mg/kg, ip, 30 min) did significantly increase circulating iB-endorphin, the response was modest (~50%) and not dose-dependent. Unlike the mixed D1, D2 receptor agonists, apomorphine and piribedil, SKF 38393 failed to evoke stereotypic displays in treated rats. These animals, in fact, were behaviorally indistinguishable from control rats. In another experiment, (results not shown) SKF 38393 (0.3, 1.0 and 3.0 mg/kg, 60 min) had no effects on basal levels of prolactin

Table 5. Effects of SKF 38393 on circulating immunoreactive β-endorphin in rats

Treatment	Minutes after treatment		
	15	30	60
Vehicle	----	0.23 ± 0.02	----
0.3 mg/kg SKF	----	0.24 ± 0.03	----
1.0 mg/kg SKF	----	0.28 ± 0.02	----
3.0 mg/kg SKF	0.29 ± 0.03	0.36 ± 0.03 [a]	0.31 ± 0.04
10.0 mg/kg SKF	----	0.34 ± 0.03 [a]	----

Rats received ip injections of venicle or SKF 38393A (SKF) in the doses indicated 15, 30 or 60 min prior to decapitation. Values are group means ± SE of plasma iβ-endorphin (ng/ml); N=6-7.

[a] Significantly different ($P<0.05$) from vehicle-treated controls

(6.5 +/- 1.4 ng/ml) nor on enhanced release of prolactin (84.3 +/- 12.8) evoked by the dopaminergic antagonist, haloperidol. As will be shown below, this finding further distinguished SKF 38393 from other dopaminergic agonists. Since the endocrine and behavioral effects of SKF 38393 were clearly different from the effects of the other dopaminergic agonists, it is unlikely that the actions of apomorphine or piribedil are mediated by a D1 receptor subtype. Consistent with this view, evidence presented below indicates that classical dopamine receptor agonists evoked pituitary release iB-endorphin through their actions on the D2 type of dopamine receptor.

The D2 receptor agonist, LY141865 (Tsurata et al, 1981), like the classical dopamine agonists, increased circulating iB-endorphin and decreased prolactin in time- and dose-related fashion. Rats treated with LY141865 also displayed some degree of the classical agonists stereotypy but were, for the most part, sedated by this treatment. As shown in Figure 7, LY141865 (1 mg/kg, ip) evoked a rapid increase in circulating iB-endorphin by 7 to 15 min after administration. Simultaneously, plasma levels of prolactin were reduced 60% from control values of 12.3 +/- 4.0 ng/ml within 15 min after the LY141865 injection and remained depressed throughout the 2 h time-coursse. Dose-response effects of LY141865 (0.01-1.0 mg/kg ip, 15 min) on iB-endorphin and prolactin are illustrated in the upper and

Figure 7. Time course effects of LY141865 on circulating levels of immunoreactive beta-endorphin (iB-END) and prolactin (PRL). Rats received an ip injection of vehicle or LY141865 (1 mg/kg) at each of the times indicated prior to decapitation. Symbols and vertical lines represent the group mean +/- SE; N=6.

*Significantly different ($P<0.05$) from the zero time controls

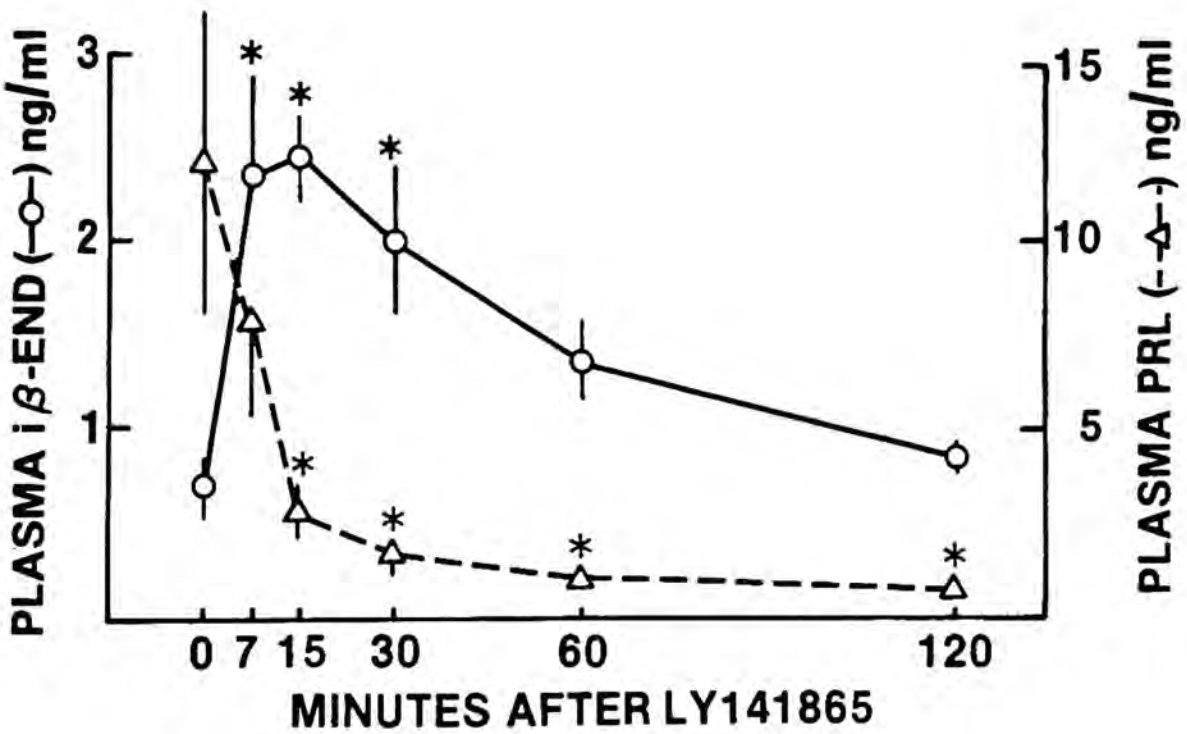
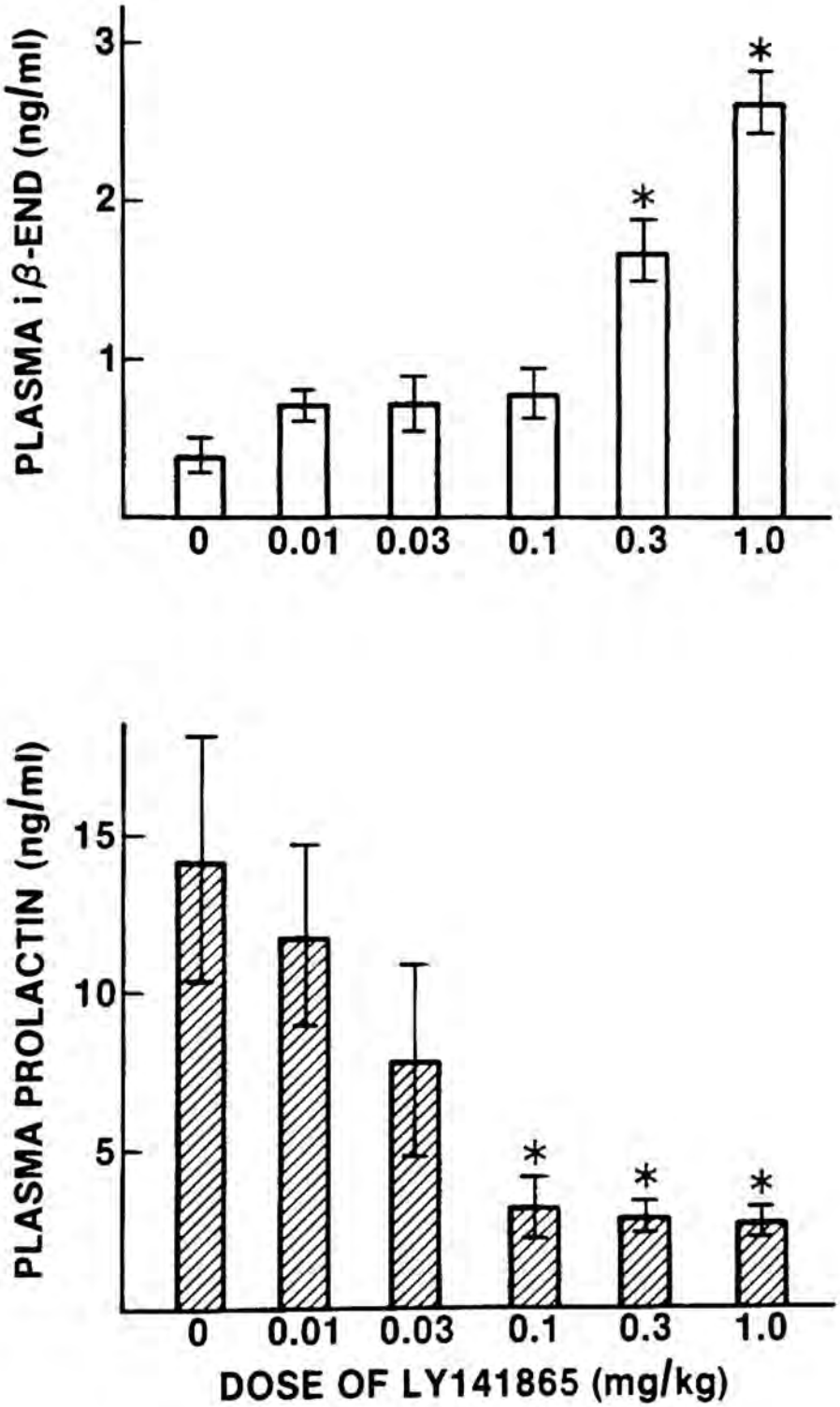

PLASMA i β-END (—○—) ng/ml
3
2
1
15
10
5
PLASMA PRL (- -△- -) ng/ml
0 7 15 30 60 120
MINUTES AFTER LY141865

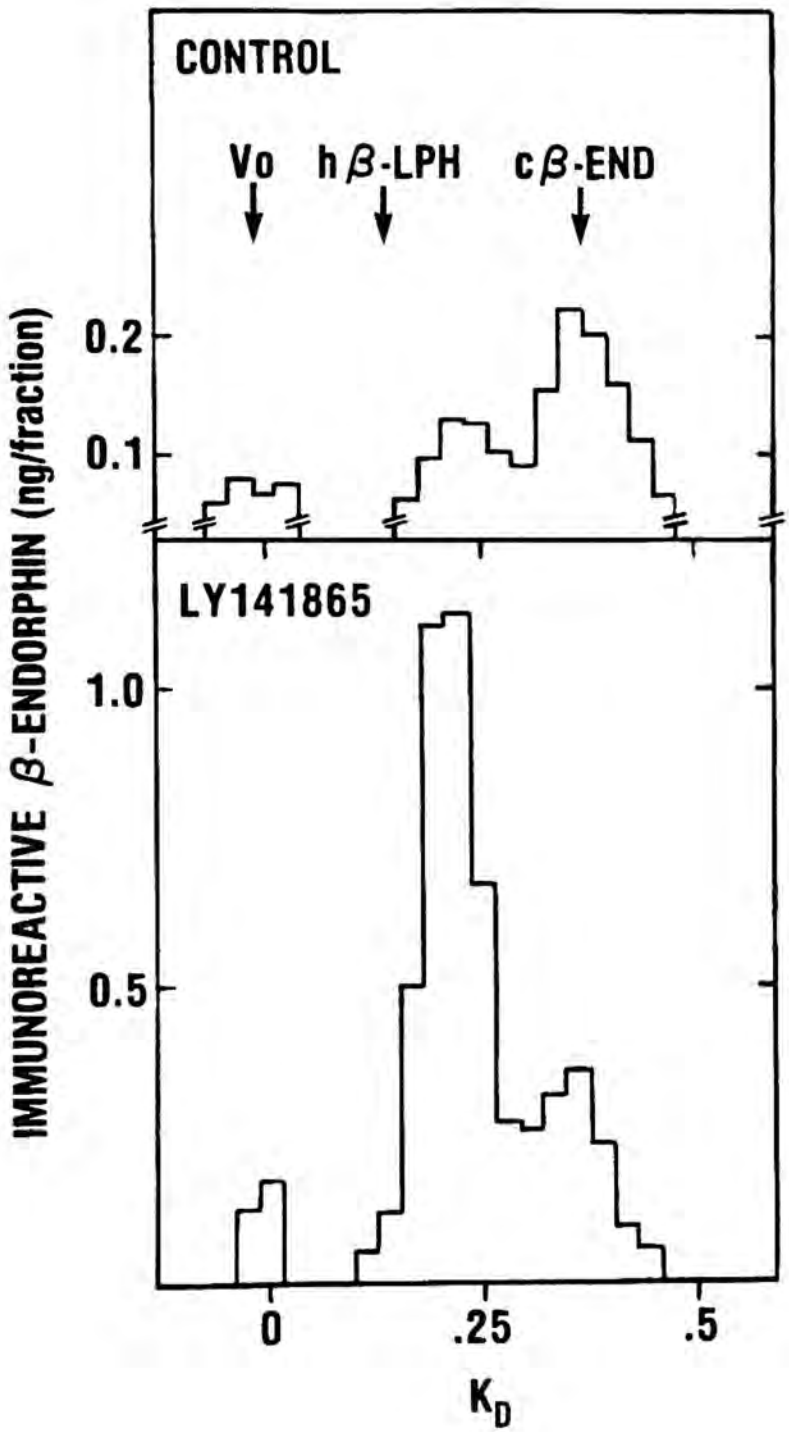
Figure 8. Dose-response effects of LY141865 on circulating levels of immunoreactive beta-endorphin (iB-END) and prolactin (PRL). Rats received an ip injection of vehicle (zero dose) or LY141865 (doses indicated) 15 min prior to decapitation. Bars and vertical lines represent the group mean +/- SE; N=6.

*Significantly different ($P<0.05$) versus zero dose controls


PLASMA iβ-END (ng/ml)
3
2
1
*
*
*
0
0.01
0.03
0.1
0.3
1.0
PLASMA PROLACTIN (ng/ml)
15
10
5
*
*
*
0
0.01
0.03
0.1
0.3
1.0
DOSE OF LY141865 (mg/kg)

lower panels of Figure 8. The dose of LY141865 which maximally suppressed basal prolactin levels (0.1 mg/kg) was in the same order of magnitude as the minimally effective dose (0.3 mg/kg) required to significantly increase circulating iB-endorphin. The highest dose of LY141865 (1 mg/kg) increased levels of total plasma iB-endorphin 7.5-fold as compared to control values (0.38 +/- 0.12 ng/ml), an increase which significantly exceeded the response to 0.3 mg/kg by 57% (top panel, Figure 8). As previously shown in the chromatographic profiles of plasma iB-endorphin from apomorphine- or piribedil-treated rats, the major molecular form of iB-endorphin appearing in plasma in response to LY141865 resembled B-LPH and accounted for 73% of total circulating immunoreactivity; B-endorphin-sized peptides represented just 22% and the remaining 5% eluted in the void volume (bottom panel, Figure 9). By 60 min after LY141865 treatment, B-endorphin-sized immunoreactivity nearly disappeared from chromatographic profiles of plasma (not shown) making the relative contribution of the B-LPH form even greater over time. Thus, the neuroendocrine effects of the D2 agonist, LY141865, were strikingly similar to those of the classical agonists, apormorphine and piribedil, whereas, the D1 agonist, SKF 38393, had little influence on pituitary iB-endorphin or prolactin release. Based on the dramatic rise in blood-borne B-LPH-sized immunoreactivity following apomorphine, piribedil and LY141865, it appears that

Figure 9. A comparison of immunoreactive beta-endorphin (B-endorphin) in gel chromatographic profiles of plasma from control and LY141865-treated rats. Pools of rat plasma (6-7 ml) were filtered on a column of Sephadex G-50, and the elution profile of immunoreactive B-endorphin was graphed (corrected to 100% recovery) with respect to a mobility coefficient, Kd. Positons of calibration peaks, i.e., blue dextran (Vo), human beta-lipotropin (hB-LPH), and camel B-endorphin (cB-END), are shown (arrows) above the control profile.


CONTROL
Vo
h β-LPH
c β-END
0.2
0.1
LY141865
1.0
0.5
IMMUNOREACTIVE β-ENDORPHIN (ng/fraction)
0
.25
.5
K_D

D2 receptors may normally stimulate hormone release by AL corticotrophs, presumably through actions in the central nervous system which result in the release of hypothalamic corticotropin releasing factor.

Bromocriptine, another D2 agonist (Markstein et al, 1978) which has proven clinically useful because of its long duration of action, was observed to lower not only circulating prolactin but also iB-endorphin. Over time, rats treated with bromocriptine became sedated relative to their control cagemates and some exhibited bouts of sniffing and gnawing. None, however, displayed vigorous and sustained episodes of stereotypic behavior so characteristic of apomorphine and piribedil treatment. Like other agonists of D2 receptors, a single injection of bromocriptine (5 mg/kg, ip) reduced plasma levels of prolactin to the limit of detectability within 15 min, and prolactin remained depressed throughout a 2 h treatment period (Figure 10). The observation that prolactin was maximally inhibited by 15 min after administration of bromocriptine indicates that the drug readily interacts with the pituitary dopamine receptors which directly mediate dopaminergic control of prolactin secretion. Despite this characteristic action of bromocriptine on pituitary prolactin release, unlike the other D2 agonist, bromocriptine did not increase plasma iB-endorphin. Instead, bromocriptine significantly reduced total levels of iB-endorphin approximately 50% as compared to control

Figure 10. Time-course effects of bromocriptine on circulating levels of prolactin. Rats received ip injections of vehicle (15 min) or bromocriptine mesylate (5 mg/kg) at the times indicated. Symbols and vertical lines represent the group mean +/- SE; N=6-7.

* Significantly different ($P<0.05$) from zero time controls

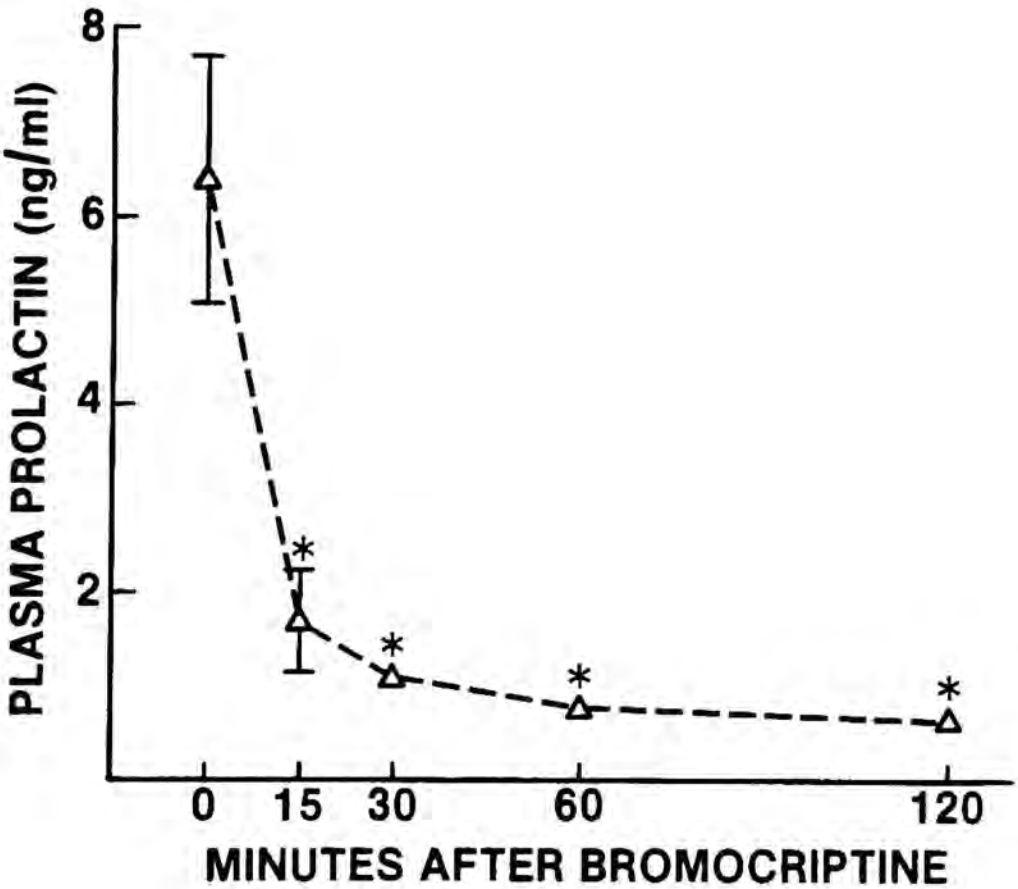
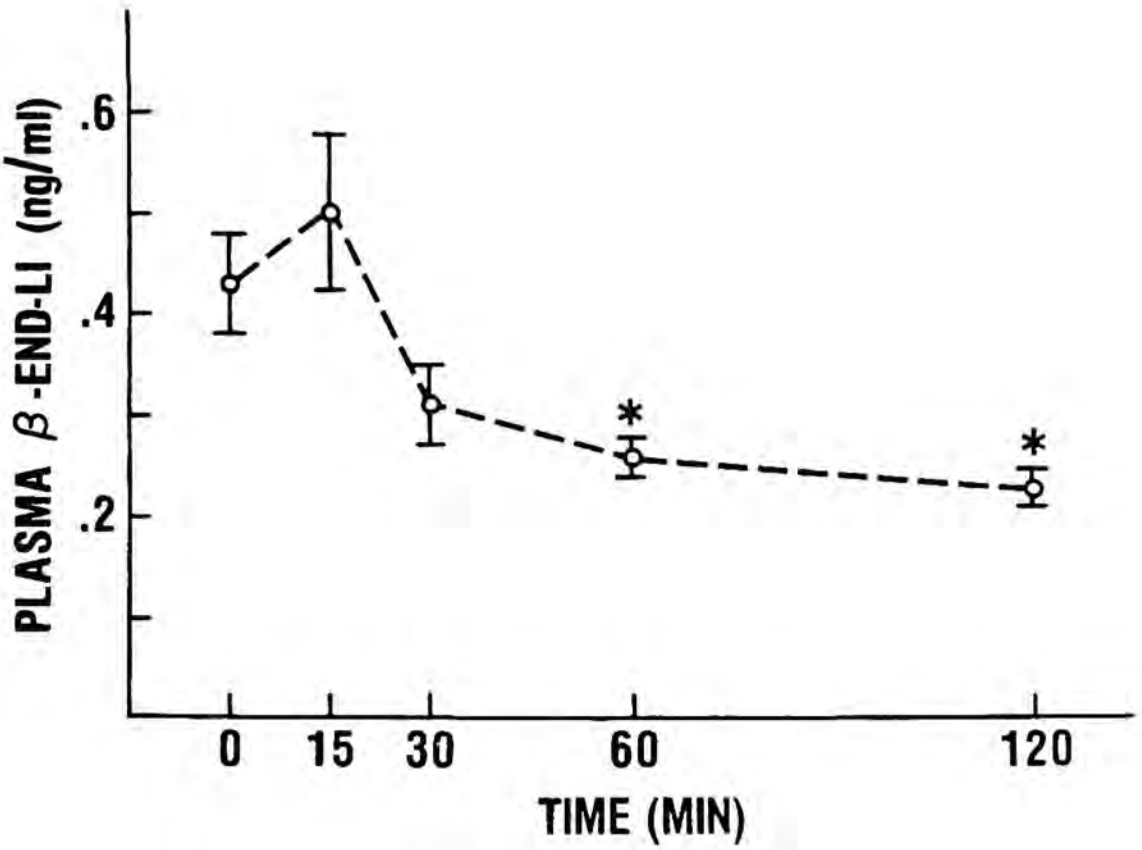

PLASMA PROLACTIN (ng/ml)
8
6
4
2
0
15
30
60
120
MINUTES AFTER BROMOCRIPTINE
*
*
*
*

Figure 11. Time-course effects of bromocriptine on circulating beta-endorphin-like immunoreactivity (B-END-LI). Rats received ip injections of vehicle (15 min) or bromocriptine mesylate (5 mg/kg) at the times indicated before sacrifice. Symbols and vertical lines represent the group means +/- SE; N=6-7.

* Significantly different ($P<0.05$) from zero time controls and the 15 min bromocriptine treatment group


PLASMA β-END-LI (ng/ml)
.6
.4
.2
0
15
30
60
120
TIME (MIN)
*
*

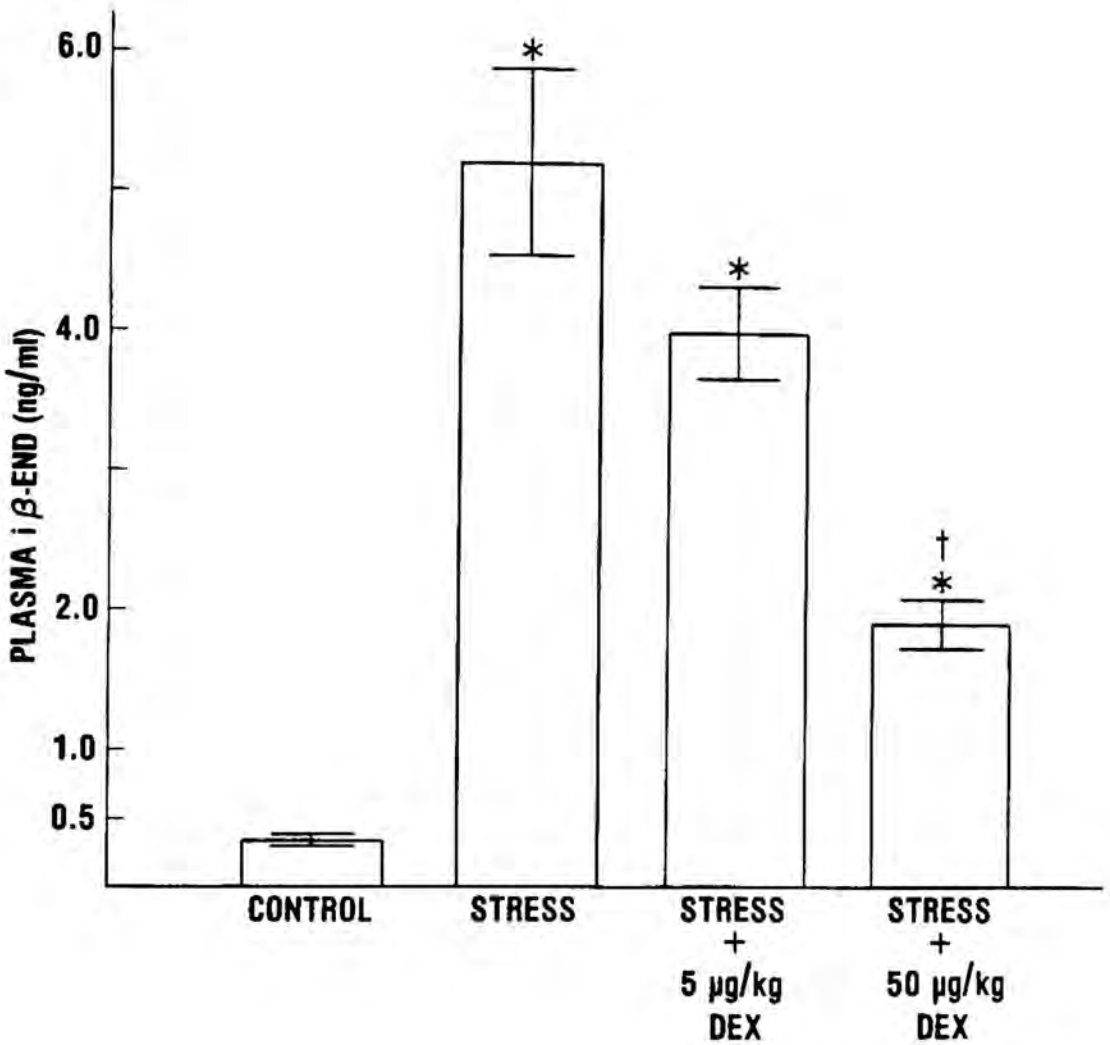
values (0.43 +/- 0.05 ng/ml) by 1 and 2 h post-injection (Figure 11). The reduction of plasma iB-endorphin in the bromocriptine time-course was primarily associated with a loss of immunoreactivity resembling B-endorphin B-LPH-sized immunoreactivity was unaffected or increased by bromocriptine treatment (not shown). Accordingly, there was a shift in the dominant form of plasma iB-endorphin to B-LPH with a net decline in total levels of iB-endorphin in blood. The decline in circulating levels of total iB-endorphin, and, particularly, the form resembling B-endorphin in molecular size, indicates that the primary action of bromocriptine on circulating iB-endorphin resulted from inhibition of IL secretion.

3.0.1.2 Effects of Dopaminergic Agonists on AL iB-endorphin *in vivo* and *in vitro*

Enhanced release of B-LPH, the form of iB-endorphin produced exclusively by the AL, and diminished release of B-endorphin-sized material in response to dopaminergic agonists suggests that dopamine neurons may normally evoke release of iB-endorphin from corticotrophs of the AL in addition to their presumed ability to suppress IL release of iB-endorphin *in vivo*. To demonstrate further that the AL is indeed the most likely source of blood-borne iB-endorphin released in response to dopaminergic stimulation, apomorphine- and LY141865-induced release of iB-endorphin was challenged by glucocorticoid

Figure 12. Effects of dexamethasone pretreatment on stress-induced release of pituitary iB-endorphin (iB-END). Rats received ip injections of vehicle or dexamethasone (DEX) 4 h prior to sacrifice; stress consisted of physical immobilization for 20 min prior to decapitation. Bars and vertical lines represent the group mean +/- SE; N=6-7.

*significantly different (P<0.05) from CONTROL
+Significantly different (P<0.05) from STRESS and STRESS + 5 mcg/kg DEX


6.0
4.0
2.0
1.0
0.5
PLASMA i β-END (ng/ml)
*
*
†
*
CONTROL
STRESS
STRESS + 5 µg/kg DEX
STRESS + 50 µg/kg DEX

pre-treatment. Glucocorticoids preferentially inhibit iB-endorphin secretion by AL corticotrophs over IL melanotrophs *in vivo* (see Roberts et al, 1982). Accordingly, administration of the synthetic glucocorticoid, dexamethasone, may be used to block the AL response without influencing IL secretion of iB-endorphin. The dose of dexamethasone used was determined from the dose which was found to significantly attenuate stress-induced release of iB-endorphin. Stress is a potent physiologic releaser of AL iB-endorphin and ACTH. As shown in Figure 12, 50 but not 5 mcg/kg dexamethasone administered 4 h before sacrifice markedly reduced levels of iB-endorphin in rats subjected to the stress of physical immobilization. As shown in Table 6, pretreating rats with the 50 mcg/kg dose of dexamethasone reduced basal levels of plasma iB-endorphin by 35% and completely prevented release of iB-endorphin (450% of control) evoked by either apomorphine or LY141865. In light of the inhibition by dopamine of IL release of iB-endorphin *in vitro* (Przewlocki et al, 1978; Vale et al, 1979), and the diminution of the B-endorphin-sized form in plasma of agonist-treated rats, these data together point strongly to the AL as the pituitary source which is specifically stimulated by dopamine receptor agonists.

In order to determine if dopaminergic agonists directly stimulate AL corticotrophs to secrete iB-endorphin, primary cultures of rat AL cells were exposed

Table 6. Effects of dexamethasone pretreatment on the elevation of circulating immunoreactive β-endorphin by dopaminergic agonists

Treatment	Pretreatment	
	Vehicle	Dexamethasone
Vehicle	0.17 ± 0.02	0.11 ± 0.01 [a]
Apomorphine	0.78 ± 0.01 [a]	0.16 ± 0.03 [b]
LY141865	0.77 ± 0.06 [a]	0.18 ± 0.02 [b]

Rats received an ip injection of vehicle or dexamethasone (50 µg/kg) 4 h prior to sacrifice; a second injection of vehicle, apomorphine (3 mg/kg sc, 10 min) or LY141865 (1 mg/kg ip, 15 min) was administered just prior to decapitation. Values are the group mean ± SE of plasma iβ-endorphin; N=6-7.

[a] Significantly different ($P<0.05$) from vehicle-treated controls

[b] Significantly different ($P<0.05$) from corresponding treatment without dexamethasone

to various concentrations of dopamine, bromocriptine or LY141865. The results of three such *in vitro* experiments are shown in Tables 7, 8 and 9. Spontaneous release of iB-endorphin from control cells varied among cultures from approximately 1 to 4 ng per plate per 2 h incubation. Within each *in vitro* experiment, however, iB-endorphin release by control cells was uniform, and release evoked by CRF or by the adrenergic agonist, epinephrine, was similar in magnitude to release provoked by these agents in other experiments. As shown in Table 7, neither dopamine nor bromocriptine at doses of 0.1 nM, 10 nM or 1 mcM altered spontaneous release of iB-endorphin by cultured AL corticotrophs. In this experiment, epinephrine (0.3 mcM) was used to evoke iB-endorphin release to levels which were double the control values. The stimulatory effect of epinephrine was significantly attenuated by 1 mcM bromocriptine which has been shown elsewhere to be an adrenergic antagonist at higher doses (U'Prichard et al, 1977; Brown et al, 1980; Galzin et al, 1982). In the next experiment (Table 8), cultured AL cells were exposed to a narrower dose range of bromocriptine (10 nM to 1 mcM) and the hypothalamic secretagogue, corticotropin releasing factor (CRF), in addition to epinephrine. Without significantly influencing spontaneous release of iB-endorphin, bromocriptine dose-relatedly reversed the 180% increase in iB-endorphin release due to epinephrine yet had no effect on the 3.5-fold increase evoked by 0.3 nM

Table 7. Effects of dopamine and bromocriptine on spontaneous and epinephrine-evoked release of immunoreactive β-endorphin from cultured anterior lobe pituitary cells

Treatment	Spontaneous	Epinephrine (3×10^{-7} M)
Control	1.43 ± 0.06 (15)	2.85 ± 0.10 [a] (10)
Dopamine, 10^{-10} M	---------	3.16 ± 0.12 [a] (5)
Dopamine, 10^{-8} M	1.75 ± 0.21 (5)	3.16 ± 0.05 [a] (5)
Dopamine, 10^{-6} M	1.56 ± 0.15 (5)	2.81 ± 0.12 [a] (5)
Bromocriptine, 10^{-10} M	1.43 ± 0.14 (4)	2.94 ± 0.10 [a] (5)
Bromocriptine, 10^{-8} M	1.79 ± 0.06 (5)	2.72 ± 0.14 [a] (5)
Bromocriptine, 10^{-6} M	1.64 ± 0.25 (5)	1.76 ± 0.14 [b] (5)

Cells were incubated for 2 h with media containing vehicle, dopamine or bromocriptine treatments with or without epinephrine. Values are the group means ± SE (ng/plate) of iβ-endorphin and the numbers in parentheses refer to number of plates per group.

[a] Significantly different ($P<0.05$) from control or corresponding dopamine or bromocriptine treatment

[b] Significantly different ($P<0.05$) from epinephrine treatment

Table 8. Effects of bromocriptine on spontaneous and evoked release of immunoreactive β-endorphin from primary cultures of rat anterior lobe pituitary

Treatment	Spontaneous	Epinephrine (3×10^{-7} M)	CRF (3×10^{-10} M)
Control	3.36 ± 0.15 (15)	6.24 ± 0.24 [a] (10)	11.60 ± 0.56 [a] (5)
Bromocriptine, 10^{-8} M	3.12 ± 0.44 (5)	4.35 ± 0.18 [ab] (5)	--------
Bromocriptine, 10^{-7} M	3.14 ± 0.60 (5)	3.65 ± 0.11 [b] (5)	10.68 ± 0.53 [a] (5)
Bromocriptine, 10^{-6} M	2.80 ± 0.20 (5)	2.85 ± 0.22 [b] (5)	12.22 ± 0.81 [a] (5)

Cells were incubated for 2 h with media containing vehicle or bromocriptine treatment with or without epinephrine or corticotropin releasing factor (CRF). Values are the group means ± SE (ng/plate) of iβ-endorphin and the numbers in parentheses refer to number of plates per group.

[a] Significantly different ($P<0.05$) from control or corresponding bromocriptine treatment

[b] Significantly different ($P<0.05$) from epinephrine treatment

Table 9. Effects of LY141865 on release of immunoreactive β-endorphin and prolactin from cultured anterior lobe pituitary cells

Treatment	N	iβ-endorphin (ng/plate)	Prolactin (ng/plate)
Control media	11	0.95 ± 0.09	152.4 ± 3.5
LY141865 10^{-8} M	5	0.95 ± 0.09	44.1 ± 1.6 [ab]
10^{-7} M	5	0.79 ± 0.06	33.3 ± 2.0 [a]
10^{-6} M	5	0.64 ± 0.05 [a]	31.4 ± 1.3 [a]
CRF 10^{-10} M	5	6.42 ± 0.24 [a]	200 ± 16 [a]

Cells were incubated for 2 h with control media or media containing LY141865 at the doses indicated; values are the group mean ± SE of the number of samples indicated under N.

[a] Significantly different ($P<0.05$) from control media release

[b] Significantly different ($P<0.05$) from other LY141865 treatments

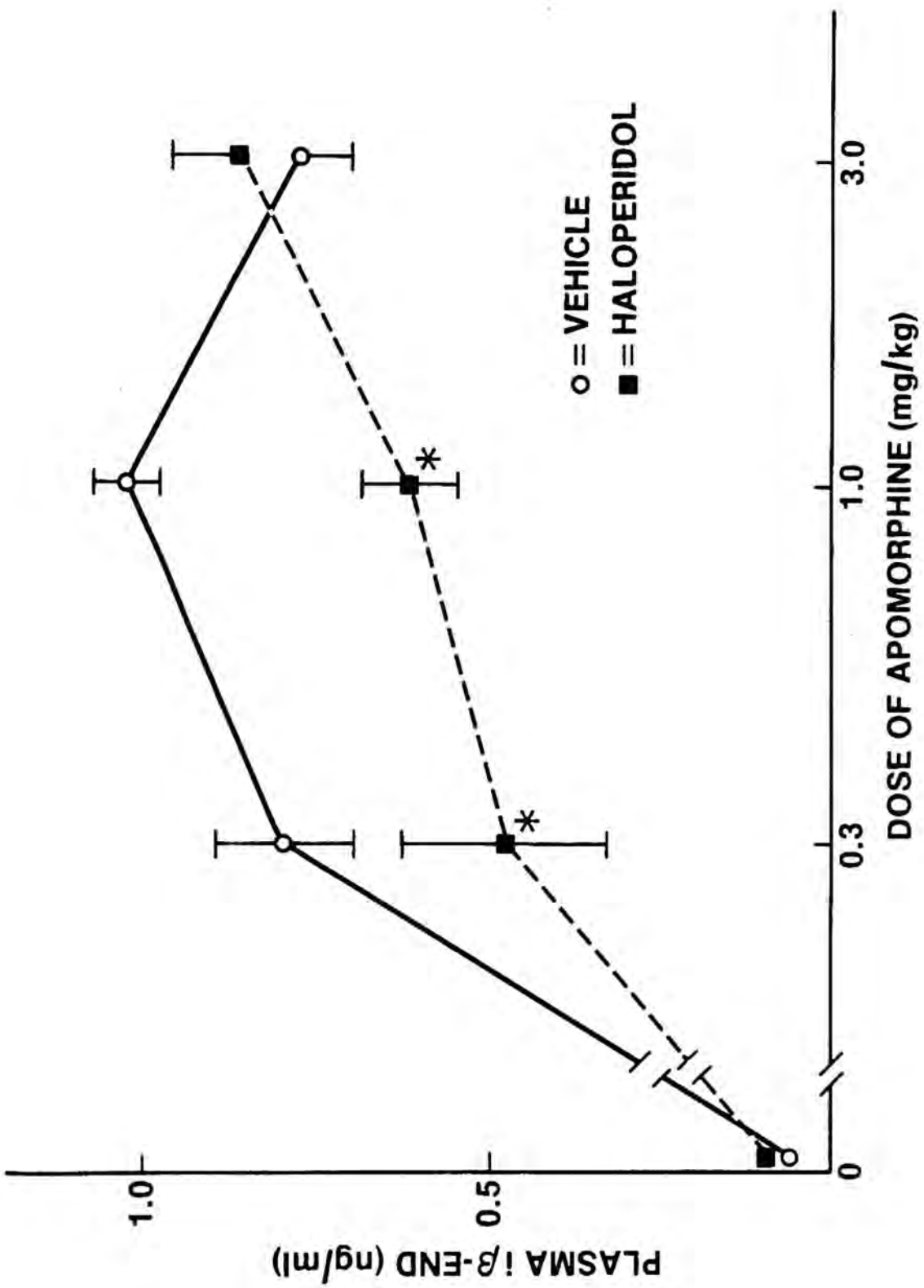
CRF. Table 9 shows that LY141865 was also unable to directly stimulate AL release of iB-endorphin. The highest dose tested (1 mcM) actually inhibited basal secretion by 33%. As anticipated, LY141865 reduced spontaneous release of prolactin in a dose-related fashion (Table 9). Together, the in vitro experiments showed first, that dopaminergic agonists do not directly stimulate AL release of iB-endorphin and, second, that at higher concentrations, these agents may actually interfere with adrenergically-evoked release. Therefore the stimulatory actions of dopaminergic agonists on AL release of iB-endorphin in vivo are most likely indirect and probably mediated by dopaminergic mechanisms which regulate CRF or some other hypothalamic secretagogue for AL corticotrophs.

3.0.1.3 Effects of Dopaminergic Antagonists on Agonist-Induced Release of iB-endorphin

In the following studies, a pharmalogical approach was used to elucidate the dopaminergic mechanisms which influence pituitary release of iB-endorphin. Prior to administration of apomorphine or LY141865, rats were pretreated with either a mixed D1, D2 antagonist, haloperidol, or with a selective D2 antagonist, sulpiride. Consistent with a dopaminergic mechanism, the stimulatory effects of both apomorphine (0.3, 1.0 and 3.0 mg/kg) and LY141865 (1 mg/kg) on pituitary iB-endorphin release were prevented by pretreatment with haloperidol, the D1, D2

Figure 13. Effects of haloperidol on apomorphine-induced release of immunoreactive B-endorphin (iB-END). Rats received an ip injection of vehicle or haloperidol (0.1 mg/kg) at 2 h and a sc injection of vehicle or apomorphine (doses indicated) 10 min prior to decapitation. Symbols and vertical lines represent the group mean +/- SE; N=6. All apomorphine groups were significantly different ($P<0.05$) from zero dose controls.

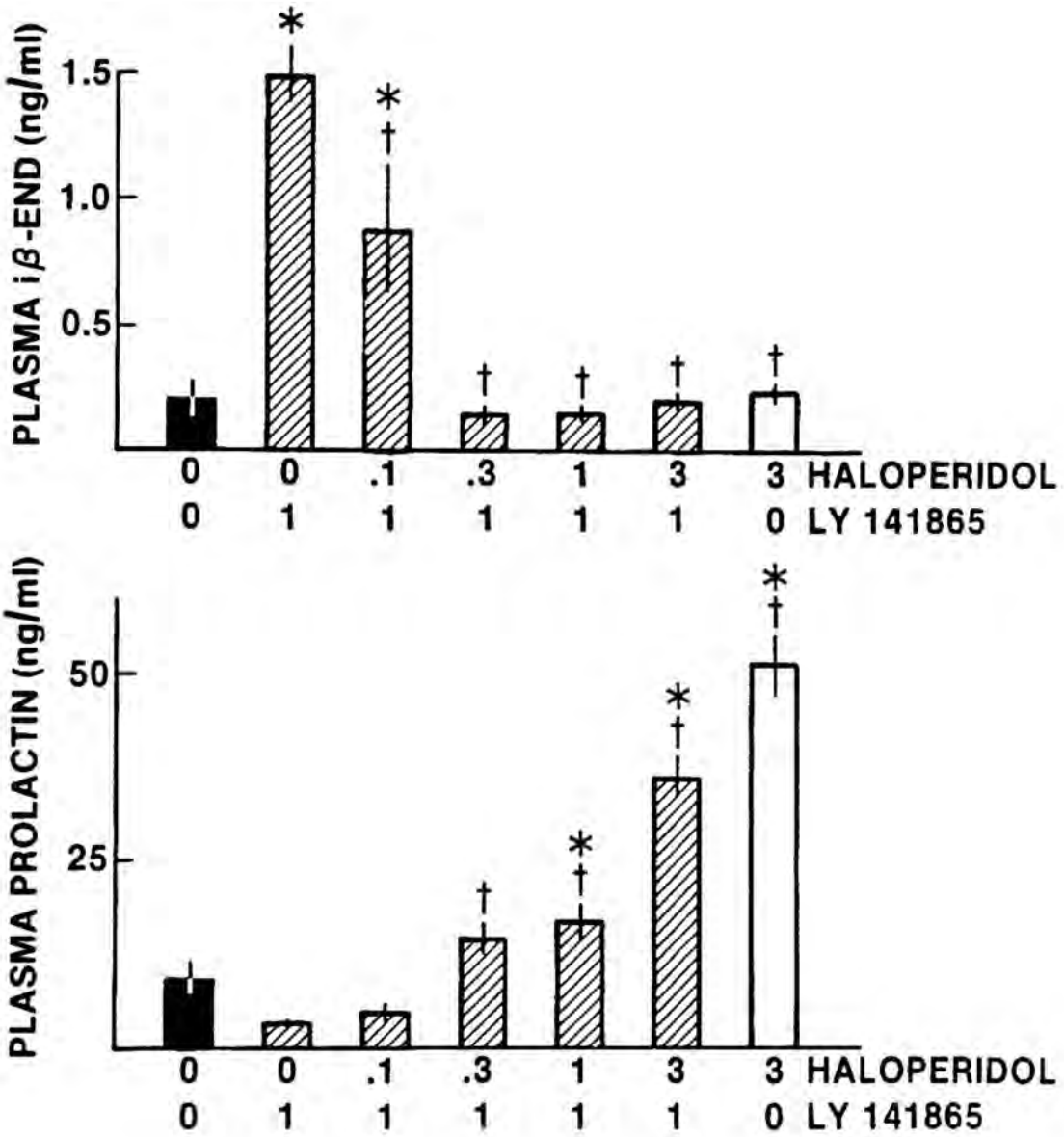
* Significantly different ($P<0.05$) from corresponding apomorphine treatment group


PLASMA iβ-END (ng/ml)
1.0
0.5
0
0.3
1.0
3.0
DOSE OF APOMORPHINE (mg/kg)
o = VEHICLE
■ = HALOPERIDOL
*
*

blocker. Pretreatment of rats with 0.1 mg/kg haloperidol (2 h) had no effect on basal levels of iB-endorphin in plasma yet reduced by 50% the stimulated release of iB-endorphin 10 min after administration of 0.3 and 1.0 mg/kg of apomorphine (Figure 13). Haloperidol also delayed the onset of stereotypic behavior induced by all doses of apomorphine. Conversely, the increase in plasma prolactin (14.1 +/- 3.4 versus 2.7 +/- 0.4 ng/ml) induced by haloperidol was reversed by all three doses of apomorphine (not shown). Pretreating rats with doses of haloperidol higher than the 0.1 mg/kg dose (used to attenuate apomorphine-induced release of iB-endorphin) completely blocked the release of iB-endorphin elicited by LY141865. As shown in the upper panel of Figure 14, a rise in circulating iB-endorphin greater than 7-fold due to LY141865 (1 mg/kg, 15 min) was reduced 40% by 0.1 mg/kg haloperidol and prevented by the 0.3 mg/kg dose. This latter pretreatment significantly increased plasma prolactin levels over those in rats treated with LY141865 alone (Figure 14, bottom graph). Higher doses of haloperidol (1.0 and 3.0 mg/kg) produced respectively greater increases in prolactin (5- and 11-fold versus 3.4 +/- 0.2 ng/ml in rats treated with LY141865 alone) and also induced ptosis, sedation, hypertonicity of limb muscles and catalepsy. In animals treated with 3 mg/kg of haloperidol alone, plasma prolactin rose to 51 +/- 4 ng/ml, a level which exceeded by 42% the levels in haloperidol-treated

Figure 14. Independent and interactive effects of haloperidol and LY141865 on circulating levels of immunoreactive B-endorphin (iB-END) and prolactin (PRL). Rats received an ip injection of vehicle or haloperidol (doses indicated, mg/kg) at 2 h and a second ip injection of vehicle or LY-141865 (1 mg/kg) 15 min prior to decapitation. Bars and vertical lines represent the group means +/- SE; N=5.

*Significantly different ($P<0.05$) from vehicle-treated controls
+Significantly different ($P<0.05$) from LY141865 alone


PLASMA iβ-END (ng/ml)
1.5
1.0
0.5
0 0 .1 .3 1 3 3 HALOPERIDOL
0 1 1 1 1 1 0 LY 141865
PLASMA PROLACTIN (ng/ml)
50
25
0 0 .1 .3 1 3 3 HALOPERIDOL
0 1 1 1 1 1 0 LY 141865

rats that also received LY141865. This suggests that although LY141865 was able to compete with haloperidol at dopamine receptors which inhibit prolactin secretion, the D2 agonist could not overcome blockade of those receptors which mediate AL release of iB-endorphin.

LY141865 is considered unique among dopaminergic agonists due to its selectivity for the D2 receptor in vitro (Tsuruta et al, 1981). Since plasma prolactin was potently inhibited by LY141865 and the receptors which mediate this inhibition have been characterized as the D2 subtype, (Kebabian and Calne, 1979), LY141865 may also stimulate AL release of iB-endorphin through a D2 receptor-mediated mechanism. In order to test this possibility in vivo, the actions of LY141865 were challenged by pretreating rats with the D2 antagonist, sulpiride (Trabucchi et al, 1976). Since sulpiride's effects in the central nervous system reportedly develop slowly after systemic administration (Costall et al, 1978; Hofmann et al, 1979; Nishibe et al, 1982), rats were pretreated with a relatively high dose of sulpiride (10 mg/kg, ip) administered either 3 h or 30 min prior to sampling. As shown in Table 10, plasma levels of iB-endorphin were increased about 2-fold after sulpiride (3 h or 30 min). Nevertheless, the sulpiride pretreatments failed to attenuate a 7-fold rise in circulating iB-endorphin due to LY141865. In fact, the combination treatment of LY141865 plus sulpiride (30 min) resulted in

Table 10. Effects of systemically administered sulpiride and LY141865 alone and in combination on circulating immunoreactive β-endorphin and prolactin

Treatment	Plasma Hormones (ng/ml) iβ-endorphin	prolactin
Vehicle + Vehicle	0.22 ± 0.04	7.8 ± 1.9
30 min Sulpiride	0.53 ± 0.10 [a]	62.1 ± 8.6 [a]
3 h Sulpiride	0.45 ± 0.06 [a]	34.8 ± 3.9 [a]
Vehicle + LY141865	1.61 ± 0.13 [a]	5.9 ± 0.7
30 min Sulp + LY141865	2.42 ± 0.50 [ab]	50.7 ± 6.5 [ab]
3 h Sulp + LY141865	2.00 ± 0.18 [ab]	20.6 ± 2.7

Rats received ip injections of vehicle or sulpiride (10 mg/kg) 3 h or 30 min before sacrifice; 15 min before decapitation, animals received a third injection of either vehicle or LY141865 (1 mg/kg). Values are the group mean ± SE; N=6-7.

[a] Significantly different ($P<0.05$) from vehicle-treated controls

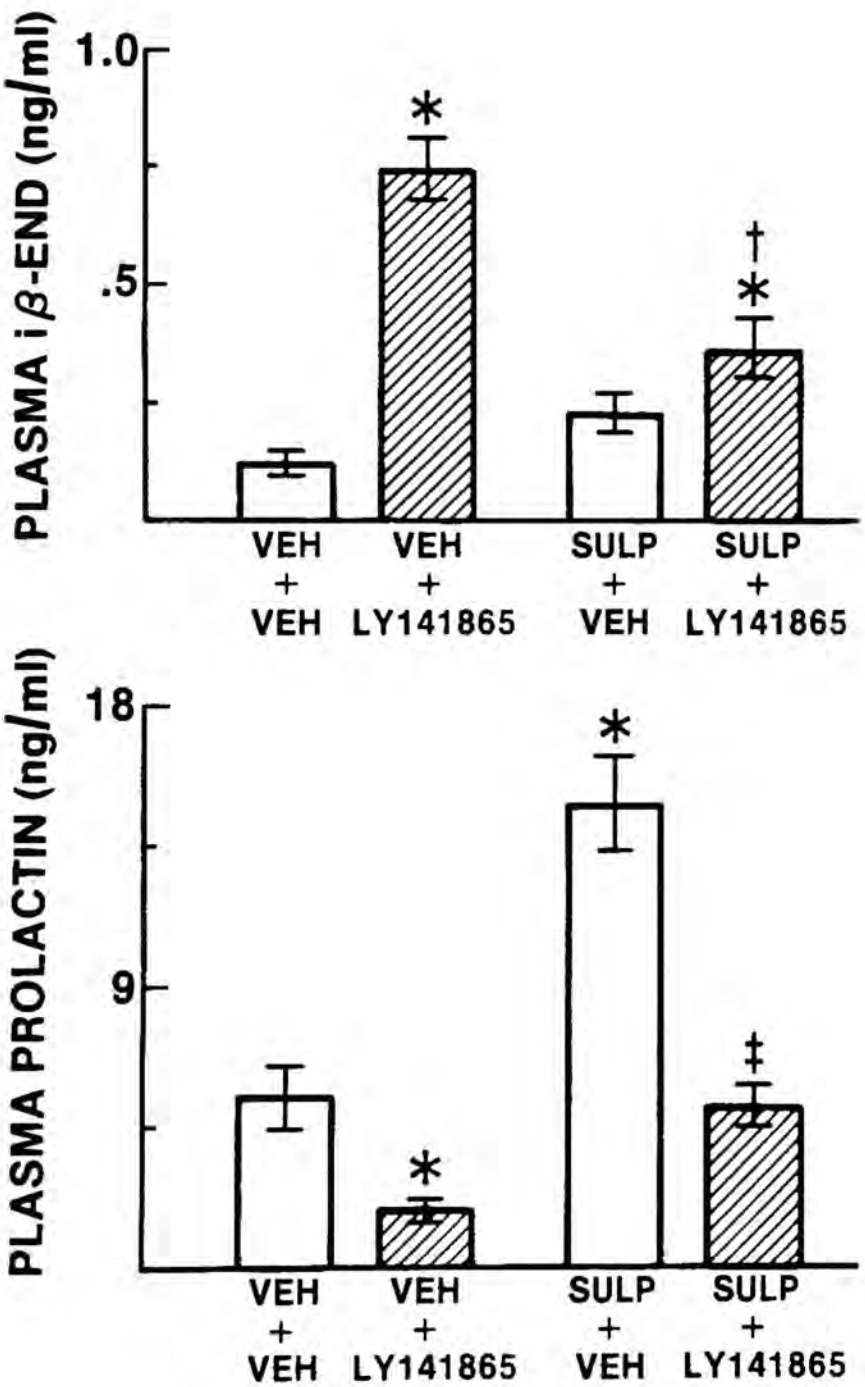
[b] Significantly different ($P<0.05$) from LY141865 treatment alone

levels of plasma iB-endorphin that exceeded by 50% those measured in plasma of rats treated with LY141865 alone.

In contrast to the failure of systemically administered sulpiride to antagonize LY141865-induced release of AL iB-endorphin, centrally administered sulpiride curtailed the increase of plasma iB-endorphin following LY141865. Conscious rats received intracerebroventricular (icv) injections of vehicle or sulpiride (100 mcg/rat, 4 h) through previously-implanted guide cannulas. Subsequently, these animals received either LY141865 or vehicle treatment. Similar to the effects of high-dose haloperidol, sulpiride alone induced a cataleptic behavioral state and halved the increase in plasma iB-endorphin due to LY141865 (Figure 15, upper panel). As shown in the bottom panel of Figure 15, the two compounds cancelled each other's effects on plasma prolactin. These results demonstrate that the endocrine actions of LY141865 can be antagonized by selective blockade of D2 receptors in vivo and again suggest that dopaminergically-stimulated secretion of AL iB-endorphin is most likely dediated through central nervous system pathways located within the blood-brain barrier. Presumably, exclusion of sulpiride by the blood-brain barrier is the reason that peripherally administered sulpiride was not effective in counteracting the releasing effects of LY141865 on pituitary iB-endorphin.

Figure 15. Effects centrally administered sulpiride on LY141865-induced release of immunoreactive B-endorphin (iB-END) and prolactin. Rats received an icv injection of vehicle (VEH, 15 mcl/rat) or sulpiride (SULP, 100 mcg/rat) at 4 h and an ip injection of vehicle or LY141865 (1 mg/kg) 15 min prior to decapitation. Bars and vertical lines represent the group mean +/- SE; N=9.

*Significantly different ($P<0.05$) versus VEH + VEH controls
+Significantly different ($P<0.05$) versus VEH + LY141865
=Significantly different ($P<0.05$) from SULP + VEH

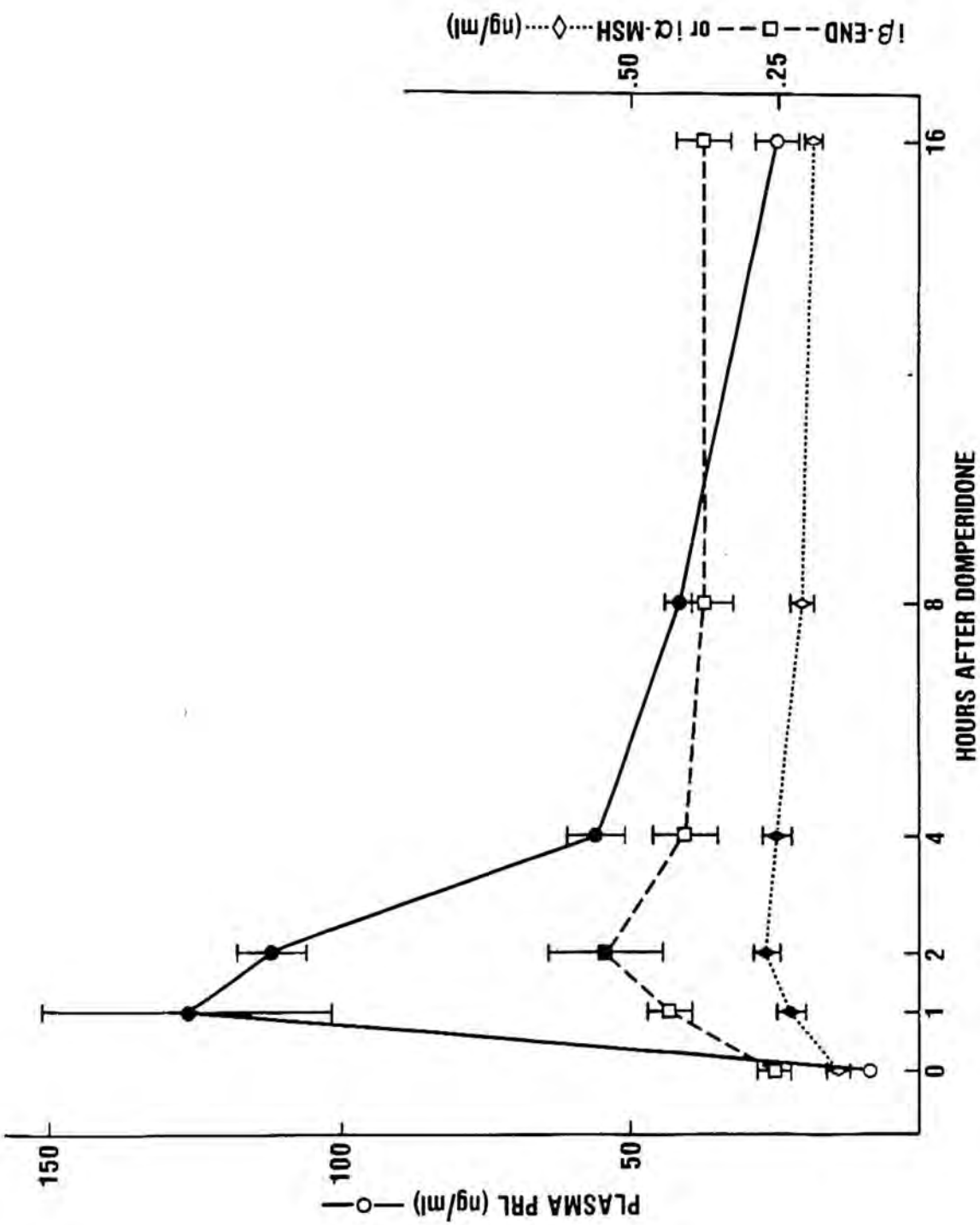

PLASMA iβ-END (ng/ml)
1.0
.5
*
†
*
VEH + VEH
VEH + LY141865
SULP + VEH
SULP + LY141865
PLASMA PROLACTIN (ng/ml)
18
9
*
*
‡
VEH + VEH
VEH + LY141865
SULP + VEH
SULP + LY141865

The data presented above revealed several characteristics about the actions of dopamine on pituitary secretion of iB-endorphin. First, the ability of agonists like apomorphine and LY141865 to evoke AL release of iB-endorphin *in vivo* is very likely mediated through a physiological dopaminergic mechanism since the stimulatory actions of agonists were dose-dependent and as sensitive to blockade by the mixed D1, D2 antagonist, haloperidol, as is disinhibition of prolactin release. Secondly, the type of dopaminergic receptor which mediates enhanced AL secretion *in vivo* may well be D2 since the D2 agonist, LY141865, evoked release of iB-endorphin through a mechanism that was attenuated by the D2 antagonist, sulpiride, albeit, only after icv administration. This introduces the third feature of agonist-induced release. Apparently, the D2 receptors which mediate increased AL secretion are located in a part of the central nervous system which is protected by the blood-brain barrier. The initial suggestion of this came from the bromocriptine results where it was observed that, unlike LY141865, bromocriptine had no apparent effect on AL release of iB-endorphin. Like sulpiride, however, bromocriptine exhibits delayed onset of central nervous system actions, therefore its failure to stimulate AL release of iB-endorphin *in vivo* may reflect limited activation of the appropriate brain receptors. Hence AL secretion of iB-endorphin may not necessarily be expected.

3.0.2 Effects of Dopaminergic Antagonists

As shown in the last studies of the preceding section, antagonists of dopamine receptors potently elevate plasma levels of prolactin. It is widely held that this response is due to disinhibition of AL lactotrophs whose secretion of prolactin is tonically inhibited by the high concentrations of dopamine in hypophyseal-portal blood (MacLeod, 1976; Meltzer et al, 1978; Gibbs and Neill, 1978). D2 receptors like those which regulate prolactin secretion have been found in the IL (see Cote et al, 1982). There, dopamine receptor stimulation blocks spontaneous and evoked release of alpha-melanotropin and iB-endorphin *in vitro* (Boner et al, 1974; Przewlocki et al, 1978; Vale et al, 1979; Cote et al, 1982). Since the IL is innervated by tonically active dopamine neurons from the basal hypothalamus *in vivo* (Bjorklund et al, 1973; Demarest & Moore, 1979), treatment of rats with blockers of, in particular, D2 receptors should increase circulating levels of iB-endorphin through disinhibition of IL secretion. Treatment with sulpiride, but not haloperidol, was seen in section 1.3 to modestly elevate plasma levels of total iB-endorphin. In order to better define the role of dopamine in regulating IL release of iB-endorphin *in vivo*, the effects of both D2 and of mixed D1, D2 antagonists on circulating iB-endorphin were examined more carefully. Results of these studies are described in the parts 1, 2

Figure 16. Time-related effects of domperidone on circulating levels of immunoreactive B-endorphin (iB-END), alpha-melanotropin (ia-MSH) and prolactin (PRL) in Long-Evans rats. Rats received sc injections of vehicle or domperidone (1.0 mg/kg) 1, 2, 4, 8 or 16 hours prior to decapitation. Symbols and vertical lines represent the group means +/- SE (N=8) with solid symbols indicating group values significantly different ($P<0.05$) from zero time controls.


PLASMA PRL (ng/ml) —o—
150
100
50
0
1
2
4
8
16
HOURS AFTER DOMPERIDONE
.50
.25
iβ-END - - □ - - or iα-MSH ····◇···· (ng/ml)

and 3 of this section. Time- and dose-related effects of the D2 antagonists, domperidone and sulpiride, as well as of the mixed D1, D2 antagonists, haloperidol and pimozide, on circulating levels of total iB-endorphin and the major molecular forms of plasma iB-endorphin are described in section 2.1. Section 2.2 reports the results of experiments designed to examine, specifically, haloperidol's influence on AL release of iB-endorphin in vivo and in vitro. Section 2.3 describes studies in which the releasing effects of haloperidol on pituitary iB-endorphin release in vivo were challenged either with the D1 agonist, SKF 38393 or with the D2 agonist, bromocriptine.

3.0.2.1 Effects of Dopaminergic Antagonists on Circulating iB-endorphin

The D2 dopamine receptor antagonists, domperidone and sulpiride, significantly increased iB-endorphin in rats. As shown in Figure 16, 2 h after administration of the long-acting D2 antagonist, domperidone (2.5 mg/kg, sc), circulating iB-endorphin was significantly increased ($P<0.05$) as compared to control values (0.25 +/- 0.03 ng/ml) and tended to be elevated throughout the 16 h time course of the experiment. The maximal rise in plasma iB-endorphin (~220%) was similar in magnitude to the rise in circulating immunoreactive alpha-melanotropin (iMSH)which was significantly increased 1, 2 and 4 h after the domperidone treatment. Compared to the effects of

domperidone on plasma prolactin, however, circulating iB-endorphin and iMSH were only moderately effected by D2 receptor blockade *in vivo*. Domperidone potently increased plasma prolactin 15-fold versus control values (8.4 +/- 0.8 ng/ml) 60 min after treatment and prolactin levels remained significantly elevated up to 8 h after domperidone. Neither domperidone nor systemically administered sulpiride produced noticeable behaviorable changes in rats relative to their vehicle-treated cage-mates. Sulpiride like domperidone, elevated circulating iB-endorphin and prolactin except that sulpiride's effects occured rapidly after its administration. The left-hand panel of Figure 17 shows that 15, 30 and 60 min after an ip injection of sulpiride (2.5 mg/kg), circulating levels of total iB-endorphin were significantly elevated to a maximum of 207% of control values at 30 min. Similar to domperidone, sulpiride maximally increased plasma prolactin to a greater degree (7-fold versus the control levels of 32.3 +/- 6.3 ng/ml) at an earlier time after treatment (15 min) than the rise in iB-endorphin. As shown in the elution profiles in the right hand panels of Figure 17, the dominant molecular form of iB-endorphin in plasma of both control and the 30 min sulpiride treatment group resembled B-endorphin in molecular size. B-endorphin-sized material contributed essentially all (80%) of the 107% increase in plasma levels of total iB-endorphin after sulpiride. In contrast, iB-endorphin resembling B-LPH in size represented just 27%

Figure 17. Effects of sulpiride on circulating immunoreactive B-endorphin (iB-END). Composite illustration showing the time-course effects (left) and the gel chromatographic profiles (right) of sulpiride versus control treatments. Rats received ip injections of vehicle (2 cc/kg, 30 min) or sulpiride (2.5 mg/kg) 15, 30 or 60 min prior to decapitation. Points and vertical lines represent the group mean +/- SE; N=5-7, in the time-course. Pools of treatment group plasma (7.5 ml) were filtered on a column of Sephadex G-50 resin and the elution profiles were graphed with respect to the relative mobility coefficient, Kd. Positions of calibration peaks, i.e., blue dextran (Vo), human B-lipotropin (B-LPH) and camel B-endorphin (B-END), are shown (arrows) above the control profile (right).

*Significantly different ($P<0.05$) from zero time controls

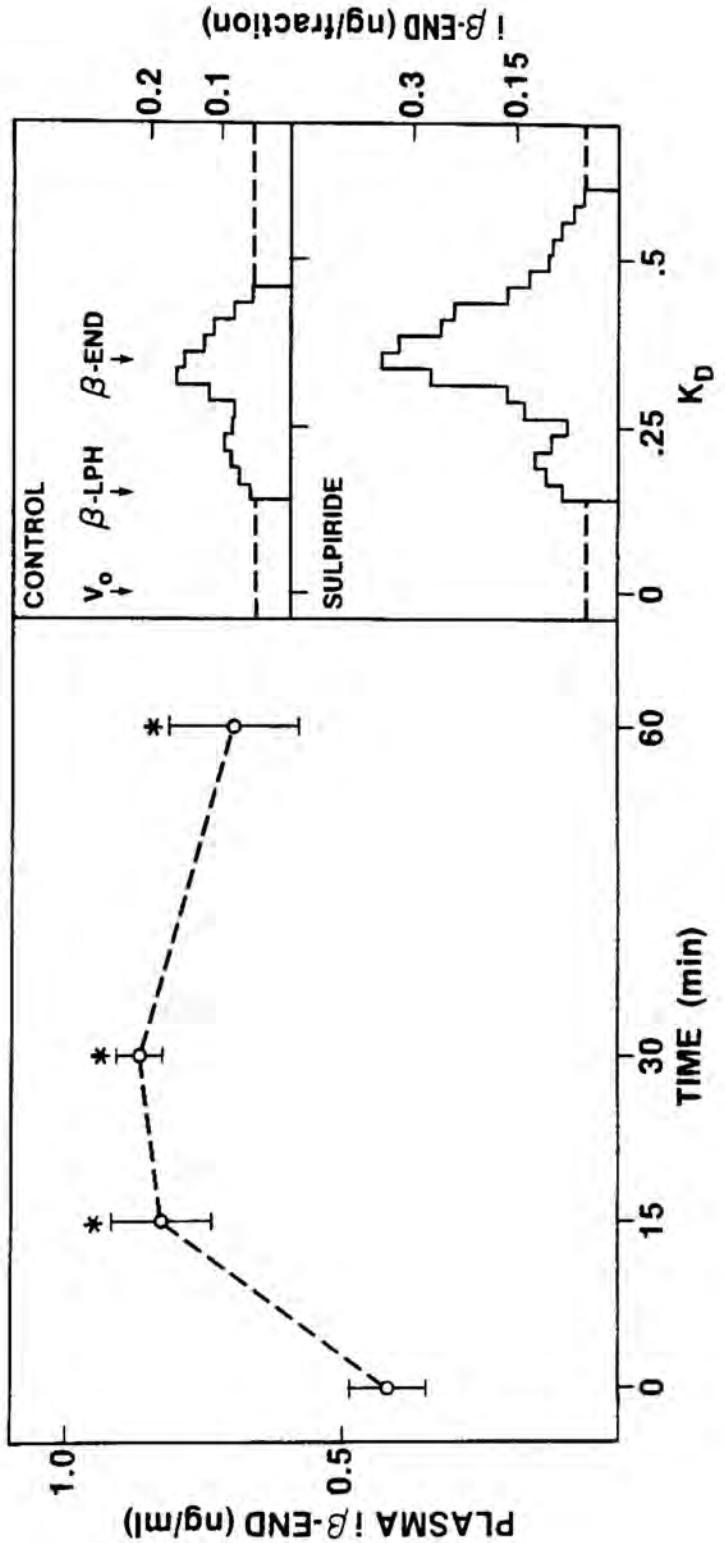

PLASMA i β-END (ng/ml)
1.0
0.5
0
15
30
60
TIME (min)
CONTROL
V_o
β-LPH
β-END
SULPIRIDE
0.2
0.1
0.3
0.15
0
.25
.5
K_D
i β-END (ng/fraction)

Table 11. Dose-response effects of sulpiride on circulating levels of immunoreactive β-endorphin

Dose of sulpiride (mg/kg)	Plasma iβ-endorphin (ng/ml)
0	0.43 ± 0.04
0.5	0.92 ± 0.12 [a]
2.5	0.80 ± 0.13 [a]
7.5	0.71 ± 0.07
25.0	1.02 ± 0.14 [a]

Rats received an ip injection of vehicle (zero dose) or sulpiride (doses indicated) 30 min prior to decapitation. Values are the group mean ± SE; N=6-7.

[a] Significantly different ($P<0.05$) from zero dose controls

of control levels and increased less than 30% after sulpiride treatment. These findings suggest that sulpiride may evoke IL release of iB-endorphin excluding a substantial increase in AL secretion. When the effects of various doses of sulpiride were examined (Table 11) it became apparent that a ceiling exists on the ability of sulpiride to induced IL release of iB-endorphin. After treating rats with doses of 0.5 to 25 mg/kg sulpiride, plasma levels of iB-endorphin were found to be increased as much by the lowest as they were by the highest doses, and the elevation was maximally 240% of control values (Table 11). Nonetheless, this modest increase approximated the rise evoked by domperidone. Considering this and the parallel elevation of ialpha-melanotropin and iB-endorphin after domperidone treatment, the evidence implicates a D2 receptor mechanism through which IL secretion of iB-endorphin is tonically inhibited.

Antagonists of both D2 and D2 receptors, haloperidol and pimozide, were also examined for their influence on plasma levels of iB-endorphin. The results demonstrate that the releasing effects of the mixed antagonists differ from the effects of D2 antagonists. Figures 18 and 19 show the time-course effects of haloperidol and pimozide on circulating levels of iB-endorphin and prolactin. A single injection of haloperidol (1 mg/kg, ip) significantly

Figure 18. Time course effects of haloperidol on plasma levels of iB-endorphin (iB-END) and prolactin (PRL). Samples were collected withen 2 h after ip injection of vehicle (zero time) or 1, 2 or 3 h after 1 mg/kg haloperidol (ip). Bars and vertical lines represent the group mean +/- SE; N=7-8. Significant differences ($P<0.05$) were determined between control levels of iB-endorphin and the 1 and 2 h haloperidol treatments, and between control prolactin levels and levels induced at all times after haloperidol treatment.

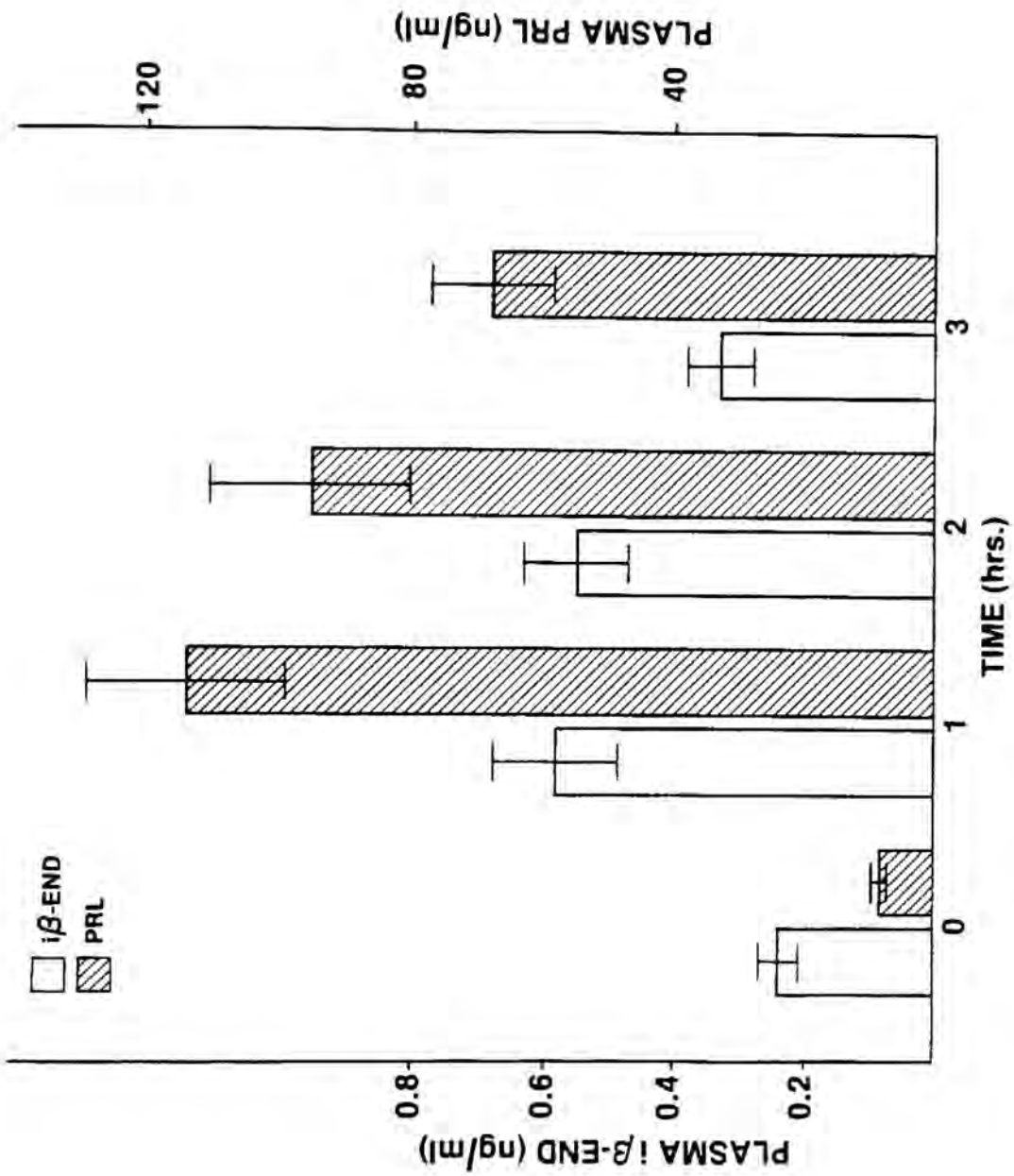

PLASMA PRL (ng/ml)
120
80
40
PLASMA iβ-END (ng/ml)
0.8
0.6
0.4
0.2
iβ-END
PRL
0
1
2
3
TIME (hrs.)

Figure 19. Time course effects of pimozide on circulating levels of beta-endorphin-like immunoreactivity (B-END-LI) (dashed line) and prolactin (PRL) (solid line). Samples were collected either 30 min after ip injection of vehicle (zero time) or 30, 120 or 360 min after pimozide (0.25 mg/kg, ip). Points and vertical lines represent group means SE; N=5-6. The 2 h pimozide treatment significantly ($P<0.05$) increased levels of B-endorphin-like immunoreactivity and prolactin versus zero time controls.

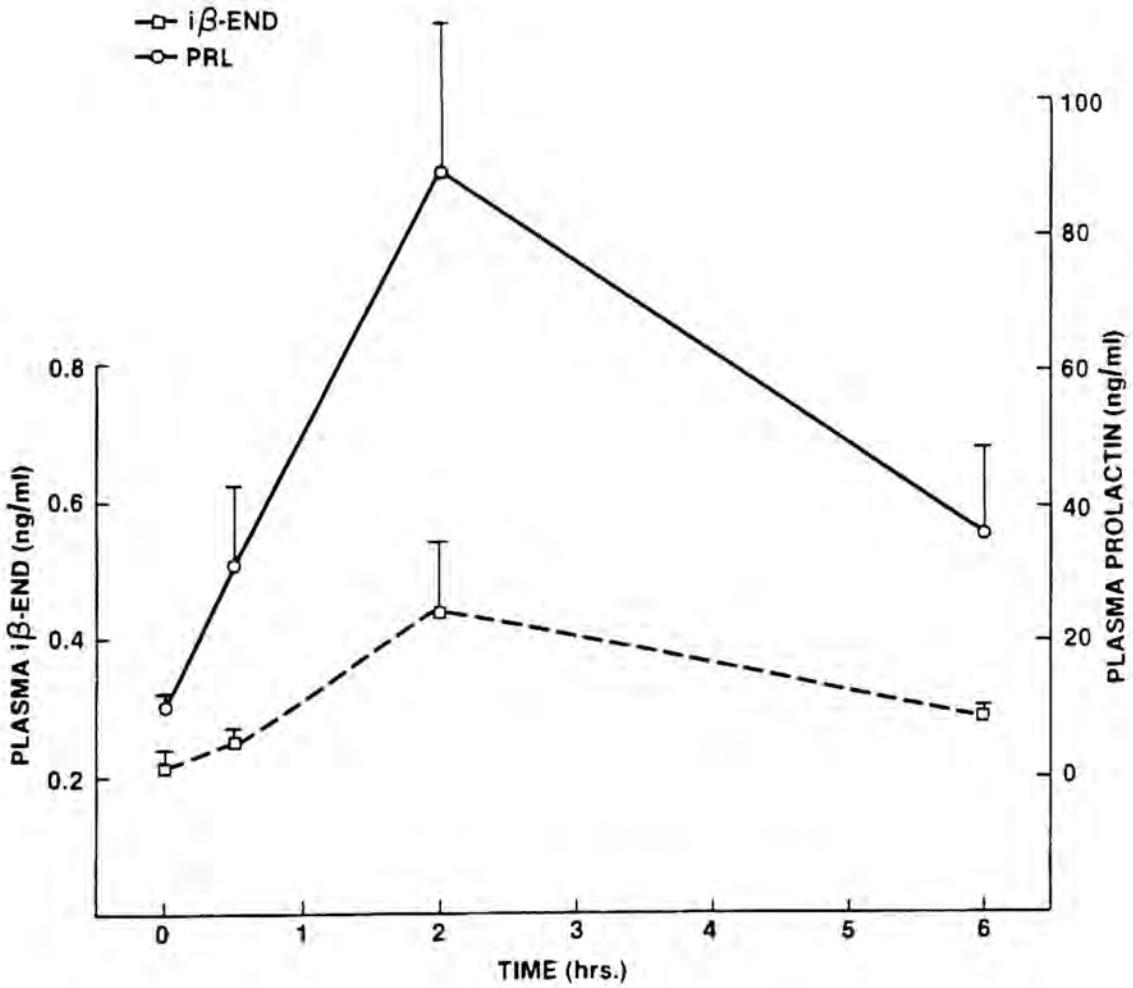

iβ-END
PRL
PLASMA iβ-END (ng/ml)
PLASMA PROLACTIN (ng/ml)
TIME (hrs.)
0.8
0.6
0.4
0.2
100
80
60
40
20
0
0
1
2
3
4
5
6

Table 12. Dose-response effects of haloperidol and pimozide on plasma levels of immunoreactive β-endorphin and prolactin

Treatment	Plasma Hormones (ng/ml) iβ-endorphin	prolactin
	Experiment I	
Vehicle	0.38 ± 0.02	13 ± 4
Haloperidol, 0.1 mg/kg	0.56 ± 0.08	59 ± 5 [a]
Haloperidol, 1.0 mg/kg	0.61 ± 0.06 [a]	79 ±10 [a]
	Experiment II	
Vehicle	0.34 ± 0.02	23 ± 5
Pimozide, 0.25 mg/kg	0.53 ± 0.10	55 ± 5 [a]
Pimozide, 2.50 mg/kg	0.74 ± 0.12 [a]	55 ± 5 [a]

Rats received ip injections of vehicle, haloperidol (2 h) or pimozide (3 h) before sacrifice. Values are the group mean ± SE; N=6-8.

[a] Significantly different ($P<0.05$) from appropriate vehicle-treated controls

increased plasma iB-endorphin 2-fold over control values by one and two hours post-treatment; after three hours, circulating iB-endorphin was no longer different from the control mean of 0.24 +/- 0.03 ng/ml (Figure 18). In these same animals, prolactin was maximally increased almost 13-fold at 1 h as compared to control levels (114 +/- 15 versus 9 +/- 1 ng/ml) and remained significantly elevated up to 3 h after injection of haloperidol. Similar to haloperidol, pimozide increased iB-endorphin and prolactin in a time-related manner (Figure 19). Plasma levels of iB-endorphin and prolactin were significantly greather than control values 2 h after ip administration of pimozide (0.25 mg/kg). As observed in haloperidol-treated rats, the prolactin increase due to pimozide was greater than the rise in levels of iB-endorphin (9-fold as compared to 2-fold at 2 h). Both haloperidol and pimozide dose-relatedly increased iB-endorphin as shown in Table 12. Compared to the appropriate control values, 1.0 mg/kg of haloperidol (2 h) and 2.5 mg/kg of pimozide (3 h) significantly incrased plasma levels of iB-endorphin 60% and 120%, respectively. Prolactin was increased to an even greater extent than the iB-endorphin by haloperidol; doses of 0.1 and 1.0 mg/kg increased prolactin 350% and 510% greater than controls, whereas, the 0.25 and 2.5 mg/kg doses of pimozide both increased prolactin by 140%. Figure 20 allows comparison of the gel chromatographic elution profiles from control and haloperidol-treated rats. As

seen in previous elution profiles, B-endorphin-sized peptides were the major component in control rat plasma. Although the B-endorphin-sized form of iB-endorphin constituted the larger peak in the plasma profiles of haloperidol-treated animals (2.5 mg/kg, ip, 45 min), the B-LPH form of immunoreactivity was also increased substantially. B-LPH constituted 37% of total immunoreactivity in control rats (0.28 +/- 0.04 ng/ml) and 43% of the total (0.85 +/- 0.10 ng/ml) in haloperidol-treated rats. This represents a 3.5-fold increase of B-LPH, whereas, B-endorphin-sized peptides increased less than 3-fold (275%) and indicates that AL release of iB-endorphin was increased at least as much as IL release after haloperidol. The data in Table 13 outline time-related effects of the 2.5 mg/kg dose of haloperidol on plasma levels of total iB-endorphin, on the ratio of B-LPH- to B-endorphin-sized material and on content of total iB-endorphin in the AL and neurointermediate lobe (NIL). Plasma iB-endorphin was maximally elevated 3.5-fold relative to control levels at 30 min yet no change in either AL or NIL content of iB-endorphin was observed at this time or up to 3 h after haloperidol treatment. Chromatography of treatment group plasma revealed that the ratio of immunoreactivity in the B-LPH versus the B-endorphin peaks decreased during the first hour after haloperidol and recovered to the control ratio by 3 h after treatment. These data indicate that during the peak

Figure 20. Effects of haloperidol on circulating levels of immunoreactive B-endorphin (iB-END). Composite illustration showing the time-course effects (left) and gel chromatographic profiles (right) of haloperidol versus control treatments. The time-course effects of 2.5 mg/kg haloperidol shown here are enumerated in Table 13. Symbols and vertical lines represent the group mean +/- SE; N=7-8. Pools of plasma (7-8 ml) from vehicle- (control) or haloperidol-treated (60 min) rats were filtered on a column of Sephadex G-50 and the elution profile was graphed with respect to the mobility coefficient, Kd. Positions of calibration peaks, i.e., blue dextran (Vo), human beta-lipotropin (B-LPH) and camel beta-endorphin (B-END), are shown (arrows) above the control profile.

*Significantly different ($P<0.05$) from zero time controls

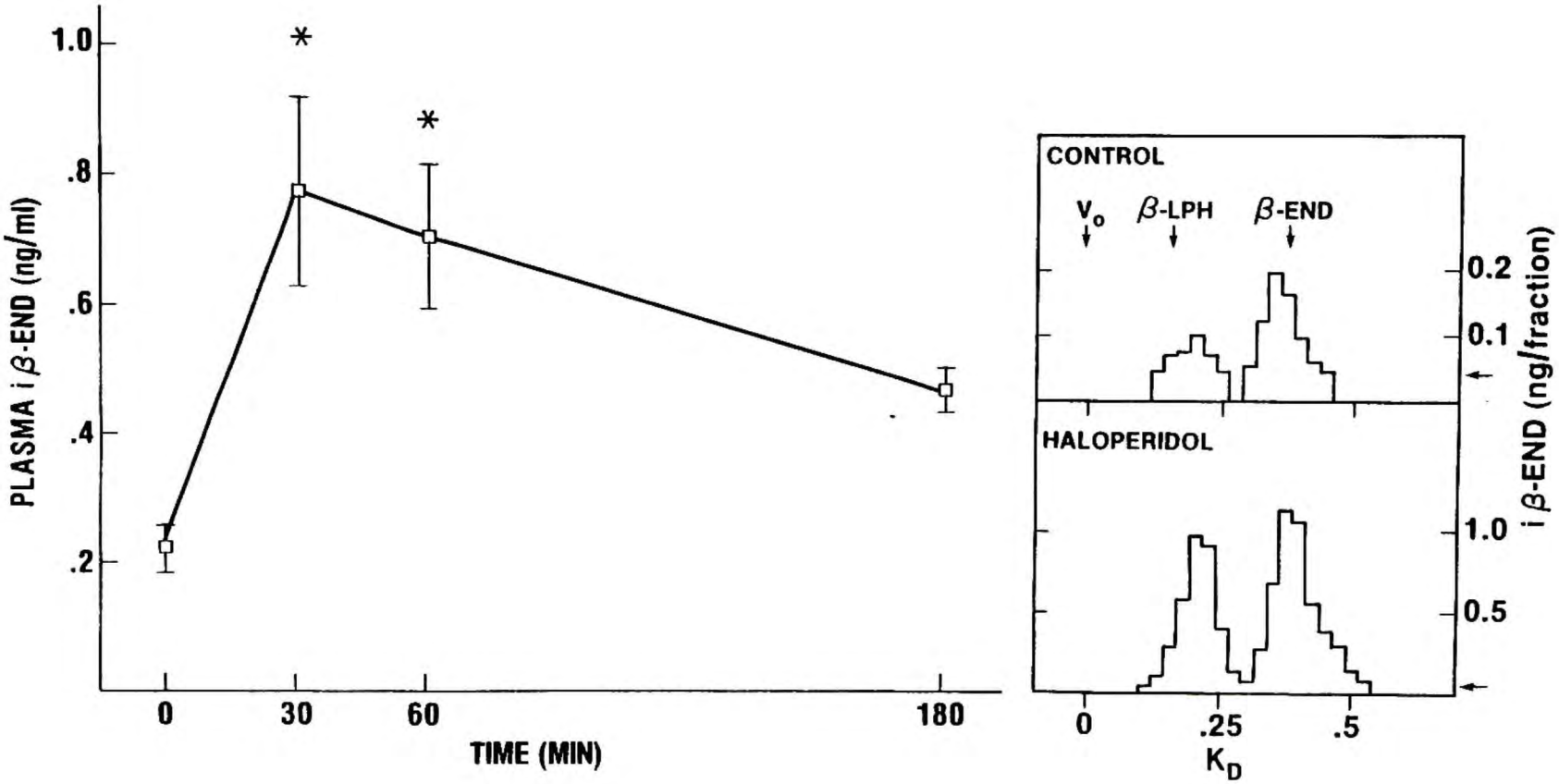

1.0
.8
.6
.4
.2
PLASMA i β-END (ng/ml)
*
*
0
30
60
180
TIME (MIN)
CONTROL
V₀
β-LPH
β-END
HALOPERIDOL
0.2
0.1
1.0
0.5
i β-END (ng/fraction)
0
.25
.5
K_D

Table 13. Time-course effects of haloperidol on plasma levels and pituitary content of immunoreactive β-endorphin in rats

Treatment	Plasma (ng/ml)	β-LPH / β-END	Pituitary (μg/mg protein) AL	Pituitary (μg/mg protein) NIL
Vehicle, 30 min	0.22 ± 0.04	.38	1.5 ± 0.2	24.2 ± 3.6
Haloperidol, 30 min	0.78 ± 0.15 [a]	.43	1.5 ± 0.2	23.6 ± 1.5
Haloperidol, 60 min	0.71 ± 0.11 [a]	.61	1.3 ± 0.1	21.5 ± 4.8
Haloperidol, 180 min	0.47 ± 0.03	.22	1.2 ± 0.1	26.3 ± 2.2

Rats received an ip injection of vehicle or haloperidol (2.5 mg/kg) at the times indicated prior to decapitation. Values are the group mean ± SE of plasma iβ-endorphin (N=7-8) except for the ratios of β-lipotropin to β-endorphin (β-LPH/β-END).

[a] Significantly different ($P<0.05$) from vehicle-treated controls

response of total circulating iB-endorphin to haloperidol, the B-LPH form of iB-endorphin was increased more than B-endorphin-sized form. This, together with the chromatographic results shown in Figure 20, further suggests that the mixed antagonist increased both AL and IL release of iB-endorphin. Similar changes in plasma levels of total iB-endorphin (1.12 +/- 0.19 versus 0.43 +/- 0.06 ng/ml) and in the underlying molecular forms (not shown) were observed after pimozide treatment (2.5 mg/kg, 2 h). Whereas higher doses of sulpiride continued to evoke no more than a doubling of total iB-endorphin, higher doses of the mixed D1, D2 antagonists induced greater relative elevations in plasma levels of iB-endorphin through additional release of the B-LPH form of circulating iB-endorphin. This AL releasing effect of the mixed D1, D2 antagonists differs from the limited ability of the D2 antagonists, sulpiride and domperidone, to elevate total levels of iB-endorphin since effects of the D2 blockers appeared, from chromatography and by concomitant release of immunoreactive alpha-melanotropin, to be restricted to the IL. As determined in section 1.3, systemically administered sulpiride is unable to reach the brain dopamine receptors which mediate the iB-endorphin releasing effects of LY141865. Likewise, domperidone has been found to have very limited access to the brain (Laduron and Leysen, 1979; Farah et al, 1983). This information and the fact that the IL is on the systemic side of the blood-brain barrier

supports the view that sulpiride and domperidone increase circulating iB-endorphin by selectively disinhibiting IL secretion.

In summary, circulating levels of total iB-endorphin were increased by two peripherally-active D2 antagonists, domperidone and sulpiride, as well as by two centrally active antagonists of both D1 and D2 receptors, haloperidol and pimozide. Due to the elevation of only B-endorphin-sized peptides in plasma of rats treated with D2 antagonists, these drugs probably enhanced only IL release of iB-endorphin. The mixed antagonists, however, increased both B-LPH- and the B-endorphin-like immunoreactivity suggesting that both AL and IL release of iB-endorphin were elevated, respectively. This was reflected in the ability of the mixed antagonists to increase total iB-endorphin in blood to a greater extent than that of the selective D2 antagonists. Before examining the mechanisms by which these differential responses occured, studies were conducted in vivo to insure that the mixed antagonists indeed increase AL release of iB-endorphin and in vitro to determine if their stimulatory effects were due to direct actions on AL corticotrophs.

3.0.2.2 Dopaminergic Antagonist Effects on AL iB-endorphin in Vivo and in Vitro

The ability of mixed D1, D2 blockers to elevate

circulating levels of total iB-endorphin was challenged by glucocorticoid pretreatment, a method described earlier for inhibiting AL but not IL secretion of iB-endorphin. As shown in Figure 21, 50 but not 5 mcg/kg dexamethasone pretreatment (4 h) lowered basal levels of total iB-endorphin (0.22 +/- 0.02 versus 0.39 +/- 0.03 ng/ml in controls) and, compared to a 3-fold increase due to haloperidol alone, dexamethasone reduced iB-endorphin release by 44%. Nonetheless, compared to dexamethasone treatment alone, haloperidol still induced nearly a 3-fold rise in total circulating levels of iB-endorphin. This 50 mcg/kg dose of dexamethasone was the same pretreatment that completely prevented agonist-induced release and reduced the rise due to immobilization by 60% (Table 6 and Figure 12, respectively). The results of combined dexamethasone and haloperidol treatments show that a substantial portion of haloperidol-induced release is glucocorticoid-suppressible but a similar amount is insensitive to exogenous glucocorticoids. These results support the chromatographic evidence (elevated iB-endorphin resembling B-LPH) that mixed D1, D2 antagonists enhance AL release of iB-endorphin in the rat.

In order to examine the remote possibility that haloperidol might directly stimulate corticotroph secretion of iB-endorphin, cultured AL cells were exposed to 0.1 mcM and 1.0 mcM concentrations of haloperidol. As shown in Table 14, neither dose of the antagonist altered

Figure 21. Effects of dexamethasone pretreatment on haloperidol-induced elevation of circulating immunoreactive B-endorphin (iB-END). Rats received an ip injection of vehicle or dexamethasone 4 h prior to sacrifice; a second vehicle injection or haloperidol (2.5 mg/kg) was administered 60 min before decapitation. Bars and vertical lines represent the group means +/- SE; N=8.

* Significantly different ($P<0.05$) from vehicle-treated controls
+ Significantly different ($P<0.05$) from haloperidol treatment alone or with 5 mcg/kg dexamethasone

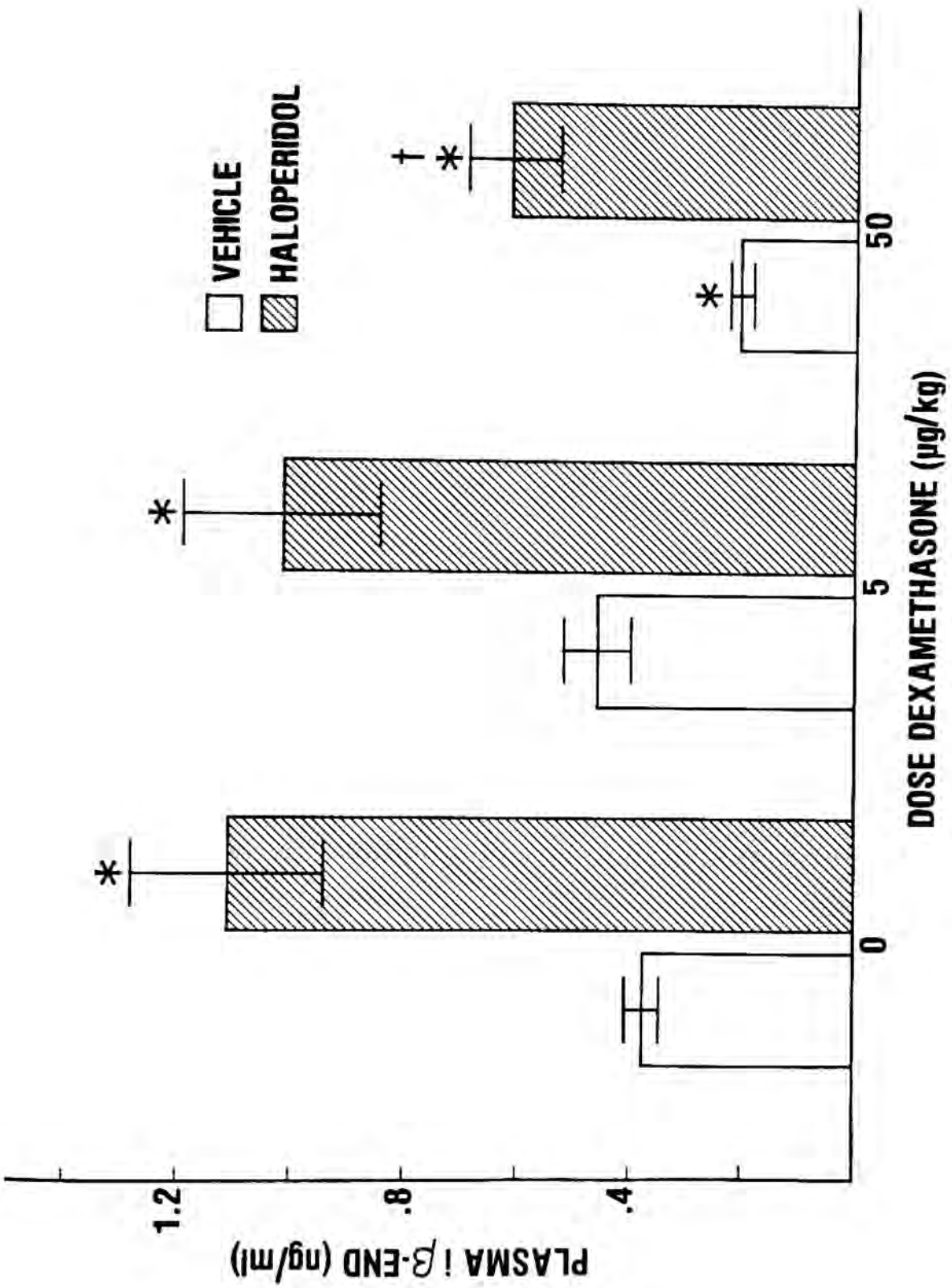

PLASMA i β-END (ng/ml)
1.2
.8
.4
VEHICLE
HALOPERIDOL
0
5
50
DOSE DEXAMETHASONE (µg/kg)

Table 14. Effects of haloperidol on spontaneous and epinephrine-evoked release of iβ-endorphin from primary cultures of rat anterior pituitary

Treatment	iβ-endorphin (ng/plate)	N
Control medium	2.49 ± 0.16	15
Haloperidol (10^{-7} M)	2.44 ± 0.18	5
Haloperidol (10^{-6} M)	2.61 ± 0.23	5
Epinephrine	7.16 ± 0.46 [a]	5
Epinephrine + Haloperidol (10^{-7} M)	6.90 ± 0.19 [a]	5
Epinephrine + Haloperidol (10^{-6} M)	3.33 ± 0.33 [b]	5

Culture AL cells were incubated for 2 h with control medium alone or medium containing 3×10^{-7} M epinephrine with and without the indicated doses of haloperidol. Values are the group means ± SE of the number of plates indicated under the column labeled N.

[a] Significantly different ($P<0.05$) from control and haloperidol

[b] Significantly different ($P<0.05$) from control, haloperidol, epinephrine and epinephrine plus 10^{-7} M haloperidol

spontaneous release of iB-endorphin. Nevertheless, the mcM dose of haloperidol blocked secretion evoked by epiniphrine (0.3 mcM) demonstrating the adrenergic inhibitory properties of higher doses of this dopaminergic antagonist (Anden et al, 1970). Clearly, however, haloperidol exhibited no ability to directly stimulate AL corticotroph secretion of iB-endorphin.

3.0.2.3 Dopaminergic Agonist Effects on Antagonist-Induced Release of iB-Endorphin

Elevated AL release of iB-endorphin <u>in vivo</u> in response to mixed D1, D2 antagonists but not to the D2 antagonists could be due to one of the followint: the ability of the mixed antagonists to block D1 as well as D2 receptors, the ability of the mixed antagonists to reach brain D2 receptors which are inaccessible to domperidone or sulpiride, or a combination of the two, namely, blockade of D1 receptors in the brain which are physically and functionally inaccessible to the D2 antagonists. In order to pharmacologically examine these possibilities, particularly that different dopaminergic receptors mediate haloperidol's ability to increase secretion both by the AL and the IL, haloperidol-induced release of iB-endorphin was challenged by pretreatment with either a D1 or a D2 receptor agonist. Since the classic agonists and LY141865 themselves so effectively stimulate pituitary release of iB-endorphin, the longer-acting D2 agonist, bromocriptine,

Figure 22. Effects of bromocriptine on haloperidol-induced elevation of circulating iB-endorphin (iB-END). Rats were pretreated with ip injections of vehicle (VEH) or 5 mg/kg bromocriptine mesylate (BROM) 60 min prior to a second injection of vehicle or haloperidol (HAL, 2.5 mg/kg); samples were collected 60 min after the second injection. Bars and vertical lines represent the group mean +/- SE; N=8.

*Significantly different ($P<0.05$) from VEH-treated controls
+Siginficantly different ($P<0.05$) from HAL treatment without BROM

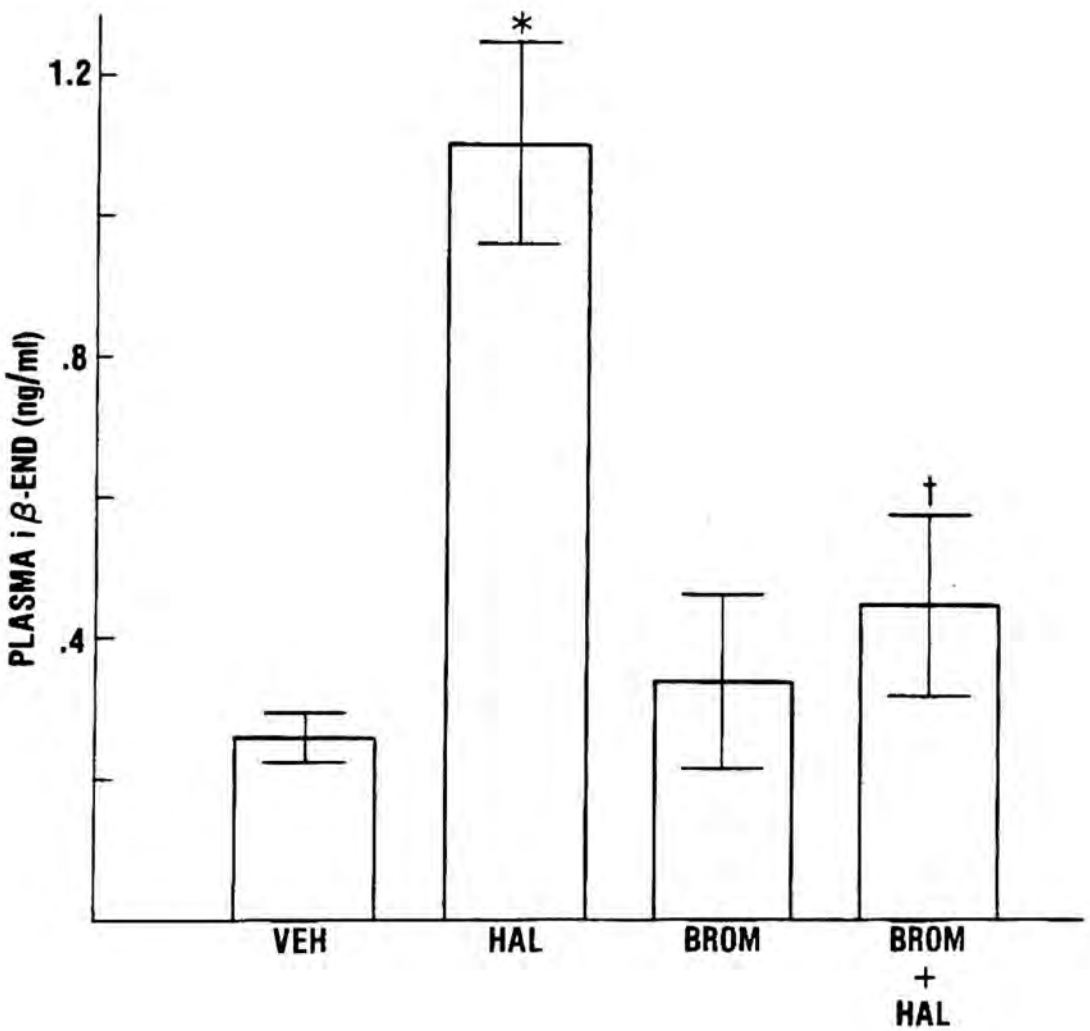

PLASMA i β-END (ng/ml)
1.2
.8
.4
*
†
VEH
HAL
BROM
BROM
+
HAL

Table 15. Effects of bromocriptine pretreatment on the haloperidol-induced rise of plasma iβ-endorphin, α-melanotropin and prolactin

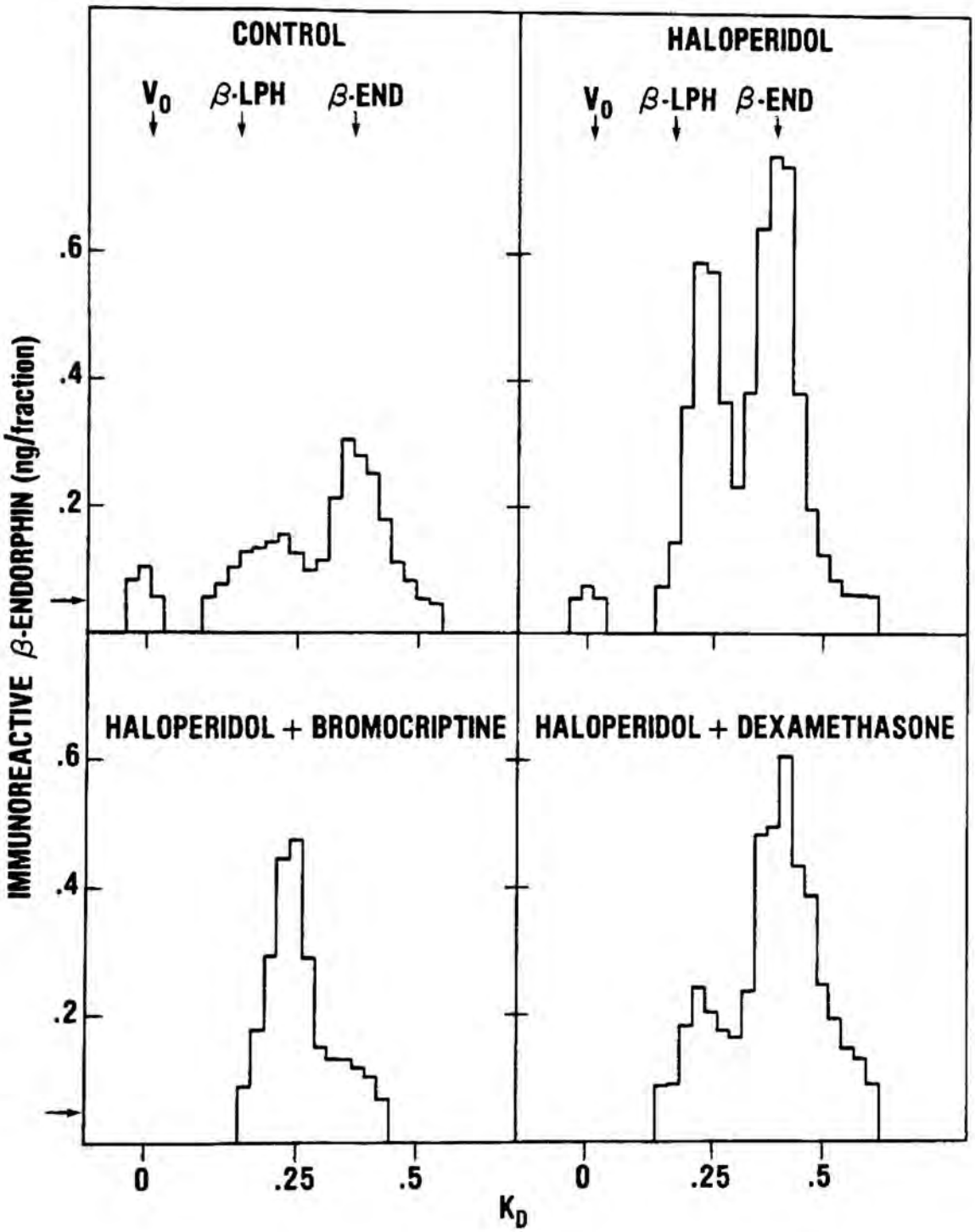
Treatment	Plasma Hormones (ng/ml) iβ-endorphin	iα-melanotropin	prolactin
Vehicle + Vehicle	0.29 ± 0.07	0.13 ± 0.01	5.4 ± 0.49
Vehicle + HAL	0.77 ± 0.09 [a]	0.26 ± 0.03 [a]	57.2 ± 9.49 [a]
0.1 mg/kg BROM + HAL	0.58 ± 0.06 [a]	0.12 ± 0.01	2.3 ± 0.27
0.5 mg/kg BROM + HAL	0.45 ± 0.09	0.13 ± 0.02	2.0 ± 0.07
1.0 mg/kg BROM + HAL	0.34 ± 0.05	0.11 ± 0.01	2.5 ± 0.14

Rats were pretreated with vehicle or bromocriptine (BROM, doses indicated, ip) 90 min before a second vehicle injection or haloperidol treatment (HAL, 2.5 mg/kg, ip); samples were collected 30 min after the second injection. Values are the group mean ± SE; N=7-8

[a] Significantly different ($P<0.05$) from appropriate control values

and the D1 specific agonist, SKF38393, were used to compete with haloperidol's actions in vivo. Figure 22 and Table 15 display the results of experiments in which bromocriptine significantly reduce the levels of iB-endorphin found in plasma of haloperidol treated rats. Haloperidol alone increases iB-endorphin 4-fold in the experiment shown in Figure 22 and this rise was reduced 60% by bromocriptine pretreatment (5 mg/kg, ip, 2 h). Doses of bromocriptine lower than 5 mg/kg also significantly reduced the elevation of circulating iB-endorphin due to haloperidol in a dose-related fashion. As shown in Table 15, 0.5 and 1.0 mg/kg bromocriptine inhibited haloperidol-induced release of iB-endorphin (260% versus controls) by 42% and 56%, respectively. Although the 0.1 mg/kg dose of bromocriptine failed to significantly alter haloperidol-induced release of iB-endorphin, this dose fully prevented haloperidol's ability to increase ialpha-melanotropin (2-fold) and prolactin (10-fold). Therefore, disinhibition of ialpha-melanotropin and prolactin secretion by haloperidol was blocked more readily by bromocriptine than was enhanced release of iB-endorphin. Gel chromatography of plasma from selected treatment groups described in Figure 21 and Table 15 are shown in Figure 23. In the control plasma profile (top left panel) the B-LPH and B-endorphin peaks represented 34% and 58%, respectively, of total iB-endorphin. As shown on the top right panel of Figure 23, the distribution of immunoreactive forms remained

Figure 23. Gel chromatographic profiles of immunoreactive B-endorphin in rat plasma. Profiles depict Sephadex G-50 elution patterns of plasma immunoreactivity from treatment groups described elsewhere in RESULTS. Profiles of immunoreactivity are graphed with respect to the mobility coefficient, Kd. Positions of calibration peaks, i.e., blue dextran (Vo), human beta-lipotropin (hB-LPH) and camel beta-endorphin (cB-END), are shown (arrows) above the control profile. The limit of detection, LD, is marked by arrows on the margin of the y-axes.


CONTROL
HALOPERIDOL
HALOPERIDOL + BROMOCRIPTINE
HALOPERIDOL + DEXAMETHASONE
V_0
β-LPH
β-END
IMMUNOREACTIVE β-ENDORPHIN (ng/fraction)
.6
.4
.2
0
.25
.5
K_D

approximately the same after haloperidol (37% B-LPH-sized, 58% B-endorphin-sized) although total plasma levels increased 250%. In contrast, attenuation of haloperidol's effects by bromocriptine was associated with a loss of immunoreactivity resembling B-endorphin but persistence of the B-LPH form which amounted to 78% of total plasma iB-endorphin (bottom left, Figure 23). This distribution was essentially inverted in plasma of haloperidol-treated rats that had been pretreated with dexamethasone. Dexamethasone's attentuation of haloperidol-induced release was associated with a loss of iB-endorphin that resembles B-LPH yet the B-endorphin-sized peptides remained, accounting for 69% of total circulating iB-endorphin. These results further support the interpretation that haloperidol evokes release of iB-endorphin from the AL (B-LPH-sized and sensitive to glucocorticoid inhibition). The reason for bromocriptine's inability to clearly reduce the contribution made by B-LPH to the rise in total iB-endorphin following haloperidol is not understood. Over a longer period of time, both bromocriptine and haloperidol have equivalent access to the brain and both have long-lasting actions on prolactin secretion. The releasing effects of haloperidol on B-LPH-sized immunoreactivity may be due to blockade of D1 receptors, a dopaminergic subtype at which bromocriptine cannot compete. To investigate this possibility, haloperidol's influence on pituitary iB-endorphin release was challenged with the D1

Figure 24. Effects of SKF 38393 on release of immunoreactive B-endorphin (iB-END) evoked by haloperidol and sulpiride. Rats received an ip injection of vehicle or SKF 38393A (SKF, 2.5 mg/kg) 30 min prior to a second injection of vehicle (VEH), haloperidol (HAL, 2.5 mg/kg) or sulpiride (SULP, 2.5 mg/kg); animals were decapitated 30 min thereafter. Bars and vertical lines represent the group means +/- SE; N=6-8.

*Significantly different ($P<0.05$ from vehicle-treated controls
+Significantly different ($P<0.05$) from HAL alone

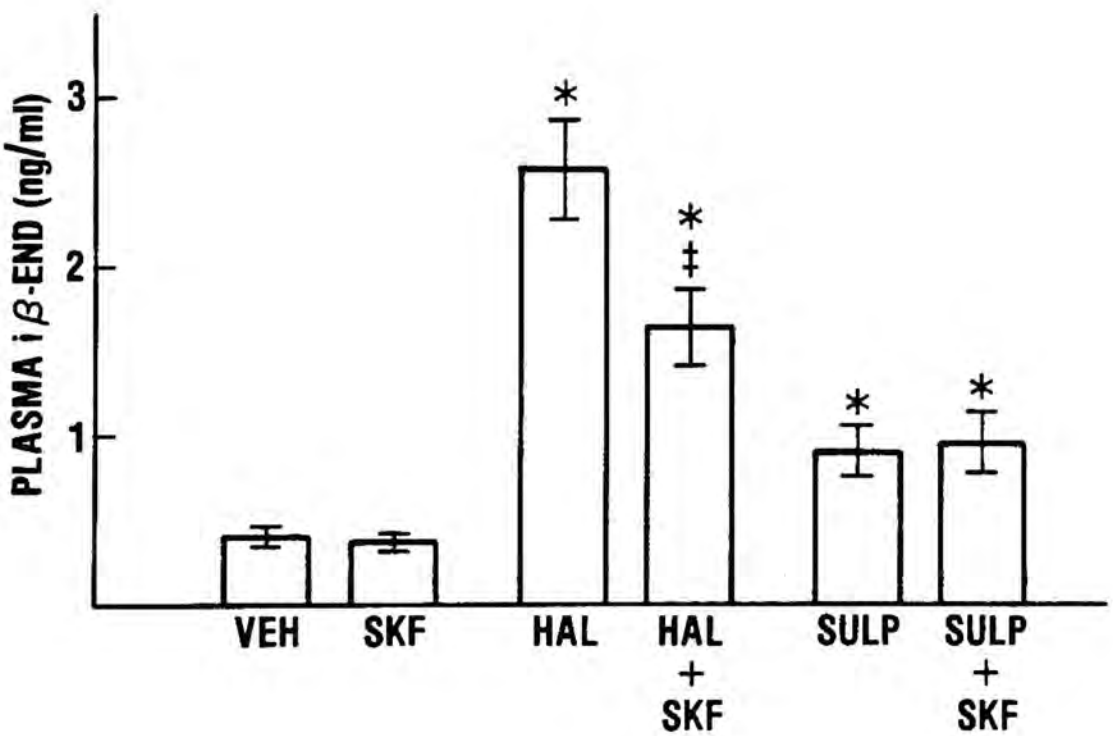
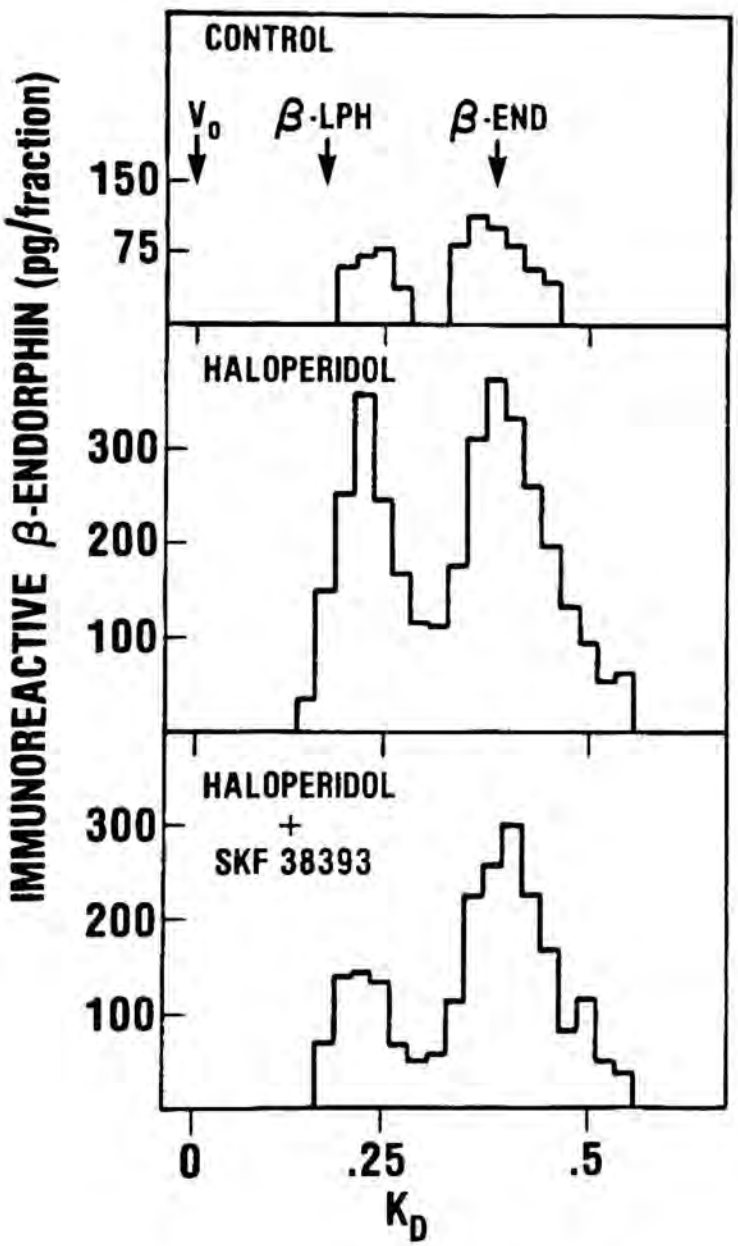

PLASMA i β-END (ng/ml)
3
2
1
*
*‡
*
*
VEH
SKF
HAL
HAL + SKF
SULP
SULP + SKF

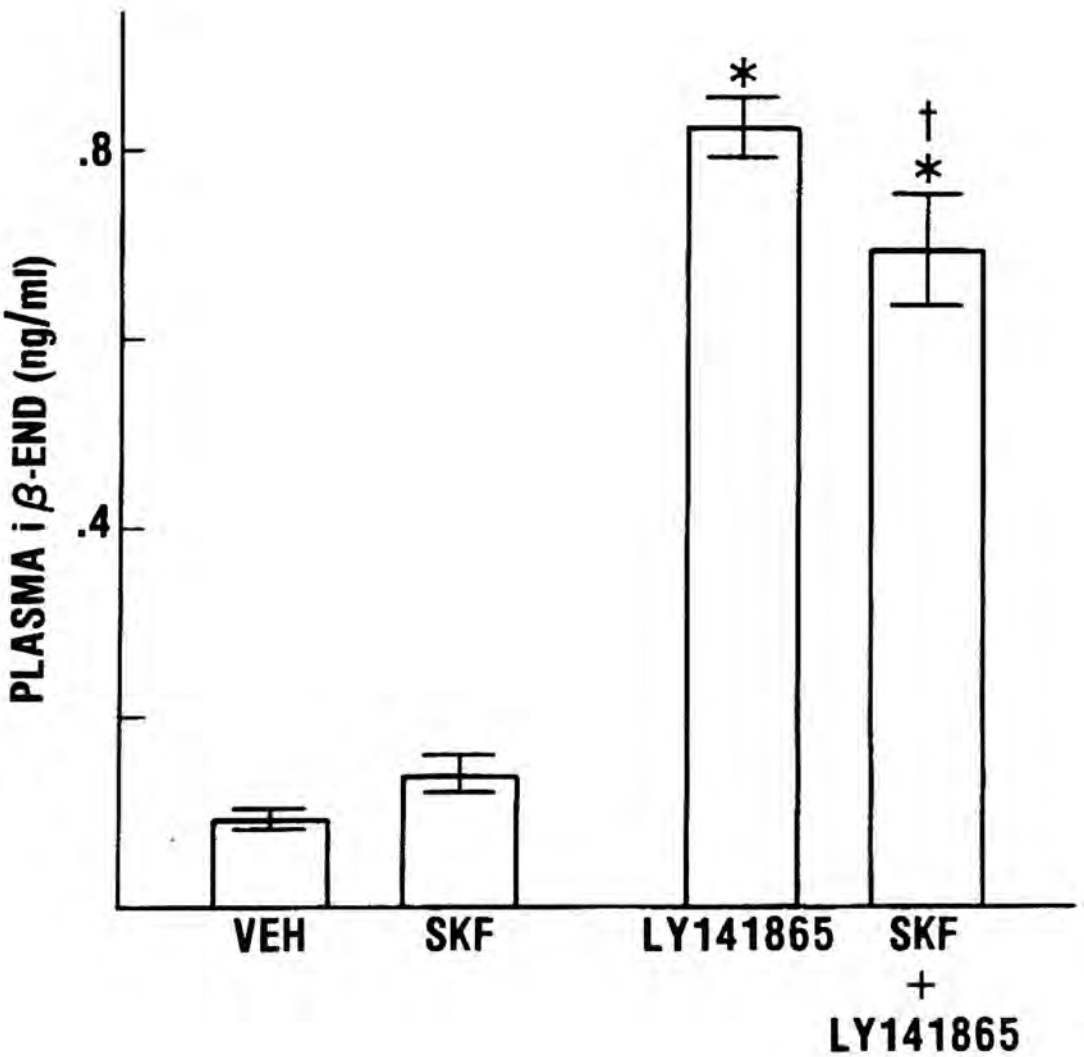
Figure 25. Comparison of gel chromatographic profiles of plasma from control, haloperidol and haloperidol plus SKF 38393 treated rats. Animals were treated as described in Figure 24. Pools of treatment group plasma were filtered on a column of Sephadex G-50 and the elution profiles are graphed with respect to the mobility coefficient, Kd. Positions of calibration peaks, i.e., blue dextran (Vo), human B-lipotropin (B-LPH) and camel B-endorphin (B-END), are indicated by arrows above the control profile (top).


CONTROL
V_0
β-LPH
β-END
150
75
HALOPERIDOL
300
200
100
HALOPERIDOL
+
SKF 38393
300
200
100
IMMUNOREACTIVE β-ENDORPHIN (pg/fraction)
0
.25
.5
K_D

agonist, SKF 38393. The results indicate that SKF 38393 was able, in part, to attenuate the increase in total iB-endorphin due to haloperidol and the effects appeared specific to the B-LPH-sized form. A 2.5 mg/kg dose of SKF 38393 was administered 30 min prior to treatment of rats with vehicle, haloperidol or sulpiride. As shown in Figure 24, both haloperidol and sulpiride significantly increased plasma iB-endorphin, but, as seen earlier, not to the same degree. Circulating iB-endorphin rose over 6-fold after haloperidol (2.5 mg/kg, 30 min) and approximately 2-fold after sulpiride treatment (2.5 mg/kg, 30 min). SKF 38393 significantly reduced the haloperidol-induced increase in plasma iB-endorphin by 35% but had no effect on the ability of sulpiride to elevate total plasma levels (Figure 24). These findings indicate that part of haloperidol's ability to increase plasma iB-endorphin may be mediated through blockade of an inhibitory D1 receptor, whereas, sulpiride-induced release of iB-endorphin is mediated exclusively by a D2 receptor. When gel chromatographic profiles of plasma from animals treated with haloperidol were compared with those from rats which received the SKF 38393 pretreatment in addition to haloperidol, the form of iB-endorphin resembling B-LPH was found to be preferentially diminished by SKF 38393. Whereas the B-LPH peak represented 40% of total iB-endorphin in plasma of haloperidol-treated rats, this same molocular form of iB-endorphin constituted only 28% of total immunoreactivity

Figure 26. Effects of SKF 38393 on release of immunoreactive B-endorphin (iB-END) induced by LY141865. Rats received ip injections of vehicle (VEH) or SKF 38393 (SKF 3 mg/kg) 45 min prior to a second ip injection of vehicle or LY141865 (1 mg/kg). Samples were collected 15 min after the second injection. Bars and vertical lines represent the mean +/- SE; N=7.

*Significantly different ($P<0.05$) from VEH-treated controls
+Significantly different ($P<0.05$) from LY141865 alone


PLASMA i β-END (ng/ml)
.8
.4
*
†
*
VEH
SKF
LY141865
SKF
+
LY141865

rats pretreated with SKF 38393 (Figure 25). Thus, SKF 38393 appears to have prevented haloperidol-induced release of AL iB-endorphin.

If, as these data suggest, an inhibitory dopaminergic mechanism regulates AL release of iB-endorphin as well as of the IL, then results generated in sections 1.1 to 1.3 suggesting that a D2 receptor mechanism stimulates AL release in vivo must be reconciled with the current interpretation. The following experiment was designed to examine the stimulatory effects of D2 receptor activation together with the inhibitory D1 effects surmised by the results of this section. As shown in Figure 26, LY141865 (1 mg/kg, 15 min) evoked a 9-fold rise in total plasma levels of iB-endorphin, whereas, the D1 agonist, SKF 38393, had no effect on basal levels. Nonetheless, release of iB-endorphin elicited by LY141865 was slightly (15%) but significantly ($P<0.05$) attenuated by the SKF 38393 pretreatment (3 mg/kg, 30 min). These findings suggest that an inhibitory D1 receptor mechanism coexists with a stimulatory D2 receptor mechanism for controlling AL release of iB-endorphin.

3.0.3 Dopaminergic Effects on Physiologically Stimulated Release of iB-endorphin

The remaining experiments were designed to examine possible involvement of dopaminergic mechanisms in the

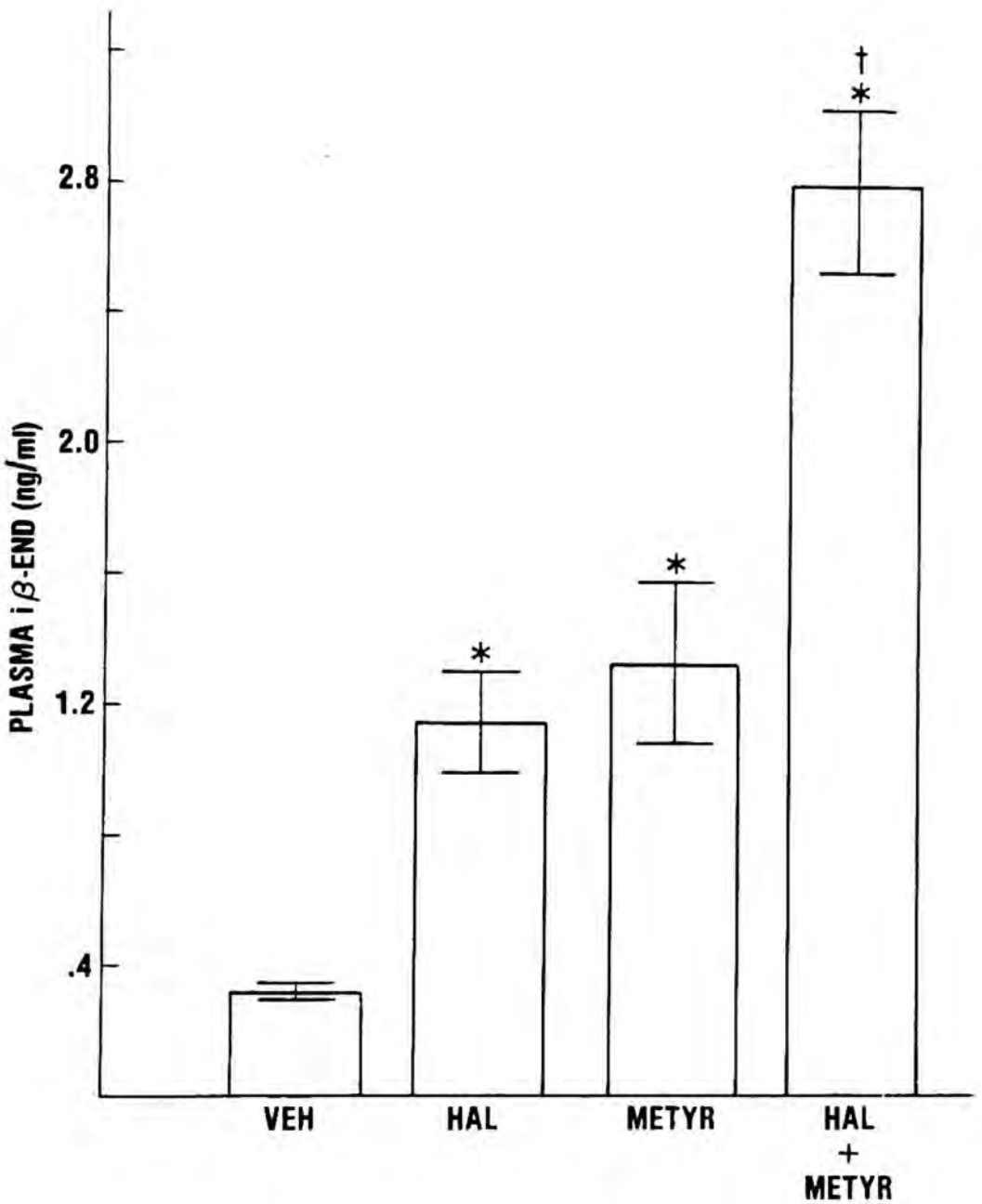
physiologic release of pituitary iB-endorphin. The two methods used to elicit physiologic release of iB-endorphin were: metyrapone treatment, which inhibits the adrenal enzyme, 11-B-hydroxylase (Chart et al, 1958), and interrupts negative feedback control of AL secretion of ACTH and iB-endorphin, and physical immobilization, which is a potent stress for rats and evokes both AL and IL release of iB-endorphin.

3.0.3.1 Dopaminergic Effects on Metyrapone-Induced Release of iB-endorphin

In order to determine if dopamine receptors participate in maintaining balance in the brain-pituitary-adrenal axis, rats were pretreated with dopaminergic drugs prior to inhibition of glucocorticoid synthesis with metyrapone. Animals received a 30 min pretreatment with haloperidol (2.5 mg/kg) then metyrapone (100 mg/kg, 30 min). As the results in Figure 27 show, treatment of rats with the combination of haloperidol and metyrapone evoked an elevation in plasma levels of total iB-endorphin (870% as compared to control) which approximated the sum of their independent releasing effects (360% and 420% due to haloperidol and metyrapone, respectively). Despite this 9-fold elevation in circulating iB-endorphin, neither AL nor IL content of iB-endorphin was altered relative to control levels (1.01

Figure 27. Effects of haloperidol and metyrapone alone and in combination on plasma immunoreactive B-endorphin (iB-END). Vehicle (VEH) or 2.5 mg/kg haloperidol (HAL) were injected ip 30 min before a second vehicle injection or metyrapone treatment (METYR, 100 mg/kg); samples were collected 30 min after the second injection. Bars and vertical lines represent the group means +/- SE; N=8.

*Significantly different ($P<0.05$) from VEH-treated controls
+Significantly different ($P<0.05$) from all other treatments


PLASMA i β-END (ng/ml)
2.8
2.0
1.2
.4
*
*
*
†
VEH
HAL
METYR
HAL + METYR

+/- 0.11 mcg/mg protein in AL and 17.8 +/- 2.4 mcg/mg protein in NIL). Although complimentary in their influence on blood-borne iB-endorphin, haloperidol and metyrapone didn't similarly effect plasma prolactin. Haloperidol induced a 12-fold increase in plasma levels of prolactin as compared to controls (9.2 +/- 2.0 ng/ml) but metyrapone alone had no effect on prolactin and did nothing to alter haloperidol-induced prolactin release. The results of this study suggest that dopamine receptor-mediated inhibition of pituitary iB-endorphin release may be as independent of glucocorticoid feedback effects on pituitary iB-endorphin as are the releasing effects of dopaminergic receptor blockade on prolactin secretion. The study which follows further separates dopaminergic influence from the regulation of the pituitary-adrenal axis.

Rats were pretreated with bromocriptine (1 or 5 mg/kg, 3 h) prior to administration of metyrapone. As shown in Table 16, bromocriptine had no effect on basal levels of total iB-endorphin and failed to influence a 5-fold increase in circulating iB-endorphin due to metyrapone. Together, these results strongly imply that dopaminergic actions on pituitary iB-endorphin secretion are independent of the mechanisms which regulate blood levels of glucocorticoids. As shown previously, however, the converse is not true. AL release of iB-endorphin following dopamine receptor stimulation (section 1.2) or inhibition (section 2.2) was readily suppressed by

Table 16. Effects of bromocriptine and metyrapone alone and in combination on plasma levels and pituitary content of immunoreactive β-endorphin

Treatment	Plasma iβ-endorphin (ng/ml)	Pituitary iβ-endorphin (μg/mg protein) AL	NIL
VEH + VEH	0.25 ± 0.03	1.6 ± 0.1	32.9 ± 3.7
1 mg/kg BROMO + VEH	0.36 ± 0.07	1.7 ± 0.2	33.2 ± 4.2
5 mg/kg BROMO + VEH	0.26 ± 0.06	1.7 ± 0.2	28.2 ± 3.2
VEH + METYR	1.31 ± 0.32 [a]	1.5 ± 0.2	25.4 ± 3.4
1 mg/kg BROMO + METYR	1.62 ± 0.29 [a]	1.4 ± 0.2	29.8 ± 4.2
5 mg/kg BROMO + METYR	1.32 ± 0.19 [a]	1.7 ± 0.3	36.1 ± 5.0

Vehicle (VEH) or bromocriptine mesylate (BROMO) were administered 90 min before a second vehicle or 100 mg/kg metyrapone injection (METYR); samples were taken 30 min after the second injection. Values are the group means ± SE; N=7-8.

[a] Significantly different ($P<0.05$) from VEH controls or corresponding BROMO treatment group without METYR

exogenous glucocorticoid treatment. The additive effects of haloperidol and metyrapone together with the lack of interaction of bromocriptine and metyrapone on plasma levels of total iB-endorphin suggest that dopamine receptor mechanisms influence pituitary release of iB-endorphin independently from those mechanisms which ultimately sustain physiological levels of circulating glucocorticoids in rats.

3.0.3.2 Dopaminergic Drug Effects on Stress-Induced Release of iB-endorphin

In order to determine how dopaminergic mechanisms might be involved with the pituitary iB-endorphin response to stress, rats were pretreated with either bromocriptine (D2 agonist), pergolide (a mixed D1,D2 agonist), or with haloperidol prior to the stress of physical immobilization. As seen in Table 17, bromocriptine failed to significantly influence basal secretion of iB-endorphin yet attenuated immobilization-induced release (6-fold versus controls) by 40%). Bromocriptine's ability to decrease total plasma iB-endorphin in stressed rats was associated with a loss of iB-endorphin resembling B-endorphin in molecular size (not shown). Circulating prolactin was also elevated in stressed rats although not to the same degree as total iB-endorphin; bromocriptine significantly reduced basal levels of prolactin and prevented immobilization-induced release of prolactin

Table 17. Effects of bromocriptine on basal and stimulated plasma levels of immunoreactive β-endorphin and prolactin in the rat

Treatment	Plasma Hormones (ng/ml) iβ-endorphin	prolactin
Vehicle	0.44 ± 0.08	25 ± 9
Bromocriptine	0.30 ± 0.05	<2 [a]
Vehicle + Immobilization	2.82 ± 0.31 [b]	63 ± 9 [b]
Bromocriptine + Immobilization	1.70 ± 0.37 [bc]	<2 [ac]

Rats received ip injections of vehicle or bromocriptine (3 mg/kg) 3 h prior to sacrifice and half of each pretreatment group was subjected to 30 min of physical immobilization prior to sacrifice. Values are the group mean ± SE; N=6-7

[a] Significantly different ($P<0.05$) from vehicle-treated controls

[b] Significantly different ($P<0.05$) from vehicle and bromocriptine groups

[c] Significantly different ($P<0.05$) from vehicle + immobilization group

Figure 28. Effects of pergolide on stress-induced release of immunoreactive B-endorphin (iB-END) in the rat. Rats received ip injections of vehicle or pergolide mesylate (2.5 mg/kg) 3 h prior to sacrifice and half of each pre-treatment group was subjected to 30 min of physical immobilization prior to sacrifice. Values are the group mean +/- SE; N=6-7. The vehicle-pretreated stress group alone was significantly different ($P<0.05$) from other group values.

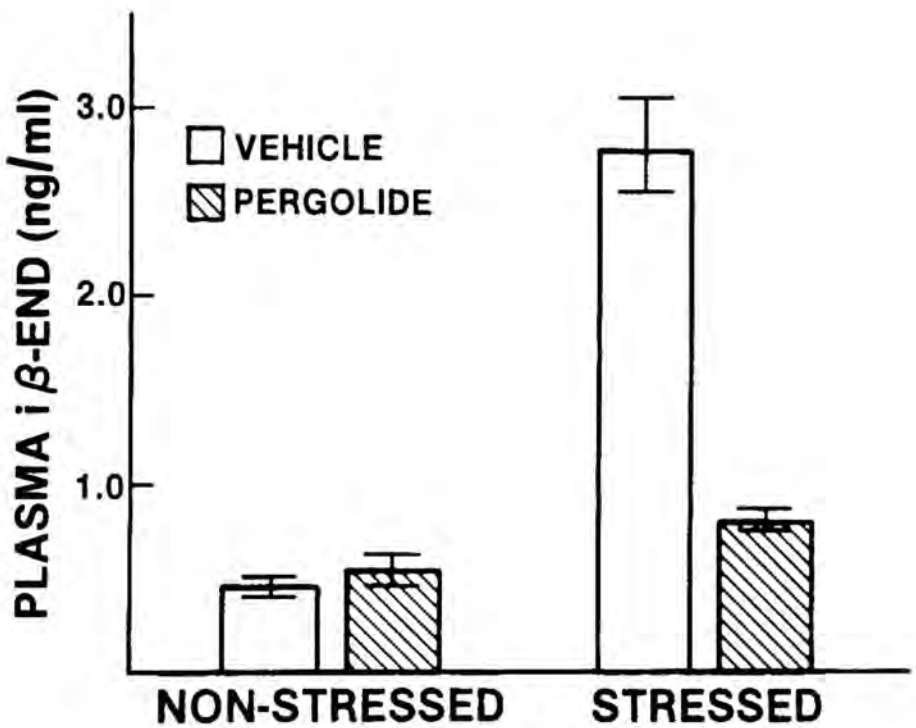

PLASMA i β-END (ng/ml)
3.0
2.0
1.0
VEHICLE
PERGOLIDE
NON-STRESSED
STRESSED

Table 18. Effects of haloperidol and immobilization on circulating levels of immunoreactive β-endorphin and prolactin in rats

Treatment	Plasma Hormones (ng/ml) iβ-endorphin	prolactin
Controls	0.23 ± 0.02	3.2 ± 0.3
Haloperidol	0.39 ± 0.03	16.5 ± 2.7 [a]
Immobilization	1.82 ± 0.25 [a]	10.9 ± 1.7 [a]
Immobilization + Haloperidol	2.44 ± 0.28 [a]	42.7 ± 5.1 [ab]

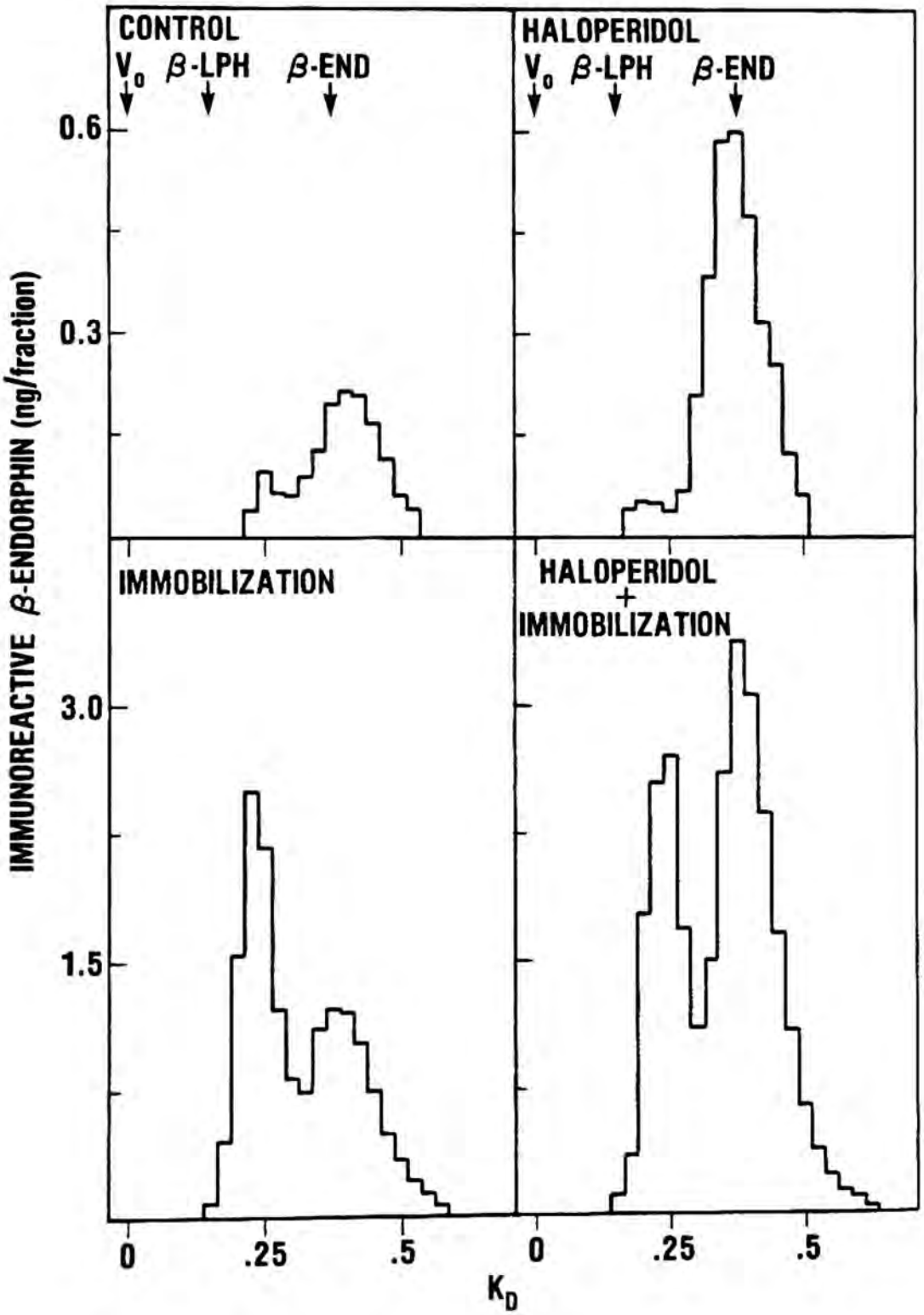
Rats received an ip injection of vehicle or haloperidol (1 mg/kg) twice daily for three days. On the morning of the fourth day, 3.5 h after the seventh injection, half of the animals were subjected to 30 min of physical immobilization and all animals were decapitated 4 h after the last injection. Values are the group means ± SE; N=8.

[a] Significantly different ($P<0.05$) from controls

[b] Significantly different ($P<0.05$) from immobilization without haloperidol

(Table 17). Pergolide, a dopaminergic agonist which, unlike bromocriptine, is an agonist of D1 as well as D2 receptors (Goldstein et al, 1980; Boissier et al, 1983), also blocked stress-induced release of iB-endorphin and prolactin, reducing by 72% the levels of total iB-endorphin provoked by immobilization (Figure 28). As revealed in chromatography of the preceding experiment, the predominant loss of iB-endorphin was the molecular form resembling B-endorphin in size (not shown) although some decrement in B-LPH was also apparent. These results suggest that dopamine neurons may participate in stress-induced release of pituitary iB-endorphin through a permissive role on the IL, i.e., withdrawal of tonic inhibitory tone. This interpretation implied that the pituitary iB-endorphin response to stress could be augmented by additional withdrawal of dopaminergic inhibitory tone. To this end, rats were repeatedly treated with the mixed dopaminergic antagonist, haloperidol (1 mg/kg every 12 h), and subjected to physical immobilization 3.5 h after the seventh haloperidol treatment. As shown in table 18, haloperidol did not effect basal levels of plasma iB-endorphin but significantly augmented stress-induced release (10-fold increment versus an 8-fold increment in stress without haloperidol). The associated changes in molecular forms of iB-endorphin are shown in Figure 29. Although resting levels of total iB-endorphin were unchanged 4 h after the final haloperidol treatment, B-endorphin-sized material

Figure 29. Gel filtration chromatography of plasma from rats exposed to haloperidol and immobilization. Rats were treated as described in Table 18 and pools of plasma from each treatment group were filtered on a Sephadex G-50 column. Immunoreactive B-endorphin in the elution fractions was graphed (corrected to 100% recovery) with respect to the mobility coefficient, Kd. Positions of calibration peaks, i.e., blue dextran (Vo), human beta-lipotropin (B-LPH) and camel B-endorphin (B-END), are indicated by arrows above the control profile (top left).


CONTROL
V_0 β-LPH β-END
HALOPERIDOL
V_0 β-LPH β-END
IMMOBILIZATION
HALOPERIDOL
+
IMMOBILIZATION
IMMUNOREACTIVE β-ENDORPHIN (ng/fraction)
0.6
0.3
3.0
1.5
0
.25
.5
0
.25
.5
K_D

represented 95% rather than 85% of circulating immunoreactivity after haloperidol treatment. Both the B-endorphin- and the B-LPH-like forms of iB-endorphin increased in response to immobilization alone but the material resembling B-LPH was increased greater than 30-fold, whereas, B-endorphin-sized immunoreactivity increased, at most, 4-fold. In stressed rats pretreated with haloperidol, B-endorphin-sized immunoreactivity was increased 200% over the amount of this molecular form in plasma of rats subjected to immobilization alone. These data reveal that stress by physical immobilization evokes a profound AL release of iB-endorphin as reflected in the elevation of B-LPH. Nevertheless, the B-endorphin-sized form of iB-endorphin, which is associated with IL secretion, was also increased by immobilization and appeared to be the only material which repeated haloperidol treatments significantly increased. Together, these findings suggest that the AL displays the greatest capacity to respond to stress but that, once relieved of tonic inhibitory tone, the IL exhibits an enhanced capacity respond to provocative stimuli like stress.

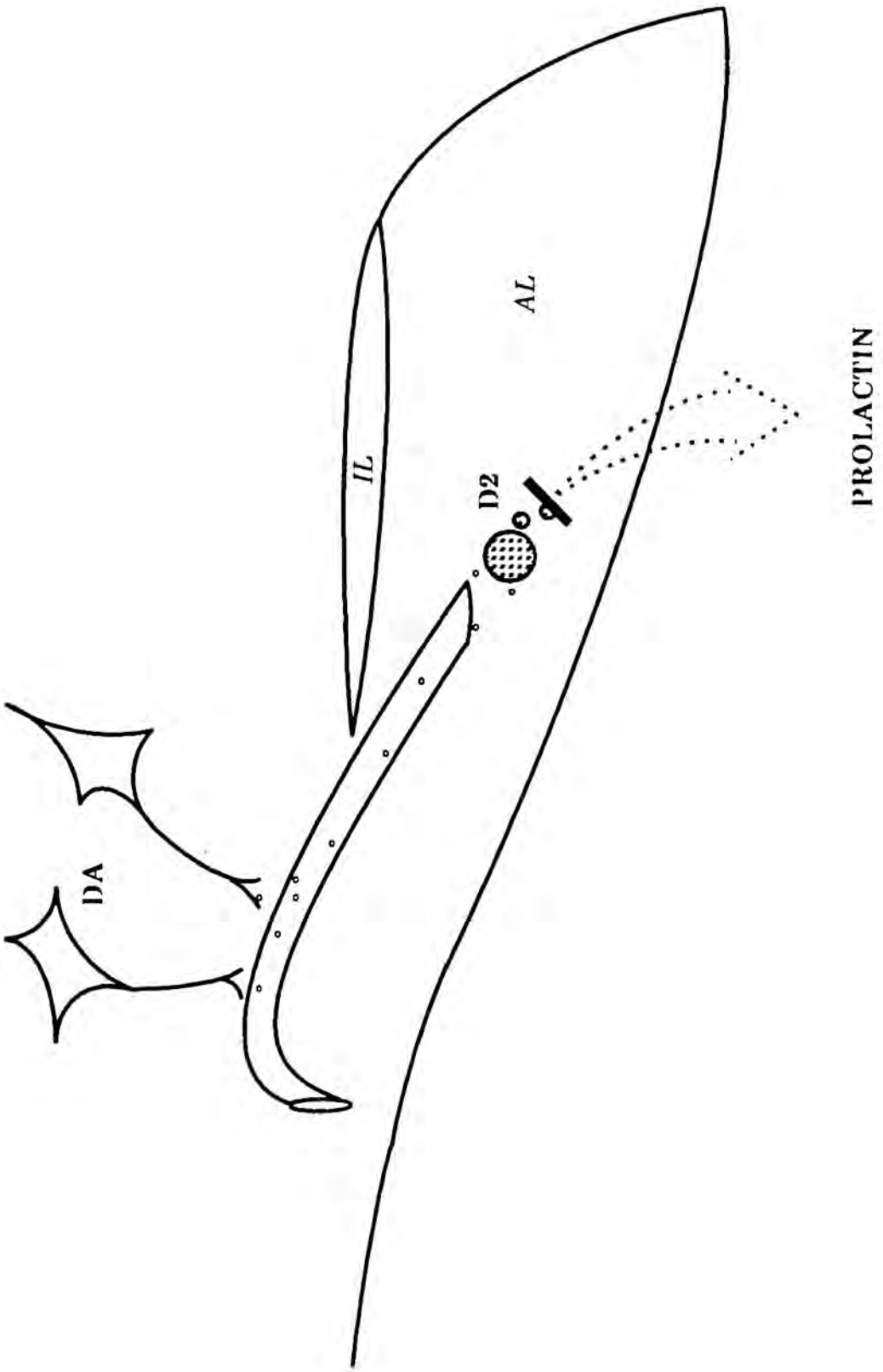
Chapter 4

DISCUSSION

The purpose of the present investigation was to examine dopaminergic mechanisms involved in controlling the secretion of pituitary immunoreactive B-endorphin (iB-endorphin). The experimental approach involved, to a large extent, the use of pharmacologic agents characterized for their receptor-specific actions on dopamine target tissues. Most of the drugs employed here had not previously been used to examine the control of pituitary B-endorphin. The present investigation was carried out using the laboratory rat, an acknowledged model species for studying neuroendocrine regulation in mammals.

Results of the present investigation indicate that dopamine neurons of the central nervous system control the release of iB-endorphin from both the anterior lobe (AL) and intermediate lobe (IL) of the pituitary gland. Regulation of AL iB-endorphin secretion by dopamine appears to involve reciprocal stimulatory and inhibitory mechanims that are most likely mediated through specific dopamine receptor subtypes located within the brain which, in turn, control the release of corticotropin releasing factor (CRF)

Figure 30. Schematic illustration modeling the regulation of prolactin secretion from the rat anterior pituitary gland. Hypothalamic dopamine (DA) neurons are shown releasing the catecholamine neurohormone into the hypophyseal-portal circulation where DA directly interacts with the D2 receptors on mammotrophs to tonically inhibit prolactin secretion from the anterior lobe (AL). Although D2 receptors are also characterized on the intermediate lobe, only the AL produces prolactin.


DA
IL
AL
D2
PROLACTIN

from the hypothalamus. Conversely, dopamine receptors located on secretory cells of the IL mediate direct inhibition of iB-endorphin release from this tissue.

As indicated in Figure 30, all dopaminergic treatments which activated the D2 receptor consistently reduced circulating levels of prolactin; antagonism of the D2 receptor reliably increased plasma levels of prolactin consistent with tonic inhibitory control of mammotrophs by hypothalamic dopamine neurons. Together, these observations, together with casual observations of behavior, greatly aided the interpretation of dopaminergic treatments, particularly with novel drug types.

4.0.1 Development of a Working Hypothesis:

Based on earlier reports that dopamine inhibits alpha-melanotropin (MSH) secretion from the IL <u>in vivo</u> (Tilders and Smelik, 1977; Tilders and Smelik, 1978), it was anticipated that dopaminergic agonist drugs would similarly depress plasma levels of iB-endorphin since B-endorphin peptides and MSH are co-secreted by IL melanotrophs. It was observed, however, that administration of the classic dopaminergic agonists, apomorphine and piribedil, resulted in rapid two- to five-fold increases in plasma levels of total iB-endorphin (Figures 5 & 6, and Table 4). Furthermore, this unexpected release of pituitary iB-endorphin due to dopamine receptor

activation was associated with inverse changes in the two major molecular constituents of blood-borne iB-endorphin, immunoreactivity resembling B-endorphin and B-LPH in size. Under basal conditions, circulating iB-endorphin normally consists of approximately 70% B-endorphin-sized material with the remainder resembling B-LPH, B-endorphin's immediate precursor. Following apomorphine or piribedil, the rise in total plasma iB-endorphin was exclusively due to B-LPH, whereas, the form resembling B-endorphin 1-31 in size was reduced by approximately 25%. These changes in the underlying molecular forms of total circulating iB-endorphin indicate that AL release, characterized by B-LPH, is stimulated by dopaminergic agonists, whereas, the decline in plasma immunoreactivity resembling B-endorphin 1-31 in size is consistent with inhibition of IL release since the IL secretes only forms of iB-endorphin resembling B-endorphin 1-31. Based upon these initial observations, the hypothesis was developed that two dopaminergic control mechanisms exist for independently regulating AL and IL secretion of iB-endorphin. According to this working hypothesis, AL release of iB-endorphin is subject to dopaminergic stimulation, whereas, IL release is inhibited by dopamine receptor activation.

4.0.2 Testing and Modification of Working Hypothesis

The present finding that dopamine agonists diminish the form of plasma iB-endorphin secreted by the IL is

consistent with earlier proposals that hypothamic dopaminergic neurons exert an inhibitory tone over melanotroph secretion (Tilders and Smelik, 1977; 1978; Penny and Thody, 1979). By contrast, the findings reported here which indicate dopaminergic stimulation of AL iB-endorphin release were largely unanticipated. The prevailing opinion in the literature on dopamine's role in ACTH regulation suggests that dopamine might, if anything, exert an inhibitory influence on AL iB-endorphin release (Fuxe et al, 1970; Van Loon, 1973; Ganong et al, 1976). Present findings to the contrary thus led to additional experiments designed to confirm that the AL is the source for elevated circulating iB-endorphin following administration of apomorphine or piribedil. Results of these studies support the hypothesis that a dopaminergic mechanism indeed stimulates AL release of iB-endorphin. Interestingly, further experiments also revealed that a second, independent dopaminergic mechanism exists for tonic inhibitory control of AL iB-endorphin release. That dual dopaminergic mechanisms oppositely control AL iB-endorphin secretion may explain the uncertainty that exists in the literature about the role of dopamine neurons in controlling ACTH secretion.

Pretreating rats with a low dose of haloperidol, a broadly acting dopamine receptor antagonist, attenuated pituitary release of iB-endorphin due to apomorphine, shifting the dose-response curve of apomorphine's effects

to the right (Figure 13). Stimulation of iB-endorphin release by apomorphine and inhibition of this response by haloperidol is consistent with a dopaminergic receptor mechanism mediating stimulatory control of pituitary iB-endorphin. Evidence that this stimulatory dopaminergic regulation is exerted specifically over AL corticotroph release is indicated by the ability of the synthetic glucocorticoid, dexamthasone, to completely block apomorphine-induced release of iB-endorphin (Table 6). Together, these findings further demonstrate that, consistent with the working hypothesis, a dopaminergic mechanism exists for stimulating AL release of iB-endorphin. In accord with this hypothesis, Fuller and Snoddy (1981a) observed that a number of agents that enhance dopaminergic neurotransmission increase serum corticosterone in rats. Since adrenal secretion of corticosterone is stimulated by ACTH (released together with iB-endorphin from the AL), their results also support dopaminergic stimulation of corticotrophs. Likewise, pharmacotherapy of Parkinsonism with L-DOPA and a peripheral decarboxylase inhibitor has been found to elevate adrenal glucocorticoid secretion in man (Bartholini and Pletscher, 1969).

It is well-accepted that hypothalamic dopamine neurons tonically inhibit alpha-MSH secretion from melanotrophs of the IL (Tilders and Smelik, 1977; 1978; Tilders et al, 1979). A large part of the evidence for this

inhibitory control of the IL has been the repeated demonstration that dopaminergic antagonists like haloperidol increase plasma levels of alpha-MSH in experimental animals (Usategui et al, 1976; Tilders and Smelik, 1978; Penny and Thody, 1979). In light of these earlier reports, the present observation that low dose haloperidol fails to increase basal release of iB-endorphin (Figure 13 & 14) was puzzling. Presuming that the haloperidol treatment paradigm was inappropriate for demonstrating disinhibition of IL iB-endorphin release, higher doses and shorter sampling times were examined. Consistent with previous work showing that in vivo dopaminergic blockade results in enhanced alpha-MSH secretion, iB-endorphin levels in plasma are significantly elevated when higher doses of haloperidol were administered and shorter sampling times were used (Figure 18 and Tables 12 & 13). Upon chromatographic analysis of plasma from haloperidol-treated animals, however, it became clear for the first time that haloperidol increases immunoreactivity resembling both B-endorphin and B-lipotropin in molecular size (Figure 20). Therefore, in addition to elevated IL secretion of iB-endorphin as indicated by the B-endorphin-sized material, haloperidol treatment also appears to evoke AL release of iB-endorphin as evidenced by increased circulating B-lipotropin. Dexamethasone's attenuation of haloperidol's effects through selective reduction of material released resembling B-lipotropin

(Figure 23) further supports the view that dopamine neurons tonically inhibit release of iB-endorphin from the AL as well as from the IL *in vivo*.

To summarize, when administered under appropriate conditions both a dopaminergic receptor blocker (haloperidol) and dopamine receptor activating drugs (apomorphine and piribedil) stimulate release of iB-endorphin from the AL. This suggests that either the AL iB-endorphin responses are not specific to dopaminergic agents or that the AL is subject to dual control by a dopaminergic stimulatory and a dopaminergic inhibitory mechanism.

Reports that higher doses of haloperidol interact with both adrenergic and serotonergic receptors (Anden et al, 1970; Peroutka and Snyder, 1980) raised the possibility that the effects of haloperidol on AL iB-endorphin release may have been due to non-dopaminergic actions of the compound. This, however, does not appear to be the case. Evidence against adrenergic mechanisms is that pimozide, a dopaminergic blocker with greater selectivity than haloperidol for dopamine receptors (Anden et al, 1970), also increases circulating levels of total iB-endorphin (Figure 19), and, like haloperidol, elevates both B-endorphin- and B-lipotropin-sized immunoreactivity in blood (not shown). Furthermore, the iB-endorphin releasing effects of haloperidol were found to be additive to those

of the adrenergic antagonist, prazosin (Mueller, Maiewski and Farah, unpublished observation) indicating independent dopaminergic and adrenergic regulation of pituitary iB-endorphin. Together, these data do not support an adrenergic action for haloperidol on AL iB-endorphin release.

Similarly, actions of haloperidol on serotonin receptors do not appear to be involved in pituitary iB-endorphin release evoked by haloperidol. The stimulatory effect of haloperidol on AL iB-endorphin release was unaffected by cinanserin, a serotonin receptor blocker which completely prevents AL iB-endorphin release in response to the serotonin receptor agonist, quipazine (Sapun-Malcolm, Farah & Mueller, in press). Thus, the ability of dopaminergic blockers to increase AL iB-endorphin release do not appear to be mediated indirectly through either serotonergic or adrenergic mechanisms.

The ability of antagonists as well as agonists of the dopamine receptor to evoke release of AL iB-endorphin supports the view that an inhibitory dopaminergic mechanism exists in parallel with dopaminergic stimulatory control over corticotrophs. Accordingly, the working hypothesis was modified to also include a dopaminergic mechanism for tonic inhibition of AL iB-endorphin release.

Dopaminergic agonists and antagonists alike

stimulate AL release of iB-endorphin despite the presence of endogenous mechanisms opposing the actions of each. This raised the possibility that such seemingly contradictory actions might result from activation and blockade of separate dopamine receptor subtypes each capable of independently controlling AL secretion of iB-endorphin. In 1979, Kebabian and Calne proposed that dopaminergic receptors can be divided into two categories that are distinguishable, in part, by their differential affinities for dopaminergic ligands, in much the same way that alpha- and beta-adrenoceptors display differential selectivity for adrenergic agonists. The proposed D-1 receptor subtype has limited affinity for dopaminergic agents, whereas, D-2 receptors exhibit much higher affinity for dopaminergic compounds (Kebabian & Calne, 1979). As observed here, the ability of only higher doses of haloperidol to evoke AL release of iB-endorphin suggests that a D-1 receptor mechanism might normally inhibit AL secretion of iB-endorphin. In contrast, the finding that low doses of haloperidol are capable of inhibiting apomorphine-induced release of iB-endorphin indicates that dopaminergic stimulatory control of AL iB-endorphin secretion might be mediated by a D-2 receptor mechanism. Recently available dopaminergic drugs which differentiate between these two receptor subtypes were employed to examine this question of dual dopaminergic mechanisms for regulating AL release of iB-endorphin. The results of

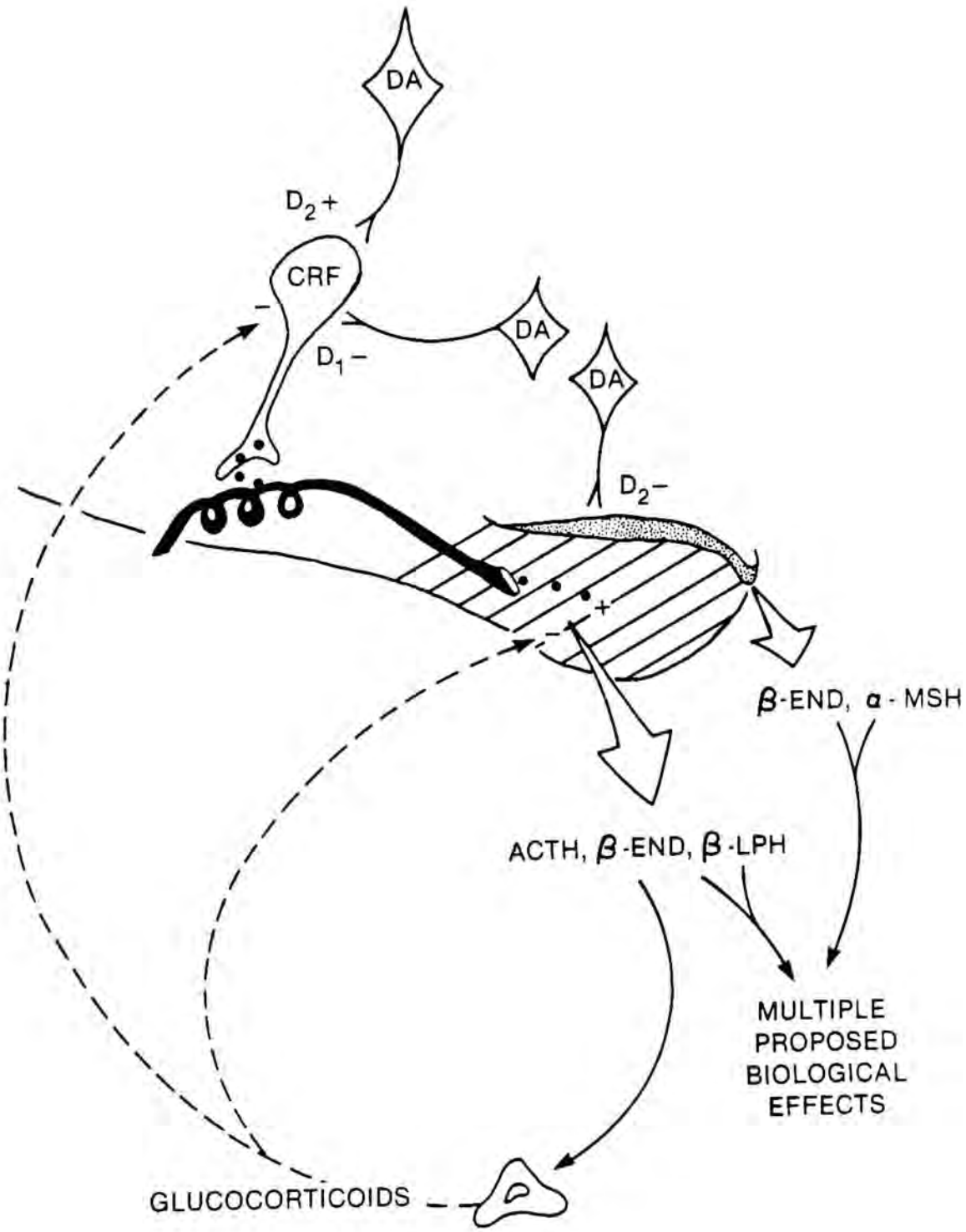
these experiments led to the development of a model for dopaminergic regulation of pituitary iB-endorphin

4.0.3 A Model for Dopaminergic Regulation of Pituitary B-endorphin Secretion

4.0.3.1 Overview

For the purpose of unifying the present discussion, a model is proposed for dopaminergic control of pituitary iB-endorphin. As diagrammed in Figure 31, brain dopamine neurons are involved in basal and dynamic regulation of iB-endorphin secretion from the pituitary gland through combined stimulatory and inhibitory actions over AL and by direct inhibition of IL secretion of iB-endorphin. Also shown is the "long loop" inhibitory control of AL corticotrophs by glucocorticoids. Secretion of adrenal glucocorticoids is stimulated by adrenocorticotropin (ACTH) which is co-released with iB-endorphin from corticotrophs. Findings presented here indicate that dynamic stimulatory control of AL secretion by dopamine is mediated through a D2 receptor mechanism that evokes the release of corticotropin releasing factor(s) (CRF) into portal blood. In all the present examples of D2 receptor actions, this receptor subtype inhibits a biological activity of the target cell (see Kebabian and Calne, 1979; Stoof and Kebabian, 1981; Wong et al, 1983). Hence, it is possible that D2 stimulation of AL iB-endorphin release occurs by

Figure 31. Schematic illustration modeling the regulation of beta-endorphin secretion from anterior and intermediate lobes of the rat pituitary. Dopamine (DA) neurons directly innervate the IL and tonically inhibit (-) melanotroph secretions of B-endorphin peptides (B-END) and alpha-melanotropin (∝-MSH) via a D2 receptor mechanism. Dopamine neurons directly or indirectly innervate corticotropin releasing factor (CRF) neurons. Through inhibitory (-) D1 receptor mechanisms and stimulatory (+) D2 mechanisms that govern CRF secretion into the portal vasculature, these brain dopamine neurons participate in controlling AL release of B-END, beta-lipotropin (B-LPH) and adrenocorticotrophin (ACTH). Adrenal glucocorticoids exert negative feedback control of the CRF-AL-adrenal axis with actions in the brain as well as on corticotrophs.


DA
D2+
CRF
DA
D1−
DA
D2−
+
−
β-END, α-MSH
ACTH, β-END, β-LPH
MULTIPLE
PROPOSED
BIOLOGICAL
EFFECTS
GLUCOCORTICOIDS

disinhibition of hypothalamic secretion of the AL secretagogue, CRF. By a separate mechanism, dopamine acts on brain D1 receptors to inhibit AL iB-endorphin secretion via blockade of CRF release. This latter dopaminergic mechanism appears to tonically inhibit release of iB-endorphin from the AL. For convenience of representation, dopamine neurons are shown interacting directly with CRF neurons, however, dopaminergic influences over CRF may be secondary to other neural events which ultimately control CRF release. For example, dopamine neurons could exert actions on CRF through acetylcholine which is known to be intimately involved with control of hypothalamic CRF (Hillhouse et al, 1975; Jones et al, 1976; Jones & Hillhouse, 1977; Buckingham, 1980).

In contrast to the more remote central nervous system mechanisms which control iB-endorphin release from the AL, dopaminergic inhibition of IL release is direct (Przewlocki et al, 1978b; Vale et al, 1979; Vermes et al, 1980b) and mediated through the tuberohypophyseal dopamine neurons that innervate this lobe (Bjorklund et al, 1973; Tilders and Smelik, 1977). Results of the present study are entirely consistent with this aspect of the model and extend recent <u>in vitro</u> evidence which demonstrates that inhibitory control of the IL is mediated principally by a D2 receptor mechanism (see Cote et al, 1982).

4.0.3.2 a. D2 Stimulation of AL iB-endorphin Secretion:

The most novel finding in the present study is that dopaminergic agonists stimulate pituitary release of iB-endorphin. As proposed in the model, this occurs through D2 receptor activation which, in turn, enhances hypothalamic release of CRF. Results indicating that dopamine stimulates AL iB-endorphin release are discussed above. Evidence extending this finding to indicate a specific D2 receptor mechanism for the response of iB-endorphin is three-fold. First, the selective D2 agonist, LY141865, evokes a rise in circulating iB-endorphin which is due primarily to the release of immunoreactivity corresponding to B-lipotropin in size. Importantly, this response is blocked by premedication with glucocorticoids. Second, in addition to blockade by a general D1 and D2 receptor antagonist (haloperidol), the LY141865-induced release of iB-endorphin is prevented by the specific D2 blocker, sulpiride. And third, in contrast to the actions of LY141865, the D1 agonist, SKF 38393, has little, if any, significant affect on pituitary release of iB-endorphin under basal conditions. Together, these observations indicate that D2 receptor activation results in the release of iB-endorphin from corticotrophs of the AL. Furthermore, this explains the ability of mixed D1, D2 agonists (apomorphine and piribedil) to increase plasma levels of iB-endorphin (B-lipotropin) while inhibiting the secretion of B-endorphin peptides from the IL.

Indirect evidence from one other laboratory supports D2 stimulation of AL corticotroph secretions. Fuller and colleagues (1983) observed that LY141865 increases serum corticosterone in rats and, further, that this response was prevented by pretreament with haloperidol. Since adrenal secretion of corticosterone is normally stimulated by ACTH which is co-released with iB-endorphin from corticotrophs, both of their findings are consistent with D2 receptor stimulation of CRF and subsequent secretion of ACTH and iB-endorphin from the AL.

4.0.3.3 b. D1 Inhibition of AL iB-endorphin Secretion:

In addition to dopaminerigic stimulation of AL iB-endorphin secretion through a D2 receptor, evidence presented here and elsewhere indicates that dopamine also tonically suppresses AL release of iB-endorphin. The mechanism mediating AL inhibition probably involves D1 receptors which appear to be tonically activated by dopamine under basal conditions. This conclusion is based on the follwing observations. General dopaminergic antagonists capable of blocking both D1 and D2 receptors (haloperidol and pimozide) increase circulating levels of B-lipotropin-sized immunoreactivity together with B-endorphin-sized material (Figures 20 & 23). Since the appearance of B-lipotropin in blood indicates AL release of iB-endorphin, these findings support the view that AL secretion of iB-endorphin is subject to tonic inhibition

through dopaminergic mechanisms. This hypothesis is supported by similar findings in rats (Hollt and Bergmann, 1982) and human subjects (Genazzani et al, 1984) and by the observation that haloperidol evokes the concomitant release of both ACTH and iB-endrophin in rats (Giraud et al, 1980). The unique finding of the present study is that the mechanisms by which dopamine tonically inhibits AL iB-endorphin secretion probably involves activation of D1 receptor subtypes.

Pituitary release of iB-endorphin due to haloperidol is attenuated by pretreatment with the D1 receptor agonist, SKF 38393 (Figure 24). SKF 38393 principally suppresses release of immunoreactivity resembling B-lipotropin in molecular size (Figure 25). Since this form of circulating iB-endorphin is the lesser component of the overall haloperidol-evoked release (B-lipotropin plus B-endorphin peptides), the reduction of total iB-endorphin release by SKF 38393 pretreatment is not dramatic (Figure 24). Unlike SKF 38393, the D2 agonist, bromocriptine, substantially diminishes the net increase in circulating levels of total B-endorphin due to haloperidol principally by inhibiting release of B-endorphin-sized material (Figures 22 & 23, and Table 15). Together, these findings indicate that AL release of iB-endorphin (marked by B-lipotropin-sized immunoreactivity) is tonically inhibited by dopamine acting through a D1 receptor mechanism.

Further evidence indirectly supporting this conclusion is the lack of influence of specific D2 antagonists on AL iB-endorphin. Although both sulpiride and domperidone increase plasma levels of total iB-endorphin, neither compound enhances the release of immunoreactivity resembling B-LPH in molecular size. Consequently, in vivo blockade of D2 receptors appears to have no effect on basal secretion of iB-endorphin from AL corticotrophs.

In summary, these findings indicate that tonic dopaminergic inhibition of AL iB-endorphin release is mediated through a D1 receptor mechanism, whereas, a D2 dopaminergic mechanism mediates stimulation of AL iB-endorphin secretion. Experimental approaches for differentiating between D1- and D2-mediated actions on AL secretion of iB-endorphin have recently been aided by development of selective D1 antagonists like bulbocapnine (Shepperson et al, 1982) and SCH 23390 (Irio et al, 1983; Hyttel, 1983). Preliminary release studies of bulbocapnine's actions in vivo support the present model for D1 inhibition of AL iB-endorphin release (Mueller, personal communication). Thus, D1-selective antagonists afford valuable pharmacologic methods for examining inhibitory dopaminergic control of AL iB-endorphin secretion independent of D2 mechanisms.

4.0.3.4 c. Dopaminergic Control of AL iB-endorphin May Be

Mediated by CRF

Among the agents that control corticotrophs directly, corticotropin releasing factor (CRF) appears most likely to mediate the effects of dopamine on AL iB-endorphin secretion. Although dopamine is present in high concentrations in portal blood (Gibbs and Neill, 1978), neither dopamine nor dopaminergic agents exert any direct effects on basal secretion of iB-endorphin from the AL *in vitro*. This is shown in the results of Tables 7, 8 and 9. Further, despite numerous actions of dopamine in the periphery (Goldberg, 1972; Thorner, 1975; Snider and Kuchel, 1983; Aguilera and Catt, 1984), there is no evidence to suggest that dopamine directly influences adrenocortical secretion of glucocorticoids in any manner that could account for dopaminergic actions on AL iB-endorphin (Ontjes, 1980). Accordingly, dopaminergic actions on iB-endorphin release from the AL *in vivo* are most likely mediated through the CNS and since virtually all evidence indicates that CRF is the final common mediator of CNS actions on corticotrophs, both dopaminergic stimulation and inhibition of AL iB-endorphin probably occur through mechanisms located within the brain.

Evidence that supports control of AL iB-endorphin secretion by CNS dopamine neurons is discussed separately for stimulatory and inhibitory dopaminergic influences. The present finding that central but not systemic

pretreatment with sulpiride inhibits release of iB-endorphin due to LY141865 (Table 10 & Figure 15) indicates that the D2 mechanism for stimulating corticotrophs resides at some site within the blood-brain barrier. Indirect support for this view comes from Fuller's investigations of dopaminergic influences on adrenocortical secretions in vivo. Whereas haloperidol prevents the rise in serum corticosterone evoked by the dopamine agonist, pergolide, pretreatment of rats with the peripheral dopaminergic antagonist, domperidone, failed to suppress pergolide-induced release of adrenal glucorticoids (Fuller and Snoddy, 1981a). It is likely that, as observed in the present results with sulpiride, systemically-administered domperidone does not readily reach D2 receptors within the brain that mediate CRF release.

Additional support for central versus peripheral dopaminergic stimulation of CRF release has emerged from studies conducted in man. Infusion of L-dihydroxyphenylalanine (L-DOPA) along with the peripheral decarboxylase inhibitor, carbidopa (alpha-methyldopa-hydrazine) (Bartholini and Pletscher, 1969), elevates serum cortisol in man, whereas, infusions of dopamine or L-DOPA alone have no significant effect on circulating adrenal glucocorticoids (Wilcox et al, 1975). Since dopamine cannot cross the blood-brain barrier (Bertler et al, 1966) and L-DOPA treatment in the absence

of carbidopa more effectively enhances peripheral rather than central dopaminergic transmission, these observations indicate that dopaminergic stimulation of cortisol secretion in man occurs through CNS rather than peropheral mechanisms which enhance ACTH secretion. Together, the data presented here along with supporting evidence from other sources strongly suggests that dopaminergic mechanisms for stimulating AL iB-endorphin secretion are located in areas of the CNS protected by the blood-brain barrier.

Although there is no equivalent evidence for placing D1 inhibitory control of AL iB-endorphin release within the CNS, the lack of direct dopaminergic inhibition of corticotrophs suggests that the most likely location for D1 control mechanisms is in the hypothalamus. Interestingly, both D1 binding sites and a D1-stimulated phosphoprotein have recently been identified in the basal hypothalamus (Fuxe et al, 1984; Walaas and Greengard, 1984; Ouimet et al, 1984). Although a direct relationship between the neurons that contain these D1 receptor constituents and CRF has yet to be established, at least the biochemical substrates for D1 inhibitory control of AL iB-endorphin secretion are situated in areas of the hypothalamus closely associated with neurohumoral control of corticotrophs.

In summary, both dopaminergic stimulation (D2) and inhibition (D1) of AL iB-endorphin release <u>in vivo</u> are

likely to be mediated by central mechanisms which control CRF release. Precedent for the coexistence of both stimulatory and inhibitory dopaminergic control mechanisms exists in electrophysiological responses of hypothalamic neurons. Moss and colleagues (1975) found that some tuberoinfundibular neurons are stimulated by dopamine, whereas, others are inhibited by the catecholamine. Likewise, there is evidence of both stimulatory and of inhibitory actions for other catecholamines in the control of AL corticotrophs (Ganong et al, 1976; Millan et al, 1982c; Berkenbosch et al, 1981a, 1981b; Pettibone and Mueller, 1982a, 1982b).

Opposing influences of brain dopamine on AL secretion of iB-endorphin suggests that different populations of dopamine neurons participate in controlling CRF. Since the release of CRF represents the sum of integrated CNS inputs for influencing corticotroph function, participation of multiple dopaminergic pathways in CRF control implies that dopamine neurons relay more than a single physiological parameter important to the secretion of AL iB-endorphin and other POMC peptides.

Of all the agents that directly effect corticotrophs (see Figure 3), CRF is the only secretagogue which profoundly stimulates AL iB-endorphin secretion <u>in vitro</u> in a manner comparable to the release evoked by LY141865 or classical dopamine agonists <u>in vivo</u> (Vale et al, 1979; Vale

et al, 1983). Although there is no evidence presently supporting a direct stimulatory action of dopamine on CRF neurons, dopamine may increase CRF release through acetycholine, a known CRF modulator with which dopamine has been shown to interact in controlling other neuroendocrine functions (Lichtensteiger and Keller, 1974; Lichtensteiger, 1975). Certainty about the relationship of dopamine neurons and CRF is likely to be established in the near future since changes in portal blood content of CRF can now be estimated by radioimmunoassay (Gibbs and Vale, 1982). Levels of hypophyseal-portal CRF vary as anticipated in response to stress and adrenalectomy (Suda et al, 1983; Plotsky and Vale, 1984). Accordingly, the physiological relationship of dopaminergic neurotransmission and secretion of this hypothalamic hormone may now be examined directly.

A second hypothalamic peptide which may also play a role in D2 stimulation of AL iB-endorphin secretion is arginine-vasopressin (AVP). Although not particularly active on its own, AVP has been shown to potentiate the actions of CRF <u>in vitro</u> (Gillies et al, 1982; Turkelson et al, 1982) suggesting that a physiologic function of AVP is to modulate corticotrophic responses to CRF (Anhut et al, 1981; Rivier and Vale, 1983). Reports that the AVP content of the median eminence increases and decreases after adrenalectomy and glucocorticoid treatments, respectively, supports this view (Dube et al, 1973; Silverman et al,

1981). Additionally, Millan and colleagues have reported that loss of hypothalamic AVP due to lesions of the paraventricular nucleus correlates well with concomitant decline in plasma levels of iB-endorphin (Millan et al, 1984).

Even if AVP were to act primarily through potentiation of CRF stimulation as suggested by Rivier and Vale (1983), the role of AVP as a participant in corticotroph regulation is particularly interesting since others have shown that dopamine neurons stimulate the release of hypothalamic AVP (Milton and Paterson, 1973; Bridges et al, 1976). Together, these results raise the possibility that AVP may also be involved in stimulatory actions of dopamine on AL release of iB-endorphin.

For some of the reasons discussed previously regarding CRF's pivotal role in governing corticotrophs, D1 inhibition of AL iB-endorphin release is also likely to be mediated by CRF. In this case, however, a dopaminergic mechanism reduces rather that augments neurosecretory release of CRF from the median eminence. Without influencing basal efflux of CRF, dopamine has been found to attenuate the stimulated release of CRF from hypothalamus <u>in vitro</u> (Edwardson and Bennett, 1974; Hillhouse et al, 1975). Thus, <u>in vivo</u>, dopamine neurons may act through a D1 receptor to limit the release of CRF into portal blood that is normally evoked by a spontaneously active pathway.

Given acetylcholine's well-described stimulatory control of CRF (Buckingham, 1980) and the ability of dopamine to inhibit acetylcholine-induced CRF release from the hypothalamus (Hillhouse et al, 1975), the D1 mechanism could modulate CRF secretion through inhibition of cholinergic neurotransmission in the hypothalamus (see Lichtensteiger and Keller, 1974; Lichtensteiger, 1975).

Based on the present study, it is difficult to predict the relationship between mechanisms that underly D1 inhibitory and D2 stimulatory control of AL iB-endorphin secretion. The slight but significant reduction of D2-stimulated iB-endorphin release by SKF 38393 (Figure 26) indicates that D1 inhibition of CRF may occur at a CNS site distal to D2 stimulatory regulation of CRF. Additional co-treatments with D1- and D2-selective agents are necessary for better modeling of the opposing dopaminergic influences on CRF (hence AL B-endorphin) secretion.

4.0.3.5 d. Direct D2 Inhibition of IL iB-endorphin Secretion

All dopaminergic antagonists used in the present study elevated plasma levels of iB-endorphin. This occurred, in large part (haloperidol and pimozide) or exclusively (sulpiride and domperidone) due to increased release of immunoreactivity resembling B-endorphin 1-31 in molecular size. These findings build upon previous reports

that dopaminergic blockers elevate alpha-MSH secretion *in vivo* (Usategui et al, 1976; Tilders and Smelik, 1977, 1978; Tilders et al, 1979; Penny and Thody, 1979). Two other laboratories have recently confirmed the ability of both haloperidol and domperidone to elevate pituitary release of iB-endorphin resembling B-endorphin in molecular size (Hollt and Bergmann, 1982; Sharp et al, 1982a). These observations together support the conclusion that tonically active dopamine neurons inhibit IL secretion of iB-endorphin and other POMC peptides. The particular neurons that mediate this inhibitory control of melanotrophs are the tuberohypophyseal dopaminergic system which directly innervate the IL (Bjorklund et al, 1973).

Present results demonstrating that dopaminergic agonists acutely reduce circulating levels of B-endorphin-sized immunoreactivity (Figures 5, 6 and 9) indicate that endogenous dopaminergic inhibition of melanotrophs is not sustained at a maximal level under basal conditions. In support of this view are reports that chronic administration of dopaminergic agonists reduces basal release of iB-endorphin *in vivo* accompanied by diminished IL content of B-endorphin-related peptides (Locatelli et al, 1983; Millington, O´Donohue and Mueller, personal communication). Since tuberohypophyseal dopamine neurons also exhibit neurochemical changes in response to similar experimental treatments (Demarest and Moore, 1982), the entire neuroendocrine unit possesses the ability to

vary according to physiological needs of the system whether this entails up or down regulation of hormones secretioned from the IL.

The common feature of dopaminergic agents that influence IL release of iB-endorphin *in vivo* is their ability to interact with the D2 subtype of dopamine receptors. This is predicted by the *in vitro* results from Kebabian and coworkers who have shown that dopaminergic inhibition of alpha-MSH release from acute IL explants is mediated by D2 receptors located directly on melanotrophs (Munemura et al, 1980; Cote et al, 1982).

At the cellular level, dopaminergic agonists directly decrease the spontaneous electrical depolarization of melanotrophs (Douglas and Taraskevich, 1978) and inhibit adenylate cyclase activity (Meunier and Labrie, 1982; Cote et al, 1982). It is likely that these two actions of dopamine on melanotrophs result in acute as well as long term changes in secretory cell activity. Without depolarization and the accompanying calcium influx, exocytosis of iB-endorphin is inhibited (Douglas and Taraskevich, 1982). In addition to rapidly preventing vesicular release, dopamine probably exerts longer term influence on the ability of the IL to synthesize B-endorphin and related POMC peptides. Reduced intracellular production of cyclic adenosine monophosphate by D2 receptor activation probably interrupts a chain of

intracellular events which maintains normal transcription and translation of the POMC gene in melanotrophs. This notion is supported by studies which have shown that chronic bromocriptine not only reduces IL content of iB-endorphin (Locatelli et al, 1983) but also reduces the content of POMC mRNA and the enzyme which acetylates MSH and endorphin peptides in the IL (Chen et al, 1983; Millington, Chappel, O'Donohue and Mueller, personal communication). Thus, dopaminergic inhibition of two processes, calcium-mediated exocytosis and cAMP-mediated induction of the POMC gene and processing enzymes, effectively reduces both the moment-to-moment secretory output of the gland and the ongoing capacity of melanotrophs to secrete iB-endorphin. Precedent for multiple levels of dopaminergic neuroendocrine control exists in the manner in which dopamine inhibits prolactin secretion. A D2 receptor mechanism in lactotrophs not only inhibits release of prolactin (Kebabian and Calne, 1979) but also reduces cellular content of prolactin mRNA through inhibition of prolactin gene transcription (Maurer, 1980; Maurer, 1981; Maurer 1982).

4.0.3.6 e. Other Findings Related to Dopaminergic Control of

Pituitary iB-endorphin Secretion

Based on results of the present study, it is proposed that dopamine neurons regulate pituitary secretion

of iB-endorphin through three independent receptor mechanisms. Tonic D1 inhibition and dynamic D2 stimulation account for the actions of dopamine on the AL, whereas, D2 inhibition controls IL secretion of iB-endorphin (see Figure 31). Most of the findings presented here have contributed to the development of this model, however, a few of the results are not obviously consistent with the proposed model. The apparent inconsistencies arise from the hormonal effects of the dopaminergic agonist, bromocriptine, and the dopaminergic reuptake inhibitor, nomfensine.

Unlike other compounds which activate D2 receptor mechanisms, bromocriptine does not significantly increase circulating levels of total iB-endorphin, apparently due to its inability to substantially enhance AL release of iB-endorphin. The dissimilarity that exists between the hormonal actions of bromocriptine and of other D2-stimulatory agonists may actually shed light on the precise nature of the D2-receptor mechanism that controls CRF release. Bromocriptine is known to have a slow onset of CNS actions after systemic administration. For example, bromocriptine does not begin to reduce dopamine turnover in the CNS or evoke contralateral turning in rats with unilateral striatal lesions until an hour or more after peripheral injection of the agonist (Corrodi et al, 1973; Johnson et al, 1976; Markstein et al, 1978). This delay occurs despite the drug's rapid peripheral effects [e.g.,

inhibition of prolactin secretion within fifteen minutes (see Figure 10)]. Considering the rapid and transient stimulation of AL iB-endorphin release by other dopaminergic agonists, bromocriptine's inability to similarly evoke corticotroph secretions may be due to the compound's failure to swiftly activate a rate-sensitive D2 receptor mechanism required to promote hypothalamic release of CRF. There is precedence for rate-sensitive dopaminergic mechanisms since D2-mediated effects on other CNS processes have been shown to exhibit properties (similar to D2-stimulated iB-endorphin release) of short-latency and rapid adaptation (Titus et al, 1983; Wong et al, 1983). Corrodi and colleagues, for example, attributed the low incidence of stereotypy in bromocriptine-treated animals to the drug's inability to activate rate-sensitive processes in the CNS (Corrodi et al, 1973). This phenomenon may be generalized to include the absence of effects of bromocriptine on CRF and AL iB-endorphin release.

Although the indirect dopaminergic agonist, nomfensine, did not significantly influence plasma levels of total iB-endorphin, there was a tendency for basal levels of total iB-endorphin to increase (Table 3). Perhaps of more importance was the shift in the underlying molecular forms of circulating iB-endorphin. As compared to control profiles, B-LPH emerged as the dominant form of iB-endorphin in nomfensine-treated rats. This change resembles the effects of classical dopaminergic agonists,

indicating that AL secretion of B-endorphin-related peptides increased concomitant with decreased IL release. Diminished IL release of iB-endorphin is expected based on nomfensine's ability to enhance dopaminergic transmission in the neurointermediate lobe (Racke and Muscholl, 1983). However, results of the present study provide no basis for a stimulatory dopaminergic pathway that tonically enhances AL iB-endorphin release. Therefore, stimulation of AL iB-endorphin release by nomfensine is difficult to reconcile with known dopaminergic actions of the antidepressant and the present model. Since nomfensine has been shown to inhibit inactivation of norepinephrine almost as effectively as it blocks reuptake of dopamine (Hunt et al, 1974), the drug's effects on AL iB-endorphin secretion might represent confounding enhancement of an adrenergic stimulatory pathway for CRF secretion.

4.0.4 Role of Dopamine Neurons in Physiologic Release of iB-endorphin

Evidence for dopaminergic regulation of AL as well as IL release of iB-endorphin led to experiments designed to investigate the role of dopamine neurons in governing the physiological secretion of pituitary iB-endorphin. The two most potent releasers of AL iB-endorphin <u>in vivo</u> are conditions of stress and interruption of negative feedback

control by glucocorticoids. The present findings discussed below, indicate that dopamine neurons may regulate IL responses to stress yet do not participate in the mechanisms by which glucocorticoids mediate feedback inhibition of AL iB-endorphin secretion.

4.0.4.1 a. Effects of Dopaminergic Treatments on Stress-Induced Release of iB-endorphin

As shown in Figure 12, physical immobilization profoundly stimulates release of pituitary iB-endorphin. The dramatic rise of B-LPH-sized immunoreactivity and the ability of glucocorticoids to prevent stress-induced release of total iB-endorphin indicates that AL corticotroph secretions comprise the major response to stress (Figures 12 & 29). Nonetheless, up to 40% of the immunoreactivity released in response to immobilization corresponds to B-endorphin-sized material. This being the only molecular weight range of melanotroph B-endorphin peptides suggests IL as well as AL involvement in the hormonal response to stress. Further support for this hypothesis is the ability of bromocriptine to reduce immobilization-induced release by about 40%. The remaining molecular form of iB-endorphin in bromocriptine-pretreated animals subjected to restraint resembles B-LPH in molecular size (not shown) suggesting that the AL response to stress is not dramatically affected by bromocriptine.

The long-acting dopaminergic agonist, pergolide, like bromocriptine, also inhibited stress-induced release of iB-endorphin. Unlike bromocriptine, however, pergolide blocked the iB-endorphin response to restraint entirely. This indicates that, in addition to IL release, pergolide also inhibits iB-endorphin secretion from the AL during physical stress. Furthermore, pergolide is known to stimulate both D1 and D2 receptors (Goldstein et al, 1980b; Boissier et al, 1983). Since both D1 inhibitory and D2 stimulatory mechanisms appear to be involved in AL iB-endorphin release, pergolide's inhibition of stress-induced release requires explanation. As indicated previously, the D1 inhibitory mechanism seems to operate at a site distal to D2 stimulatory control. However, the potency of D1 inhibition does not appear great enough to account for complete blockade by pergolide of corticotroph secretions elicited stress. The most likely explanation for blockade of stress-induced AL iB-endorphin release by pergolide involves the principal physiologic inhibitor of corticotrophs, corticosterone. Others have shown that doses of pergolide lower than those used in the present study rapidly increase serum corticosterone to levels comparable to stress-evoked concentrations (Fuller and Snoddy, 1981a). In the present study, levels of corticosterone were still undoubtedly high at the time that rats were exposed to immobilization (90 min post-pergolide). During this period, a second major

release of ACTH (and presumably AL iB-endorphin) becomes most susceptable to feedback inhibition by sustained high levels of glucocorticoids (Dallmand and Yates, 1969; Dallman et al, 1972). Indeed, Dallman and Jones have shown that non-stressful elevation of corticosterone to serum concentrations that approximate stress levels inhibits subsequent stress-evoked release of ACTH (Dallman and Jones, 1973). It is likely, therefore, that feedback inhibition of the CNS-pituitary-adrenal axis by glucocorticoids secondary to stimulation of ACTH release probably accounts for a large part of the inhibition of stress-induced AL iB-endorphin release in pergolide-pretreated animals. Consequently, pergolide's ability to completely block stress-induced iB-endorphin secretion from corticotrophs reflects the drug's transient activation of D2 stimulatory mechanisms that lead to glucocorticoid feedback control together with prolonged activation of inhibitory D1 regulation of the AL. To better understand how D1 and D2 mechanisms might influence stress-induced secretions from corticotrophs, different experimental designs will be needed than used in the present study. For instance, since D2 stimulatory control of AL iB-endorphin appears to be a rate-sensitive mechanism that rapidly adapts to receptor activation, dopamine neurons may influence the rate of release of CRF hence iB-endorphin secretion by corticotrophs rather than the maximal level of release. To examine this possibility

requires comparison of time-course related effects of D2 agonists on stress-induced iB-endorphin secretion.

Consistent with the IL-directed effects of dopaminergic agonists during immobilization are results which show that haloperidol treatments augmented stress-induced release of iB-endorphin by enhancing secretion of B-endorphin-sized immunoreactivity (Figure 29). Since haloperidol had been administered for several days in succession prior to restraint stress, disinhibition of melanotrophs probably increased the secretory reserve of the IL. These findings together with the actions of dopaminergic agonists on stress-induced secretion of iB-endorphin indicate that physiological release of iB-endorphin from the IL is highly sensitive to dopaminergic transmission and highlights the importance of tuberohypophyseal innervation to the IL and the D2 receptor mechanisms therein.

4.0.4.2 b.Effects of Dopaminergic Treatments on Metyrapone-Induced Release of iB-endorphin

Administration of metyrapone to rats causes a rapid decline in circulating corticosterone (Chart et al, 1958 ;deNichola and Dahl, 1971) which is accompanied by an equally rapid increase in pituitary release of iB-endorphin (Pettibone and Mueller, 1984; Mueller et al, submitted). Unlike the release evoked by stress, however, metyrapone

almost exclusively elevates AL secretion of iB-endorphin (Pettibone and Mueller, 1984; Mueller et al, submitted), a finding which is consistent with the selective inhibitory control of corticotrophs by glucocorticoids. Judging from the additive release of pituitary iB-endorphin in response to combined treatment with metyrapone and haloperidol (D1, D2 blocker), glucocorticoid feedback control and dopaminergic inhibitory regulation of AL iB-endorphin secretion appear to be mediated through separate, perhaps parallel, mechanisms (Figures 27, 31). This conclusion is further supported by the failure of bromocriptine to alter metyrapone-induced release of iB-endorphin (Table 16). The present lack of evidence for dopaminergic influence on CNS-pituitary-adrenal feedback mechanisms is somewhat surprising because others have shown a functional association between hypothalamic dopamine neurons and adrenal glucocorticoids. Metyrapone's acute effects on circulating corticosterone are quite similar to those of adrenalectomy. The activity of hypothalamic dopamine neurons reportedly increases soon after adrenalectomy (Versteeg et al, 1984) which is consistent with the longer term increase in hypothalamic content of dopamine observed after either adrenalectomy or hypophysectomy (Smith and Fink, 1972; Olsen et al, 1972; Konstantinova and Danilova, 1975). Notwithstanding those studies, the present results clearly militate against interactions of dopaminergic mechanisms with adrenocortical feedback control of

corticotrophs.

4.0.5 SUMMARY AND CONCLUSIONS

The purpose of the present study was to determine what role brain dopamine neurons have in the regulation of B-endorphin secretion from the pituitary gland. Through results obtained from pharmacologic treatments of adult male rats, dopamine receptors appear to participate in controlling secretion of B-endorphin peptides from both the AL and IL of the pituitary gland. Whereas dopamine exclusively inhibits IL iB-endorphin release through a D2 subtype of the dopamine receptor, D1 and D2 receptors oppositely influence AL secretion. A D1 mechanism inhibits AL secretion of iB-endorphin, whereas, a D2 mechanism is capable of potently stimulating AL iB-endorphin release. Since dopamine has no direct influence on AL corticotrophs, the opposing effects of D1 and D2 activation are likely to be mediated by actions of dopamine on CRF neurons. In contrast to the more remote and opposing influences of dopamine on AL B-endorphin secretion, hypothalamic dopamine neurons directly inhibit the release of IL B-endorphin. This conclusion is entirely consistent with the known innervation and actions of dopamine at inhibitory D2 receptors located on IL melanotrophs. Together, these differential dopaminergic mechanisms for controlling AL and IL secretion of B-endorphin peptides indicate that brain dopamine neurons play an important role in the physiology

of the opiomelanocortin endocrine system.

Interestingly, under conditions of stimulated iB-endorphin release, dopaminergic treatments had little apparent effect on AL secretion. It may be necessary to employ more selective dopaminergic agents (e.g., LY141865 or SCH 23390) or use different treatment paradigms than those used in the present studies. Alternatively, the multiplicity of control mechanisms involved with hypothalamic CRF release could rapidly compensate for acute perturbations in CRF release caused by dopaminergic treatments. Under basal conditions, however, when control of CRF is not marshalled by the powerful stimuli of stress or decreased glucocorticoid feedback control, the reciprocal actions of D1 and D2 mechanisms on CRF secretion are evident.

APPENDIX

Radioimmunoassay [RIA] Protocol

Reagents for the radioimmunoassay were pipetted on ice into prelabeled 12 x 75 mm borosilicate glass culture tubes in the order presented below. Two tubes received tracer alone (total counts tubes), two received tracer plus heat-inactivated horse serum (GIBCO) and assay buffer to volume (non-specific binding tubes) and two received all reagents except for standards or unknowns (maximum specific binding tubes). Routinely used standards (10, 30, 60,...1000 pg/0.1 ml) were stored at -20 C in convenient aliquots and assayed in duplicate. Unknowns were assayed in duplicate at two dilutions. After vortexing and covering with Parafilm, the assay was allowed to equilibrate for 60-72 h at 4 C.

RIA Reagent Mix

ASSAY REAGENT	Volume
Heat-Inactivated Horse Serum (except in prolactin assay)	0.05 ml
Standards or unknowns	0.01-0.25 ml
Assay Buffer	as needed
Antiserum (pre-diluted)	0.10 ml
Tracer (~15,000 cpm)	0.10 ml
total volume	0.50 ml

The beta-endorphin (B-endorphin) and alpha-MSH assays were terminated by adsorption of unbound antigen into charcoal. A suspension of charcoal and bovine serum albumin (0.5% and 0.05% by weight, respectively, in 0.05 M sodium phosphate, pH 7.4) was maintained by vigorous stirring as 1 ml of the suspension was added to each assay tube. Assay tubes were then vortexed, incubated at 4 C for 30 min and centrifuged at ~3,000 rpm (Sorval RC-3B, Dupont Instruments, Newtown, CT) for another 30 min at 4 C. Supernatants were decanted into appropriately-labeled 12 x 75 mm glass tubes counted for 1 min/tube at 80% efficiency (Automatic Gamma System Model 1185, Tracor Analytic, Atlanta, GA).

The prolactin radioimmunoassays were pipetted in 10 x 75 mm glass culture tubes with standards ranging from 60 to 6000 ng tube. Unlike the other hormone assays, the prolactin assays were terminated by second antibody precipitation of antigen bound to the primary antiserum as follows. After a 60-72 hr equilibration period at 4 C all assay tubes (except the total count tubes) received a 0.1 ml aliquot of goat anti-rabbit antiserum (diluted 1:20 in assay buffer) and vortexed and allowed to incubate for an additional 12-24 h at 4 C. The second antibody-primary antibody complex was precipitated by addition of 1 ml deionized water and centrifugation as described above. Supernatants were discarded and the pellets were counted.

REAGENTS

ASSAY BUFFER: 0.05 M sodium phosphate (pH 7.4)/0.05% bovine serum albumin/0.02% sodium azide/5 mg% bacitracin

The reagents below were routinely dissolved in 4 liters of deionized and filtered water (Millipore Corporation, Freehold, NJ) with a resulting pH of 7.3-7.4.

Recipe for 4 liters of Assay Buffer:

6.62 g hydrated sodium monobasic phosphate, $NaH_2PO_4:H_2O$ (No.S-368; Fisher Scientific Company, Fairlawn, NJ)
21.57 g anhydrous sodium dibasic phosphate, Na_2HPO_4 (No.S-374; Fisher)
2.00 g bovine serum albumin, fraction 5 (No.A-4503; Sigma Chemical Company, St. Louis, MO)
0.80 g sodium azide, NaN_3 (No.S-2002; Sigma)
0.20 g bacitracin, 56,300 Units/g (No.B-0125; Sigma)

ANTISERA:

Anti-rat prolactin antiserum was provided through the National Hormone Distribution Program of the NIADDK. Other antisera were developed in rabbits as follows:

A peptide-thyroglobulin conjugate (see below) was emulsified in a mixture of Freunds Complete and Incomplete Adjuvant (DIFCO Laboratories, Detroit, MI) for the initial inoculation and subsequently in Incomplete Adjuvant to a concentration of approximately 0.3 mg of conjugate per ml of emulsion. The inoculum was injected intradermally into 10-20 sites along the hind quarters of female New Zealand albino rabbits every three or four weeks. Three months after the initial inoculation and two weeks after every subsequent booster inoculation, rabbits were bled (~15 cc)

from the dorsal ear vein (vasodilated with xylene and nicked using a straight razor blade) and the blood was allowed to clot overnight at 4 C. Serum was obtained by centrifugation and stored at -20 C. Titers of antiserum were estimated by incubating serial dilutions of the serum (in assay buffer) with peptide tracer (see below). Appropriate dilutions of antisera for routine use were made in assay buffer and stored at -20 C.

Peptide-thyroglobulin Conjugate:

Peptide (0.5 mg of camel B-endorphin 1-31 or alpha-MSH [alpha-N-acetyl ACTH 1-13 amide] from Peninsula Laboratories, San Carlos, CA) was mixed with 5.0 mg of bovine thyroglobulin (No.T-1001;Sigma) in 1.0 ml of deionized water. The reaction was performed on ice with constant stirring by the dropwise addition of 0.5 ml 100 mg/ml carbodiimide {EDAC (1-ethyl-3-[dimethylaminopropyl] carbodiimide HCL), No.153-0990, Bio-Rad Laboratories, Richmond, CA} in deionized water; the mixture was stirred on ice for 10 min and then brought to room temperature (~23 C) for 20 min with stirring. Reagents were separated from the peptide-thyroglobulin conjugate by extensive dialysis (12,000 mw cut off) against 4 liters of water at 4 C. The dialyzate was diluted to a final volume of 5cc (~1 mg conjugate/cc) and stored at -20 C. As needed, one part conjugate was emulsified with 2-3 parts adjuvant (Freunds Complete or Incomplete)

TRACERS:

Camel B-endorphin 1-31, N-Acetyl B-endorphin 1-27, alpha-MSH (Peninsula Laboratories) and rat prolactin (National Hormone Distribution Program) were radioisotopically labeled with 125-I in a chemical oxidative reaction by sequential addition of the following reagents into a vial containing ~1 mCi sodium 125-I in 10 microliters of carrier-free sodium hydroxide (Amersham Corporation, Arlington Heights, IL).

20 microliters-0.5 M sodium phosphate buffer, pH 7.4

20 microliters-0.1 mg/ml peptide (~ 2 mcg) in 0.05 M phosphate buffer, pH 7.4

10 microliters-1 mg/ml Chloramine-T (N-chloro-p-toluene-sulfonamide sodium; No.C-9887, Sigma Chemical Company) in 0.05 M phosphate buffer, pH 7.4

The oxidation mixture was vortexed for 30 sec then the reaction was quenched by addition of:

20 microliters-0.66 mg/ml sodium bisulfite (No.S-9000, Sigma Chemical Co) in 0.05 M phosphate buffer, pH 7.4.

The iodination mixture was then transferred for chromatographic purification using a tuberculin syringe containing 0.3 ml of either 0.05% triflouroacetic acid (B-endorphin or alpha-MSH tracers) or 0.05 M phosphate buffer (prolactin tracer).

125 I-labeled B-endorphin or alpha-MSH peptides were crudely purified by reverse-phase chromatography on

commercially-available C18 cartridges (Sep-Pak, Waters Associates, Millford, MA). Sep-Pak cartridges were prepared by rinsing first with 2-3 ml of acetonitrile containing 0.05% triflouroacetic acid (TFA) followed by 2-3 ml of water containing 0.05% TFA. Radioisotopically-labeled peptide (see above) were applied to the column in 0.05% TFA and rinsed with a 2 ml fraction of the aqueous 0.05% TFA solution. The 125 I-labeled peptide was eluted with stepwise rinses of 25%, 50% and 100% acetonitrile containing 0.05% TFA. B-endorphin and MSH tracers were routinely eluted from the Sep-Pak cartridges in the 50% acetonitrile-TFA fraction. The tracer was kept at 4 C with 0.2 ml of absolute ethanol added as a free radical scavenger.

125 I-labeled prolactin was purified by gel filtration chromatography. Briefly, the iodination mixture was applied to a 1 x 50 cm column of Sephadex G-50 (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated and eluted with the sodium phosphate assay buffer. Fractions (~1.5-2 ml) were collected with an automatic sampler (Gilson Medical Electronics, Middleton, NJ) and the prolactin tracer routinely eluted between fractions 9 and 15. Peak tubes of radioactivity were covered and stored at 4 C with 0.2 ml of absolute ethanol.

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