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Selective Post-Translational Processing of Opioid Peptides in Cardioregulatory Mechanisms of the Dorsal Medulla

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Chapter 25

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I. INTRODUCTION

The hypotensive properties of morphine and other opiate drugs have been well documented for over a century,[1] yet only in the past two decades have we begun to understand the neuronal mechanisms responsible for these ofttimes deleterious effects. The history of these discoveries has been widely disseminated; stereoselective opioid receptors were first identified in the early 1970s, followed soon thereafter by isolation of their endogenous ligands, the opioid peptides β-endorphin, met- and leu-enkephalin, and dynorphin.[2] All three opioid peptide families are expressed by neurons in the nucleus tractus solitarius (NTS) and other cardioregulatory brain sites, providing an anatomical basis for the cardiovascular side effects of opiate drugs.

The selectivity of opioid peptides for the parallel trilogy of opioid receptor subtypes was initially thought to follow a relatively discrete equation — β-endorphin, as well as the enkephalins, act at mu and delta receptors, whereas dynorphin is relatively selective for kappa receptors. However, it has since become evident that opioid peptide prohormones are multihormonal precursors which generate a complex array of peptide derivatives with varying degrees of selectivity for each of the three classical opioid receptors and, perhaps, nonopioid binding sites, as well. As a result, despite the wealth of anatomical and biochemical data available, predicting the exact physiological role of opioid peptides in cardiovascular regulation has turned out to be a rather complicated undertaking. This review will provide a brief overview of the anatomy and physiology of opioid peptides in the NTS, focusing on the role of posttranslational processing in defining their receptor selectivities and cardioregulatory actions.

II. ANATOMY OF OPIOID PEPTIDE NEURONS IN THE NTS

The NTS is an important regulatory site for integrating baroreflex stimuli. As reviewed elsewhere in this volume, the NTS receives sensory afferent fibers from baroreceptors in the aortic arch and carotid sinus which ascend in the glossopharyngeal (9th) and vagus (10th) nerves, terminating primarily in medial and commissural subnuclei of the caudal NTS.[3-5] The baroreflex includes both short- and long-loop components. The short-loop circuit consists of NTS neurons which project directly to preganglionic sympathetic neurons in the intermediolateral cell column of the thoracolumbar spinal cord and parasympathetic neurons in the motor

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nucleus of the vagus and nucleus ambiguus.[4] NTS neurons also project to two adjacent medullary regions: a pressor, or vasomotor area in the rostral ventrolateral medulla (RVLM), composed of the A5 and C1 catecholamine cell groups and adjacent reticular nuclei, and a depressor area in the caudal ventrolateral medulla (CVLM), which includes the A1 noradrenergic cell group.[6] The RVLM projects to the intermediolateral cell column, modulating sympathetic outflow, whereas the CVLM operates, at least in part, by tonically inhibiting RVLM projection neurons. The long-loop baroreflex pathway is composed of reciprocal projections to forebrain structures, including the hypothalamus and amygdala, as well as other brainstem sites, the locus ceruleus and parabrachial, raphe, and Kölliker-Füse nuclei.[3,4] Thus, arterial blood pressure and heart rate are regulated by short- and long-loop pathways, both of which contain opioid peptide neurons, explaining, in part, the multiple actions of opioid peptides in cardiorespiratory homeostasis.

A. PROOPIOMELANOCORTIN (POMC) PROJECTIONS FROM THE ARCUATE AND SOLITARY TRACT NUCLEI

Two distinct populations of POMC neurons play important roles in both short- and long-loop baroreflex pathways. The first, and best characterized, is localized in the arcuate nucleus and surrounding regions of the medial-basal hypothalamus.[7] These neurons project extensively throughout the brain, both rostrally, innervating hypothalamic and limbic structures, and caudally to the mesencephalon, brainstem, and spinal cord. Caudal projections also innervate brainstem nuclei including the rostral NTS, particularly the dorsomedial border of the nucleus, as well as the dorsal motor nucleus of the vagus, nucleus ambiguus, and both the RVLM vasomotor and CVLM depressor areas.[8,9] Arcuate POMC processes reach the brainstem through two descending pathways; a dorsomedial pathway projecting to the rostral NTS and RVLM and a ventrolateral pathway innervating the CVLM and nucleus ambiguus.[8] Thus, anatomically distinct POMC neuronal projections innervate the medullary pressor and depressor areas.

The arcuate nucleus was initially thought to be the sole source of POMC peptides in the brainstem, but subsequent studies revealed a second POMC cell group localized in the caudal NTS, primarily in the commissural nucleus.[8-11] These neurons emanate a dense intrinsic axonal network within the commissural nucleus and also project rostrally in the NTS, particularly to lateral subnuclei.[8,9] Although they do not innervate forebrain structures, efferent projections do course ventrolaterally, sending an arching bundle of immunoreactive axons to the ventrolateral medulla, innervating both the RVLM and CVLM as well as the nucleus ambiguus.[8] Like the arcuate cell group, NTS POMC neurons also innervate other brainstem nuclei, including the parabrachial and Kölliker Füse nuclei, consistent with evidence that commissural nucleus projections are importantly involved in respiratory control.[12] Thus, with the exception of the commissural nucleus[8] itself, cardiorespiratory brainstem nuclei receive a dual POMC neuronal innervation, originating from both the NTS and arcuate nucleus, suggesting that the two POMC systems mediate different specific functions.

B. RECIPROCAL ENKEPHALIN PATHWAYS

Like POMC, enkephalin neurons interconnect brainstem nuclei. Enkephalin immunoreactive perikarya and axonal processes are distributed throughout the rostrocaudal extent of the NTS, particularly within the medial, intermediate, ventro- and dorsolateral subnuclei.[10,13,14] A subpopulation of enkephalin neurons also express tyrosine hydroxylase immunoreactivity, presumably the A2 noradrenergic cell group which overlaps with medial NTS subnuclei.[15] Retrograde tracing studies have further revealed that NTS enkephalin neurons densely innervate the parabrachial area,[16] and unlike NTS POMC neurons, project further rostrally to forebrain structures, including the bed nucleus of the stria terminalis, amygdala,[14] and paraventricular nucleus.[17] Enkephalin immunoreactive axons also project to the RVLM[18] where, evidently, they synapse directly on catecholaminergic neurons which project, in turn, to the intermediolateral cell column.[19] RVLM enkephalin neurons also project back to the NTS, reciprocally interconnecting the two nuclei;[20] the NTS receives an afferent enkephalin projection from the paraventricular nucleus, as well.[21] Thus, enkephalin neurons apparently function as both intrinsic, local circuit neurons within the NTS and in the reciprocal connectivity with other brainstem and forebrain cardioregulatory sites.

C. INTRINSIC AND EFFERENT DYNORPHIN PROJECTIONS

Dynorphin immunoreactive axons and cell bodies are also found in the NTS, particularly in the medial, ventrolateral, and dorsolateral subnuclei.[13,14,22-24] Less is known about their anatomic projections, however. Fodor et al.,[24] using discretely placed surgical

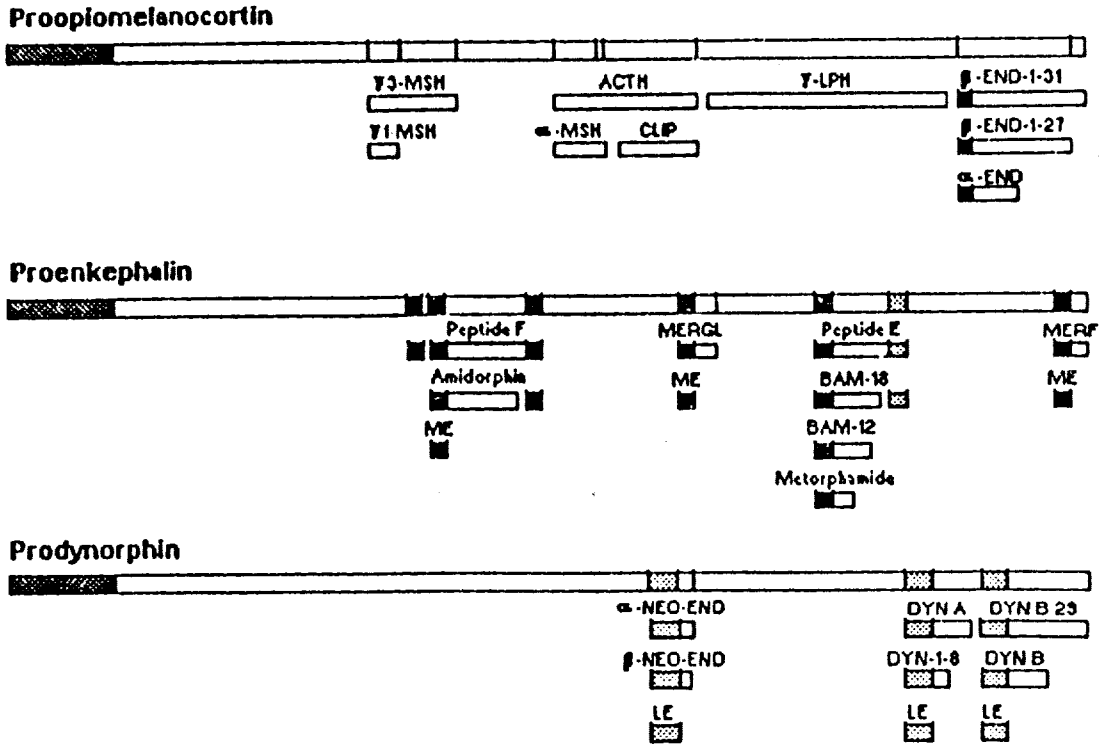

Figure 1 The posttranslational processing of opioid peptide prohormones.

transections, found little evidence that NTS dynorphin neurons innervate extrinsic targets although a small subset in the commissural nucleus apparently project ventrolaterally to other medullary sites. Retrograde tracer studies have shown, however, that dynorphin perikarya in the rostral NTS project to the hypothalamus, amygdala, medial parabrachial area, and other forebrain regions.[14] These findings, as well the relatively high levels of dynorphin immunoreactivity localized in the NTS,[25,26] support the concept that dynorphin neurons are an integral component of intrinsic NTS circuitry and may participate, to some extent, in the reciprocal innervation of forebrain structures.

The historical observation that morphine lowers blood pressure is clearly compatible with the anatomical localization of opioid peptide neurons in the NTS and other brainstem nuclei. A precise understanding of the cytoarchetectonic organization of opioid neurons is difficult to formulate, however, because the relative contribution of afferent, local circuit, and efferent projections has not been thoroughly elucidated. However, current evidence does support the conclusion that β-endorphin, enkephalin, and to a lesser extent, dynorphin neurons participate in both short- and long-loop baroreflex pathways. Nevertheless, the anatomical distribution of opioid neurons is not a sufficient basis for predicting their physiological role because the specific peptides they synthesize must also be considered.

III. OPIOID PEPTIDE PROCESSING AND RECEPTOR SELECTIVITY

Molecular cloning and protein sequencing studies have fully elucidated the peptide structures that can be generated from each of the three opioid prohormones. However, sequence information merely provides a menu of possibilities, for peptide processing is regionally specific and predicting the physiological actions of opioid peptides must be based on a thorough analysis of the individual peptide derivatives actually expressed within specific brain areas. This is particularly important because the relationship between opioid peptide structure and receptor selectivity is far more complex than generally assumed; indeed, the receptor selectivity of posttranslationally derived opioid peptides is often quite promiscuous and may involve not only mu, delta, and kappa receptors, but additional, nonclassical binding sites as well.

A. PROOPIOMELANOCORTIN (POMC)

POMC is a multihormonal prohormone which serves as the precursor, not only for β-endorphin, but for a variety of other peptides as well (Figure 1).[27-30] Unlike other opioid precursors, POMC

contains but a single copy of the enkephalin sequence, and although met-enkephalin, itself, is not an end product, multiple forms of β-endorphin are generated from β-endorphin-1-31, including β-endorphin-1-27, β-endorphin-1-26, γ-endorphin (β-endorphin-1-16), and α-endorphin (β-endorphin-1-16). β-Endorphin peptides also undergo α-N-acetylation, a relatively specific processing step largely restricted to POMC neurons. Like other opioid peptides, β-endorphin processing is regionally specific in brain; β-endorphin-1-31 is converted to β-endorphin-1-27 and β-endorphin-1-26 to a limited extent in the hypothalamus, for example, although C-terminally shortened, N-acetylated forms predominate in other brain regions.[29-34]

The opioid receptor selectivity of β-endorphin continues to be a subject of controversy. There is little doubt that β-endorphin-1-31 has a high affinity for mu receptors, with equivalent, or somewhat lower, affinity for delta and limited affinity for kappa receptors.[35-38] Bioassays, using the rat vas deferens,[38,39] as well as brain receptor binding experiments,[36] suggest that β-endorphin-1-31 interacts with an additional population of binding sites, originally termed the epsilon receptor, that differs from mu and delta receptors. Although the existence of the epsilon receptor in rat vas deferens has since been questioned,[40] Tseng and his colleagues have recently generated extensive evidence that β-endorphin-induced antinociception is mediated by receptors which do not correspond to classical opioid receptor subtypes.[41] Nonclassical β-endorphin binding sites have also been identified in peripheral tissues, including cellular elements of the immune system.[42] Whether these sites constitute the putative epsilon receptor, a kappa, or other opioid receptor subtype, or an as yet uncharacterized binding site remains to be determined. Nevertheless, structure-activity studies also provide evidence that β-endorphin peptides act at binding sites other than classical mu, delta, or kappa receptors.[38,43,44]

Receptor binding and bioassay experiments clearly demonstrate that both C-terminal proteolysis and N-acetylation virtually eliminate β-endorphin-1-31 opioid receptor affinity and dramatically reduce its antinociceptive potency.[29,45,46] Yet, these 'nonopioid' β-endorphin forms are not biologically inactive. β-Endorphin-1-27, for example, retains agonist activity in the rat vas deferens[39] although in brain it acts as an antagonist, blocking β-endorphin-induced antinociception, while exhibiting little agonist activity.[41,46,47] β-Endorphin-1-27 does not inhibit antinociception induced by selective mu, delta, or kappa receptor agonists, however, prompting Suh et al. to conclude that it acts as a selective epsilon receptor antagonist,[41] although the brain receptor certainly differs from that in the rat vas deferens where β-endorphin-1-27 acts as an agonist.[39]

Unlike β-endorphin-1-27, β-endorphin-1-26 lacks opioid antagonist activity, and although its physiological role in brain has yet to be established, it retains agonist activity at rat vas deferens[39] and vascular β-endorphin binding sites.[48] Both γ- and α-endorphin display limited affinity for opioid receptors, but based on their unique behavioral actions, are thought to activate a novel class of nonopioid binding sites.[49] *N*-acetyl-β-endorphin-1-31 lacks opioid receptor potency but reportedly exhibits nociceptive,[50] endocrine,[51] and immune[42] activities, although again, the binding sites responsible for these responses remains enigmatic. Clearly, β-endorphin processing redirects its receptor selectivity, generating both agonist and antagonist forms, and invoking an ever more perplexing array of putative β-endorphin binding sites.

B. PROENKEPHALIN

Unlike POMC, proenkephalin contains multiple copies of the enkephalin sequence — six met- and one leu-enkephalin — amplifying the opioid "message" derived from the prohormone (Figure 1). Met- and leu-enkephalin are not, in all cases, the final products of proenkephalin processing, however; C-terminally extended peptides are also generated, particularly in the adrenal medulla.[27,28] In brain, the major end product of the three enkephalin sequences near the prohormone's N-terminal appears to be the pentapeptide, itself, although this is almost certainly not the case for the remaining met-enkephalin sequences which are expressed, at least in part, as C terminally extended forms.[28] Thus, both met-enkephalin-Arg-Gly-Leu (ME-RGL) and met-enkephalin-Arg-Phe (ME-RF) are abundant in brain and low levels of the peptide E derivatives, BAM-22,[52] BAM-18,[28,53] BAM-12,[54] and metorphamide (BAM-8)[55] are also present; BAM-18, rather than its larger precursors or metorphamide, appears to predominate, however.[27,28] Like POMC, proenkephalin processing is regionally specific in the brain, and relative amounts of BAM-18, ME-RGL, ME-RF, and met-enkephalin vary extensively among brain regions,[53] suggesting that C-terminally extended forms may be selectively expressed to mediate yet unidentified functions. Nevertheless, the greater abundance of met-enkephalin indicates that the pentapeptide, itself, rather than larger forms, is the predominant product of proenkephalin processing in the brain.

The receptor selectivity of met- and leu enkephalin is well documented — they are relatively selective for delta, with significant, but lower affinity

for mu and limited affinity for kappa receptors — but C-terminally extended enkephalin peptides exhibit markedly reduced delta selectivity, with enhanced affinity for mu and/or kappa receptors.[35,37] Thus, ME-RP retains significant delta receptor selectivity, but ME-RGL is equipotent at delta and kappa receptors and still longer forms, peptide E and its derivatives, are generally more potent at mu and kappa than delta receptors.[35,37,56-58] Peptide F, on the other hand, appears to lack significant affinity for any opioid receptor subtype, yet it is a relatively potent antinociceptive agent[59] prompting the speculation that it, as well as peptide E and BAM-22, may activate the putative epsilon receptor.[57,58] Hence, predicting the physiologic role of endogenously released enkephalins must take into account the variable receptor affinities of larger enkephalin forms.

C. PRODYNORPHIN

Prodynorphin processing is relatively uncomplicated compared to POMC and proenkephalin.[27,28] The prohormone contains three copies of leu-enkephalin clustered near its C-terminal, each of which forms the N-terminal of a different set of dynorphin peptides; α-neo-endorphin, dynorphin A (dynorphin-1-17), and dynorphin B-29 (leumorphin). These, in turn, serve as precursors to yet smaller forms. Hence, α-neo-endorphin is converted to β-neo-endorphin through removal of its C-terminal arginine residue, and dynorphin-1-17 undergoes endoproteolytic cleavage at a single arginine residue, forming dynorphin-1-8. Dynorphin B-29 is similarly processed at a single arginine, forming a 13-amino-acid peptide, dynorphin B (rimorphin). Leu-enkephalin may also be a product of prodynorphin processing, at least in certain brain regions.[60] Like other opioids, the ratio of dynorphin peptides varies regionally, although dynorphin-1-8, dynorphin B, and α-neo-endorphin predominate in many brain areas.[25,26,28,61]

Although the assumption is often made that dynorphin-related peptides are selective ligands for kappa receptors, this holds true only for larger derivatives. The structural determinants for kappa selectivity appear to require an arginine residue at position 7 and a second basic amino acid at position 10 or 11.[27] Virtually all dynorphin peptides fulfill these criteria except dynorphin-1-8 and β-neo-endorphin, which lack a second basic residue. As one might predict, these derivatives, as well as α-neo-endorphin, exhibit lower potency and less selectivity for kappa receptors, with significant, if not higher affinity for mu and delta receptors.[37,56,57] As is the case for other opioid peptides, the differing receptor affinities, as well as regional differences in presynaptic processing, emphasize the difficulty of predicting the physiological role of endogenously released dynorphin.

IV. CARDIOREGULATORY ACTIONS OF OPIOID PEPTIDES

A. β-ENDORPHIN AND THE ENKEPHALINS: THE MU/DELTA CONUNDRUM

The role of mu and delta receptors in cardioregulation has been studied extensively, yet remains somewhat contentious.[62,63] Receptor autoradiographic experiments have localized both receptors subtypes in the NTS, although mu receptors are most abundant, particularly in the medial, dorsolateral, and commissural subnuclei.[64-66] Interestingly, vagal transection substantially reduces mu binding densities, suggesting that mu receptors are primarily localized on baroreceptor nerve terminals.[66] Delta receptors have also been identified in caudal NTS subnuclei, albeit in lower densities.[64,65] Dashwood et al.[66] have questioned this conclusion, however; they found no detectable delta binding in the cat NTS, attributing prior data to limited radioligand selectivity. Hence, mu receptor binding sites, no doubt, are present in relative abundance, particularly within NTS subnuclei that receive baroreceptor afferents, but comparable agreement has not been reached regarding delta receptors.

Physiological studies also provide ample evidence that NTS mu receptors serve an important role in cardioregulation. Thus, selective mu receptor agonists, such as D-Ala^2-MePhe^4-Gly-ol^5 enkephalin (DAGO) have repeatedly been shown to increase mean arterial pressure (MAP) and heart rate following both intraventricular (icv)[67,68] and intra-NTS[69-71] injection. This response is reversed by the selective mu receptor antagonist, β-funaltrexamine,[67] as well as naloxone[69,71] and is reproducible in both conscious[67] and artificially ventilated anesthetized rats.[70] The delta receptor agonist, D-Ala^2-D-Leu^5 enkephalin (DADLE) also induces a pressor response, but at a 10- to 100-fold lower potency than DAGO, suggesting that it, too, may act through mu rather than delta receptors.[68,69] DAGO also significantly attenuates the baroreflex depressor response evoked by aortic nerve stimulation consistent with the reported localization of mu receptors on vagal nerve afferents.[66] Hence, these studies clearly indicate that mu and perhaps delta agonists elevate MAP and heart rate by activating receptors in the NTS.

In contrast, β-endorphin has consistently been shown to produce hypotension and bradycardia

when injected icv,[72,73] intracisternally (ic),[74] or directly into the caudal NTS.[75,76] The response is reversed by NTS naloxone,[75] as well as β-funaltrexamine injection,[76] evidence that mu receptors mediate the response. Hypotension and bradycardia are induced by quite low β-endorphin doses — 280 fmol is maximally effective — although higher doses, 3 pmol or more, induce a pressor response.[75,76] Petty and de Jong[75] have further shown that NTS met-enkephalin injection also produces a pressor response, concluding that mu receptors mediate the depressor and delta receptors the pressor effects of β-endorphin.[75] This conclusion appears to be the fulcrum of an ongoing controversy because, of course, other investigators report that mu selective agents produce a pressor, rather than depressor response. The pressor response to DAGO occurs at higher NTS doses (30 pmol or more), however,[70,71,77] implying that DAGO may generate its effects by activating delta receptors.[73] Alternatively, Hassen et al. hypothesized that the hypotensive response to β-endorphin may be mediated by the putative epsilon receptor.[77] As discussed previously, β-endorphin-induced antinociception has also been attributed to epsilon receptors, in part, because it is not reversed by the mu selective antagonist, β-funaltrexamine.[47] A similar receptor does not appear to account for β-endorphin's cardioregulatory actions, however, which β-funaltrexamine effectively blocks.[76] It remains plausible that a different nonopioid binding site may be involved, although this will remain unresolved until more thorough dose-response studies are conducted with selective mu and delta agonists.

B. CARDIORESPIRATORY EFFECTS OF DYNORPHIN PEPTIDES

The cardioregulatory actions of dynorphin have been little studied and its precise role remains to be fully elucidated. Dynorphin-1-13, injected either i.c.[78] or i.c.v.[73] produces a prolonged fall in blood pressure and heart rate, consistent with evidence that kappa receptor binding sites are localized in the NTS and other brainstem nuclei.[64,66] Whether dynorphin acts within the NTS remains an open question, however, for NTS dynorphin-1-13 injection produces only minor, if any reductions in MAP.[77,79] Moreover, selective kappa receptor agonists induce a dose-related hypotension when injected into the NTS of spontaneously breathing rats, but not in artificially ventilated animals, indicating that the hypotensive response is secondary to respiratory depression.[79] Nucleus ambiguus injection remains effective, however, suggesting that this may be the site of action for i.c.v. dynorphin. Hence, based on these findings, kappa selective dynorphin peptides do not appear to serve an important cardioregulatory role in the NTS.

Nevertheless, shorter dynorphin peptides may yet be active at NTS mu or delta receptors. For example, NTS dynorphin-1-9 injection elevates MAP,[80] consistent with its relatively high affinity for mu and/or delta receptors.[35,37,56] Furthermore, C-terminally shortened dynorphin peptides appear to predominate in the NTS; for example, the molar ratio of dynorphin-1-8:dynorphin A is 1.7,[26] suggesting that dynorphin-1-8, which has some affinity for mu receptors,[57] is the predominant form released within the nucleus. Thus, although thorough structure activity studies have not as yet been conducted, analysis of the physiological role of dynorphin neurons in the NTS must consider the differing receptor selectivities of the dynorphin forms actually released within the nucleus.

V. OPIOID PEPTIDE PROCESSING AND CARDIOREGULATION

Clearly, differential posttranslational processing generates a spectrum of opioid peptides with significant affinity for mu, delta, and kappa receptors. Few investigations have comprehensively evaluated the cardioregulatory actions of the peptide end products actually synthesized in the caudal medulla, however. The paucity of structure-activity studies is particularly apparent for C-terminally extended enkephalin and C-terminally shortened dynorphin peptides although their actions may be predicted with some certainty based on their differing affinities for mu, delta, and kappa receptors. β-Endorphin peptides have been more thoroughly evaluated; the results of these studies emphasize the importance of conducting thorough structure-activity experiments and further suggest that the hemodynamic actions of opioid peptides can not always be accurately predicted from theoretical considerations.

These studies revealed that β-endorphin-1-31 is not alone in exhibiting cardioregulatory activity; β-endorphin-1-27 also acts as a potent hypotensive agent.[73,74] Indeed, β-endorphin-1-27 is even more potent than β-endorphin-1-31 after ic injection[74] (Figure 2) although the two peptides appear to be equipotent following icv administration.[73] Ic β-endorphin-1-27 is also more potent than the parent peptide in eliciting bradycardia and in stimulating adrenal medullary catecholamine secretion.[74] The equivalent, if not greater, cardioregulatory potency of β-endorphin-1-27 compared to β-endorphin-1-31 stands in marked contrast to what one might predict from studies of antinociception, in which

Peptide	Analgesia	Blood Pressure
●β-End-1-31	Agonist	Agonist
●β-End-1-27	Antagonist	Agonist
●β-End-1-26	Inactive	Inactive
Ac-β-End-1-31	Inactive	Inactive
●Ac-β-End-1-27		
Ac-β-End-1-26		

Figure 2 The posttranslational processing of β-endorphin differentially alters its analgesic and hemodynamic potency. (From Hirsch, M.D. and Millington, W.R., *Brain Res.*, 550, 61, 1991. With permission.)

β-endorphin-1-27 is a considerably less potent agonist, but acts as an antagonist.[41,46] Consistent with cardiovascular studies, however, β-endorphin-1-27 appears to be more potent than its precursor in the central mediation of certain behavioral syndromes.[82]

Further posttranslational modifications generate a markedly different outcome, greatly reducing, if not abolishing hemodynamic potency. Hence, N-acetylation virtually eliminates the hemodynamic activity of both β-endorphin-1-31 and β-endorphin-1-27;[73,74] analgesic activity is similarly affected.[46] Proteolytic conversion of β-endorphin-1-27 to β-endorphin-1-26, likewise abolishes the peptide's hemodynamic potency.[73,74] Thus, both β-endorphin-1-31 and β-endorphin-1-27 are potent hypotensive agents but further C-terminal proteolysis, as well as N-acetylation, essentially abolishes this activity.

Shorter β-endorphin fragments, including α- and γ-endorphin, also induce a transient, naloxone reversible decrease in heart rate, but no change in MAP, following icv injection.[73] Following NTS injection, however, α-endorphin, like β-endorphin, produces a U-shaped dose-response curve with lower doses inducing a fall in MAP; conversely, γ-endorphin produces a dose-related pressor effect.[83] Still smaller fragments, including β-endorphin-6-16, -10-16, -6-17, -10-17, as well as des-tyrosine α- and γ-endorphin, induce a rise in MAP which is not affected by naloxone pretreatment.[83] Thus, the β-endorphin sequence appears to contain an opioid receptor mediated depressor region, dependent upon an intact N-terminal, as well as a pressor region located in the midportion of the molecule.[81]

Biochemical analyses also support the concept that β-endorphin-1-31 and β-endorphin-1-27 function endogenously in central cardioregulation. Both peptides have been isolated from the rat dorsal caudal medulla, although β-endorphin-1-31 appears to be the predominant form; β-endorphin-1-26 is evidently not expressed.[32] Surprisingly, however, N-acetylated β-endorphin-1-31 and β-endorphin-1-27 are also abundant. Indeed, 52% of the β-endorphin peptides expressed in the caudal medulla are N-acetylated and thus appear to serve no known role in cardiorespiratory control. This implies that β-endorphin may be selectively acetylated within a specific anatomic pathway, presumably the intrinsic NTS cell group,[32] thereby imparting anatomical specificity to the β-endorphin peptides released within the caudal medulla. Conceivably, N-acetylation may serve as an inactivating mechanism promoting the action of other POMC peptides; γ-MSH, for example, induces a pressor response when centrally injected.[84]

The receptor mediating the cardioregulatory actions of β-endorphin-1-27 has yet to be identified. An opioid receptor appears to be involved because, like β-endorphin-1-31, its effects are naloxone reversible,[74] although β-endorphin-1-27 has little affinity for classical opioid receptors, retaining only about 30% of the opioid binding activity of β-endorphin-1-31.[46] The relative hypotensive potency of the two peptides is consistent with their affinity for the putative epsilon receptor, for which β-endorphin-1-27 displays a slightly greater affinity than β-endorphin-1-31, although the structural

requirements of the two receptors clearly differ because β-endorphin-1-26, which lacks hemodynamic potency, retains activity for the putative epsilon receptor.[19] Alternatively, the mu2 opioid receptor subtype has been proffered as a putative cardioregulatory site.[85] Thus, the receptor(s) responsible for the cardioregulatory actions of β-endorphin peptides remains unknown, although the aggregate results certainly imply that multiple binding sites may be involved.

VI. SUMMARY AND CONCLUSIONS

Substantial progress has been made during the past two decades towards understanding the neuronal mechanisms underlying the cardiorespiratory depression induced by opiate drugs. We now know that all three opioid peptide genes are expressed by neurons in the NTS whose efferent projections evidently participate in the short- and long-loop baroreflex circuits that interconnect the NTS with other medullary as well as forebrain cardioregulatory sites. Moreover, at least certain opioid peptides reproduce the hypotensive and bradycardic effects of opiates when centrally administered. Nevertheless, our understanding of the physiologic role of opioid peptides in the maintenance of cardiovascular homeostasis remains inconclusive. This is due, in part, to contradictions in the published literature, although disparate results may be inherent to any research area so complicated by methodologic variables.[86] Conceptual considerations also limit our ability to predict exactly how opioid peptides function endogenously. Much of our current data base is derived from studies utilizing receptor-selective ligands, and as a result we know considerably more about the cardioregulatory function of opioid receptors than of opioid peptides. Opioid peptides are far from selective, however, and as discussed in this review, opioid neurons release multiple peptides with differing opioid receptor selectivities; indeed, certain opioid peptides display significant affinity for more than one opioid receptor. Hence, predicting their action requires a detailed understanding of both the peptides and receptors present within specific brain regions, but our knowledge regarding either parameter is far from complete. Furthermore, structure-function studies with β-endorphin peptides reveal that additional, nonclassical binding sites may mediate the effects of certain opioid peptide derivatives. The potent hypotensive action of β-endorphin-1-27, for example, is quite unexpected based on its limited affinity for classical opioid receptors. Conversely, posttranslational modifications can generate peptides with no known hemodynamic action, a point well illustrated by the N-acetylation of β-endorphin in the caudal medulla. And finally, neither the processing of opioid peptides[29] nor the expression of opioid receptors[76] are fixed entities but can be modified by pathological conditions, such as stress or hypertension, adding a whole new dimension to opioid peptide physiology.

Despite the difficulties involved in establishing the precise cardioregulatory function of opioid peptides, the attempt may ultimately be a worthy one. Several lines of evidence indicate that β-endorphin, and perhaps other opioid peptides, are involved in the mechanism of action of clonidine and other antihypertensive drugs, for example.[81] Moreover, understanding the role of posttranslational processing in defining the hemodynamic actions of opioid peptides may facilitate development of opiate drugs which lack cardiorespiratory depression, a long-sought aspiration of opiate pharmacologists.

REFERENCES

1. **Hall, L.,** The influence of the force of gravity on the circulation of the blood, *J. Physiol.*, 18, 15, 1895.
2. **Kosterlitz, H.W. and Hughes, J.,** Development of the concepts of opiate receptors and their ligands, in *Advances in Biochemical Psychopharmacology*, Vol. 18, Costa, E. and Trabucci, M., Eds., Raven Press, New York, 1978, 31.
3. **Cechetto, D.F.,** Central representation of visceral function, *Fed. Proc.*, 46, 17, 1987.
4. **Palkovits, M.,** Neuronal circuits in central baroreceptor mechanism, in *Progress in Hypertension*, Vol. 1, Saito, H., Parvez, H., Parvez, S. and Nagatsu, T., Eds., VSP, Utrecht, 1988, 387.
5. **Barraco, R., El-Ridi, M., Ergene, E., Parizon, M. and Bradley, D.,** An atlas of the rat subpostremal nucleus tractus solitarius, *Brain Res. Bull.*, 29, 703, 1992.
6. **Chalmers, J.P., Badoer, E., Morilak, D., Drolet, G., Minson, J.B., Llewellyn-Smith, I.J., Somogyi, P., Kapoor, V., and Pilowsky, P.,** Afferent inputs to ventrolateral medulla, in *Central Neural Mechanisms in Cardiovascular Regulation*, Kunos, G. and Ciriello, J., Eds., Birkhäuser, Boston, 1991, 3.
7. **Khachaturian, H., Lewis, M.E., Tsou, K., and Watson, S.J.,** β-Endorphin, α-MSH, ACTH, and related peptides, in *Handbook of Chemical Anatomy*, Vol. 4, Björklund, A. and Hökfelt, T., Eds., Elsevier, Amsterdam, 1985, 216.

8. Palkovits, M., Mezey, E., and Eskay, R.L., Pro-opiomelanocortin derived peptides (ACTH/β-endorphin/α-MSH) in brainstem baro-receptor areas of the rat, *Brain Res.*, 436, 323, 1987.
9. Joseph, S.A. and Michael, G.J., Efferent ACTH-IR opiocortin projections from nucleus tractus solitarius. A hypothalamic deafferentation study, *Peptides*, 9, 193, 1988.
10. Yamazoe, M., Shiosaka, S., Shibasaki, T., Ling, N., Tateishi, K., Hashimura, E., Hamaoka, T., Kimmel, J.R., Matsuo, H., and Tohyama, M., Distribution of six neuropeptides in the nucleus tractus solitarii of the rat: an immunohistochemical analysis, *Neuroscience*, 13, 1243, 1984.
11. Bronstein, D.M., Schafer, M.K.-H., Watson, S.J., and Akil, H., Evidence that β-endorphin is synthesized in cells in the nucleus tractus solitarius: detection of POMC mRNA, *Brain Res.*, 587, 269, 1992.
12. Otake, K., Ezure, K., Lipski, J., and She, R.B.W., Projections from the commissural subnucleus of the nucleus of the solitary tract. An anterograde tracing study in the cat, *J. Comp. Neurol.*, 324, 365, 1992.
13. Fallon, J.H. and Leslie, F.M., Distribution of dynorphin and enkephalin peptides in the rat brain, *J. Comp. Neurol.*, 249, 293, 1986.
14. Riche, D., De Pommery, J., and Menetrey, D., Neuropeptides and catecholamines in efferent projections of the nuclei of the solitary tract in the rat, *J. Comp. Neurol.*, 293, 399, 1990.
15. Pickel, V.M., Chan, J., and Milner, T.A., Ultrastructural basis for interactions between central opioids and catecholamines. II. Nuclei of the solitary tracts, *J. Neurosci.*, 9, 2519, 1989.
16. Maley, B.E. and Panneton, W.M., Enkephalin-immunoreactive neurons in the nucleus tractus solitarius project to the parabrachial nucleus of the cat, *Brain Res.*, 442, 340, 1988.
17. Sawchenko, P.E., Arias, C., and Bittencourt, J.C., Inhibin β, somatostatin and enkephalin immunoreactivities coexist in caudal medullary neurons that project to the paraventricular nucleus of the hypothalamus, *J. Comp. Neurol.*, 291, 269, 1990.
18. Morilak, D.A., Somogyi, P., McIlhinney, R., and Chalmers, J., An enkephalin-containing pathway from nucleus tractus soilitarius to the pressor area of the rostral ventrolateral medulla of the rabbit, *Neuroscience*, 31, 187, 1989.
19. Milner, T.A., Pickel, V.M., and Reis, D.J., Ultrastructural basis for interactions between central opioids and catecholamines. I. Rostral ventrolateral medulla, *J. Neurosci.*, 9, 2114, 1989.
20. Millhorn, D.E., Seroogy, K., Hökfelt, T., Schmued, L.C., Terenius, L., Buchan, A., and Brown, J.C., Neurons of the ventral medulla oblongata that contain both somatostatin and enkephalin immunoreactivities project to nucleus tractus solitarii and spinal cord, *Brain Res.*, 424, 99, 1987.
21. Sawchenko, P.E. and Swanson, L.W., Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat, *J. Comp. Neurol.*, 205, 260, 1982.
22. Khachaturian, H., Watson, S.J., Lewis, M.E., Coy, D., Goldstein, A., and Akil, H., Dynorphin immunocytochemistry in the rat central nervous system, *Peptides*, 3, 941, 1982.
23. Lee, H.S. and Basbaum, A.I., Immunoreactive pro-enkephalin and pro-dynorphin products are differentially distributed within the nucleus of the solitary tract of the rat, *J. Comp. Neurol.*, 230, 614, 1984.
24. Fodor, M., Csiffáry, A., Kiss, P., and Palkovits, M., Dynorphin A-containing neural elements in the nucleus of the solitary tract of the rat. Light and electron microscopic immunohistochemistry, *Brain Res.*, 522, 251, 1990.
25. Zamir, N., Palkovits, M., and Brownstein, M.J., Distribution of immunoreactive β-neo-endorphin in discrete areas of the rat brain and pituitary gland: comparison with α-neo-endorphin, *J. Neurosci.*, 4, 1248, 1984.
26. Zamir, N., Palkovits, M., and Brownstein, M.J., Distribution of immunoreactive dynorphin A_{1-8} in discrete nuclei of the rat brain: Comparison with dynorphin A, *Brain Res.*, 307, 61, 1984.
27. Höllt, V., Opioid peptide processing and receptor selectivity, *Annu. Rev. Pharmacol. Toxicol.*, 26, 59, 1986.
28. Evans, C.J., Hammond, D.L., and Frederickson, R.C.A., The opioid peptides, in *The Opiate Receptors*, Pasternak, G.W., Ed., Humana Press, Clifton, NJ, 1988, 23.
29. Smith, A.I. and Funder, J.W., Proopiomelanocortin processing in the pituitary, central nervous system, and peripheral tissues, *Endocr. Rev.*, 9, 159, 1988.
30. Loh, Y.P., Molecular mechanisms of β-endorphin biosynthesis, *Biochem. Pharmacol.*, 44, 843, 1992.
31. Zakarian, S. and Smyth, D.G., Distribution of β-endorphin-related peptides in rat pituitary and brain, *Biochem. J.*, 202, 561, 1982.

32. **Dores, R.M., Jain, M., and Akil, H.,** Characterization of the forms of β-endorphin and alpha-MSH in the caudal medulla of the rat and guinea pig, *Brain Res.*, 377, 251, 1986.
33. **Emeson, R.B. and Eipper, B.A.,** Characterization of pro-ACTH/endorphin-derived peptides in rat hypothalamus, *J. Neurosci.*, 6, 837, 1986.
34. **Millington, W.R. and Smith, D.L.,** The post-translational processing of β-endorphin in human hypothalamus, *J. Neurochem.*, 57, 775, 1991.
35. **Garzón, J., Sánchez-Blázquez, P., Höllt, V., Lee, N.M., and Loh, H.H.,** Endogenous opioid peptides: comparative evaluation of their receptor affinities in the mouse brain, *Life Sci.*, 33, (Suppl. I), 291, 1983.
36. **Houghten, R.A., Johnson, N., and Pasternak, G.W.,** [^{3}H]-β-endorphin binding in rat brain, *J. Neurosci.*, 4, 2460, 1984.
37. **Quirion, R. and Weiss, A.S.,** Peptide E and other proenkephalin-derived peptides are potent kappa opiate receptor agonists, *Peptides*, 4, 445, 1983.
38. **Shook, J.E., Kazmierski, W., Wire, W.S., Lemcke, P.K., Hruby, V.J., and Burks, T.F.,** Opioid receptor selectivity of β-endorphin *in vitro* and *vivo*: Mu, delta and epsilon receptors. *J. Pharmacol. Exp. Ther.*, 246, 1018, 1988.
39. **Huidobro-Toro, J.P., Caturay, E.M., Ling, N., Lee, N.L., Loh, H.H., and Way, E.L.,** Studies on the structural prerequisites for the activation of the β-endorphin receptor on the rat vas deferens, *J. Pharmacol. Exp. Ther.*, 222, 262, 1982.
40. **Sheehan, M.J., Hayes, A.G., and Tyers, M.B.,** Lack of evidence for ε-opioid receptors in the rat vas deferens, *Eur. J. Pharmacol.*, 154, 237, 1988.
41. **Suh, H.H., Tseng, L.F., and Li, C.H.,** β-Endorphin-(1-27) antagonizes β-endorphin- but not morphine-, D-Pen^{2}D-Pen^5-enkephalin- and U50,488H-induced analgesia in mice, *Neuropharmacology*, 27, 957, 1988.
42. **Carr, D.J.J.,** The role of endogenous opioids and their receptors in the immune system, *Proc. Soc. Exp. Biol. Med.*, 198, 710, 1991.
43. **Goldstein, A. and James, I.F.,** Site-directed alkylation of multiple opioid receptors. II. Pharmacological selectivity, *Mol. Pharmacol.*, 25, 343, 1984.
44. **Tseng, L.F. and Collins, K.A.,** The tail-flick inhibition induced by β-endorphin administered intrathecally is mediated by activation of K- and μ-opioid receptors in the mouse, *Eur. J. Pharmacol.*, 214, 59, 1992.
45. **Deakan, J.F., Doströvsky, J.O., and Smyth, D.J.,** Influence of N-terminal acetylation and C-terminal proteolysis on the analgesic activity of β-endorphin, *Biochem. J.*, 189, 501, 1980.
46. **Nicolas, P. and Li, C.H.,** β-Endorphin-(1-27) is a naturally occurring antagonist to etorphine-induced analgesia, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 3178, 1985.
47. **Suh, H.H. and Tseng, L.F.,** Intrathecal β-funaltrexamine antagonizes intracerebroventricular β-endorphin- but not morphine-induced analgesia in mice, *J. Pharmacol. Exp. Ther.*, 245, 587, 1988.
48. **Bucher, B., Stoclet, J.-C., and Illes, P.,** Further evidence for the existence of a homogenous β-endorphin-sensitive receptor population in the rat tail artery, *Eur. J. Pharmacol.*, 147, 305, 1988.
49. **De Wied, D. and Jolles, J.,** Neuropeptides derived from pro-opiocortin. Behavioral, physiological and neurochemical effects, *Physiol. Rev.*, 62, 976, 1982.
50. **Sánchez-Blázquez, P. and Garzón, J.,** Further characterization of $^{\alpha}$N-acetyl β-endorphin-(1-31) regulatory activity. I. Effect on opioid- and α_2-mediated supraspinal antinociception in mice, *Life Sci.*, 50, 2083, 1992.
51. **Ellerkmann, E., Porter, T.E., Nagy, G.M., and Frawley, L.S.,** N-acetylation is required for the lactotrope recruitment activity of α-melanocyte-stimulating hormone and β-endorphin, *Endocrinology*, 131, 566, 1992.
52. **Höllt, V., Haarmann, I., Grimm, C., Herz, A., Cankat Tulunay, F., and Loh, H.H.,** Proenkephalin intermediates in bovine brain and adrenal medulla: Characterization of immunoreactive peptides related to BAM-22P and peptide F, *Life Sci.*, 31, 1883, 1982.
53. **White, J.D., Gall, C.M., and McKelvey, J.F.,** Proenkephalin is processed in a projection-specific manner in the rat central nervous system, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 7099, 1986.
54. **Baird, A., Ling, N., Böhlen, P., Benoit, R., Klepper, R., and Guillemin, R.,** Molecular forms of the putative enkephalin precursor BAM-12P in bovine adrenal, pituitary and hypothalamus, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 2023, 1982.
55. **Weber, E., Esch, F.S., Böhlen, P., Paterson, S., Corbett, A.D., McKnight, A.T., Kosterlitz, H.W., Barchas, J.D., and Evans, C.J.,** Metorphamide: Isolation, structure, and biologic activity of a novel amidated opioid octapeptide from bovine brain, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 7362, 1983.

56. **Wüster, M., Rubini, P., and Schulz, R.,** The preference of putative pro-enkephalins for different types of opiate receptors, *Life Sci.*, 29, 1219, 1981.
57. **James, I.F., Fischli, W., and Goldstein, A.,** Opioid receptor selectivity of dynorphin gene products, *J. Pharmacol. Exp. Ther.*, 228, 88, 1984.
58. **Höllt, V., Sánchez-Blázquez, P., and Garzón, J.,** Multiple opioid ligands and receptors in the control of nociception, *Philos. Trans. R. Soc. London, Ser. B*, 308, 299, 1985.
59. **Hollt, V., Cankat Tulunay, F., Woo, S.K., Loh, H.H., and Herz, A.,** Opioid peptides derived from pro-enkephalin A but not that from pro-enkephalin B are substantial analgesics after administration into brain of mice, *Eur. J. Pharmacol.*, 85, 355, 1982.
60. **Zamir, N., Palkovits, M., Weber, E., Mezey, E., and Brownstein, M.J.,** A dynorphinergic pathway of leu-enkephalin production in rat substantia nigra, *Nature*, 307, 643, 1984.
61. **Seizinger, B.R., Grimm, C., Höllt, V., and Herz, A.,** Evidence for a selective processing of proenkephalin B into different opioid peptide forms in particular regions of rat brain and pituitary, *J. Neurochem.*, 42, 447, 1984.
62. **Van Giersbergen, P.L.M., Palkovits, M., and de Jong, W.,** Involvement of neurotransmitters in the nucleus tractus solitarii in cardiovascular regulation, *Physiol. Rev.*, 72, 789, 1992.
63. **Ramabadran, K.,** Cardiovascular effects of opioid peptides, in *Opioid Peptides. Biochemistry and Applied Physiology*, Vol. 4, Szekely, J.I. and Ramabadran, K., Eds., CRC Press, Boca Raton, FL, 1990, 289.
64. **Sales, N., Riche, D., Roques, B.P., and Denavit-Saubie, M.,** Localization of μ- and δ-opioid receptors in cat respiratory areas: an autoradiographic study, *Brain Res.*, 344, 382, 1985.
65. **Mansour, A., Khachaturian, H., Lewis, M.E., Akil, H., and Watson, S.J.,** Anatomy of CNS opioid receptors, *Trends Neurosci.*, 11, 308, 1988.
66. **Dashwood, M.R., Muddle, J.R., and Spyer, K.M.,** Opiate receptor subtypes in the nucleus tractus solitarii of the cat: the effect of vagal section, *Eur. J. Pharmacol.*, 155, 85, 1988.
67. **Pfeiffer, A., Feuerstein, G., Zerbe, R.L., Faden, A.I., and Kopin, I.J.,** μ-Receptors mediate opioid cardiovascular effects at anterior hypothalamic sites through sympathoadrenomedullary and parasympathetic pathways, *Endocrinology*, 113, 929, 1983.
68. **Gordon, F.J.,** Central opioid receptors and baroreflex control of sympathetic and cardiovascular function, *J. Pharmacol. Exp. Ther.*, 237, 428, 1986.
69. **Hassen, A.H., Feuerstein, G., and Faden, A.I.,** μ Receptors and opioid cardiovascular effects in the NTS of rat, *Peptides*, 3, 1031, 1982.
70. **Hassen, A.H. and Feuerstein, G.,** μ-Opioid receptors in NTS elicit pressor responses via sympathetic pathways, *Am. J. Physiol.*, 252, H156, 1987.
71. **Gordon, F.J.,** Opioids and central baroreflex control: a site of action in the nucleus tractus solitarius, *Peptides*, 11, 305, 1990.
72. **Sitsen, J.M.A., van Ree, J.M., and de Jong, W.,** Cardiovascular and respiratory effects of β-endorphin in anesthetized and conscious rats, *J. Cardiovasc. Pharmacol.*, 4, 883, 1982.
73. **Van Giersbergen, P.L.M., de Lang, H., and de Jong, W.,** Effects of dynorphin A(1-13) and fragments of β-endorphin on blood pressure and heart rate of anesthetized rats, *Can. J. Physiol. Pharmacol.*, 69, 327, 1991.
74. **Hirsch, M.D. and Millington, W.R.,** Endoproteolytic conversion of β-endorphin-1-31 to β-endorphin-1-27 potentiates its central cardioregulatory activity, *Brain Res.*, 550, 61, 1991.
75. **Petty, M.A. and de Jong, W.,** Cardiovascular effects of β-endorphin after microinjection into nucleus tractus solitarii of the anesthetized rat, *Eur. J. Pharmacol.*, 81, 449, 1982.
76. **Mosqueda-Garcia, R. and Kunos, G.,** Opiate receptors and the endorphin-mediated cardiovascular effects of clonidine in rats: evidence for hypertension-induced μ-subtype to δ-subtype changes, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 8637, 1987.
77. **Hassen, A.H., Feuerstein, G., and Faden, A.I.,** Cardiovascular responses to opioid agonists injected into the nucleus of tractus solitarius of anesthetized cats, *Life Sci.*, 31, 2193, 1982.
78. **Laurent, S. and Schmitt, H.,** Central cardiovascular effects of K agonists dynorphin-(1-13) and ethylketocyclazocine in the anaesthetized rat, *Eur. J. Pharmacol.*, 96, 165, 1983.
79. **Hassen, A.H., Feuerstein, G., and Faden, A.I.,** Kappa opioid receptors modulate cardiorespiratory function in hindbrain nuclei of rat, *J. Neurosci.*, 4, 2213, 1984.
80. **Carter, D.A. and Lightman, S.L.,** α-MSH exhibits opioid antagonist-like effects in the brainstem of rats, *Peptides*, 8, 1173, 1987.

81. **Van Giersbergen, P.L.M. and de Jong, W.,** Neuro-opioid peptides and blood pressure control, in *Neuropeptides: Basics and Perspectives*, de Wied, D., Ed., Elsevier, New York, 1990, 277.
82. **Hirsch, M.D. and O'Donohue, T.L.,** Structural modifications of pro-opiomelanocortin-derived peptides alter their behavioral effects markedly, *J. Pharmacol. Exp. Ther.*, 237, 378, 1986.
83. **Petty, M.A., de Jong, W., and de Wied, D.,** The blood pressure changes resulting from the microinjection of fragments of the beta endorphin molecule into the nucleus tractus solitarii (NTS), *Life Sci.*, 33, (Suppl. I), 735, 1983.
84. **Gruber, K.A. and Callahan, M.F.,** ACTH-(4-10) through gamma-MSH: evidence for a new class of central autonomic nervous system-regulating peptides, *Am. J. Physiol.*, 257, R681, 1989.
85. **Pasternak, G.W. and Wood, P.J.,** Multiple mu opiate receptors, *Life Sci.*, 38, 1889, 1986.
86. **Feuerstein, G.,** The opioid system and central cardiovascular control. Analysis of controversies, *Peptides*, 6, (Suppl. 2), 51, 1985.

Nucleus of the Solitary Tract

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