REPORT DOCUMENTATION PAGE

AD-A247 044

Form Approved
OMB No. 0704-0188

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1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED: FINAL 01 Dec 88 TO 30 Nov 91

4. TITLE AND SUBTITLE
MEASUREMENT AND REGULATION OF CENTRAL NORADRENERGIC NEURTRANSMISSION.

5. FUNDING NUMBERS
AFOSR-89-0208
PE - 61102F
PR - 2312
TA - A2

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10. SPONSORING / MONITORING AGENCY REPORT NUMBER

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MAR 09 1992
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11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Unlimited
This document has been approved for public release and sale; its distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Over the period of the AFOSR grant we have made significant progress in clarifying the relationship between the noradrenergic system and IEG expression in the brain. A series of studies has been performed in which the effects of activation of the noradrenergic system by drugs or stress on activation of the IEG response was examined. Results strongly suggest that the noradrenergic system is involved in the activation of IEGs caused by physiological as well as pharmacological agents. Whether this is the only neuronal system involved is not yet clear as stress, yohimbine and propranolol are known to affect other neurotransmitters and their receptors in the brain. This problem will be addressed in future research. In conjunction with our biochemical studies of noradrenergic neurotransmission we have also sought to identify the postsynaptic cells in which these biochemical events accur so as to facilitate studies of long term changes caused by stress. We have made substantial progress in this area and have obtained evidence that there are 2 separate target cells, a glial cell in which cyclic AMP is synthesized in response to beta receptor activation and a neuronal cell in which the immediate early genes are produced again in response to beta receptor activation.

14. SUBJECT TERMS

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
(U)	(U)	(U)	)U)

AFOSR 89-0208 - Stone, E.A.

Final Technical Report (12/1/88-11/30/91)

Significant progess toward the goals of the project was made in three areas concerning the neurobiology of the central noradrenergic neuronal system. These involved 1) studies of immediate early gene activation by the noradrenergic system, 2) identification of the cell type of postsynaptic noradrenergic target cells, and 3) methodological advances in the measurement of noradrenergic neurotransmission involving the microdialytic collection of brain cAMP.

1) Immediate early gene (IEG) activation in cerebral cortex by noradrenergic system

Over the period of the AFOSR grant we have made significant progress in clarifying the relationship between the noradrenergic system and IEG expression in the brain. A series of studies has been performed in which the effects of activation of the noradrenergic system by drugs or stress on activation of the IEG response was examined.

In the initial study, the drug yohimbine was used to cause the release of brain NE by blockade of inhibitory presynaptic alpha-2 adrenoceptors (Bing et al., 1991 (1). Five IEGs as well as three non-IEGs were examined in cortical and hippocampal tissues. Standard methods of RNA preparation and "Northern blots" were used. Yohimbine was found to produce marked increases (4-8 fold) in all 5 IEGs examined (c-fos, nur77, zif-268, tis-7 and tis-21) but not in genes that are not IEG (HSP70, beta NGF, beta actin). In agreement with previous findings on these genes, the increases were transient, peaking at 60 min after injection.

In order to determine if yohimbine administration also increases the protein products as well as mRNA levels of these genes, we examined brain sections for the c-fos and zif-268 proteins by immunohistochemistry. We found marked increases of c-fos-like- and zif-268-like-immunoreactivity widely distribution throughout the CNS (Bing et al., submitted (2)). This indicated that the response involves both the mRNA as well as protein products of these genes.

We next looked at the ability of physiological stimuli that release brain NE to activate the IEGs. A variety of stressful stimuli were found effective in this regard. A simple i.p. injection of saline was stressful enough to cause a 2-3 fold elevation in the mRNA of all 5 IEGs in the cortex. Since this effect tended to be variable we also examined restraint stress, for 10 or 60 min. The latter stress produced a dose-dependent activation of 4 out of 4 tested IEGs with the 60 min restraint effect being as potent as the yohimbine effect (Bing et al., 1991 (1)).

The above experiments utilized drugs or physiological stimuli with complex effects to elicit increases in central noradrenergic activity. To determine if the selective stimulation of the cerebral cortex with NE would also be effective, we infused a solution of exogenous NE directly into the cortex and then examined for c-fos protein immunohistochemically. The infusion was accomplished atraumatically by the use of intracerebral microdialysis. It was found that NE infused dialytically produced a marked increase in c-fos-like immunoreactivity in the vicinity of the probe (Stone et

al., 1991 (12)). This further supported the notion that the response was controlled by the noradrenergic system.

To determine if adrenergic receptors were involved in these responses the effect of blocking agents were examined (Bing et al., 1991 (1)). dl-Propranolol, 10 mg/kg, a beta adrenoceptor antagonist, produced significant decreases in the mRNA of 3/3 tested genes that were activated by yohimbine (c-fos, nur77, tis-21) and of 3/3 tested genes that were activated by restraint or injection stress (c-fos, nur77 and zif-268). The alpha-1 antagonist, prazosin, 5 mg/kg, produced a smaller reduction in the responses of these same genes. Given together, propranolol and prazosin markedly attenuated the response of 4/4 tested IEGs to yohimbine injection (c-fos, nur77, zif-268 and tis-21). These data confirmed similar findings by others for c-fos (5) and extended them to the other IEGs.

These results have therefore strongly suggested that the noradrenergic system is involved in the activation of IEGs caused by physiological as well as pharmacological agents. Whether this is the only neuronal system involved is not yet clear as stress, yohimbine and propranolol are known to affect other neurotransmitters and their receptors in the brain. This problem will be addressed in future research.

2) Identification of postsynaptic cells affected by noradrenergic system

In conjunction with our biochemical studies of noradrenergic neurotransmission we have also sought to identify the postsynaptic cells in which these biochemical events occur so as to facilitate studies of long term changes caused by stress. We have made substantial progress in this area and have obtained evidence that there are 2 separate target cells, a glial cell in which cyclic AMP is synthesized in response to beta receptor activation and a neuronal cell in which the immediate early genes are produced again in response to beta receptor activation. These two target cells are discussed below.

a) glial-cAMP cell

The glial target cell was identified in biochemical studies using the gliotoxin, fluorocitrate (FC), to localize the cAMP response to beta adrenoceptor stimulation. The cAMP response was of interest to us because it is a direct response to beta receptor activation and also because of evidence that it is necessary for activation of certain IEGs responses. Previous research by others using cultured brain cells had shown that beta receptors and cAMP responses to beta stimulation are localized to astrocytes but not to neurons in the cerebral cortex (6,7,13). To investigate whether this was also true for the intact brain, we tested the ability of a gliotoxin, FC, to block the cAMP response to beta receptor stimulation in brain slices (a model system of "intact" brain tissue). It was found that FC virtually abolished the beta-cAMP response in various forebrain regions whereas a neurotoxin, kainic acid, had no effect (Stone et al., 1990 (11)). We verified that FC was selective for glia by demonstrating normal intracellular electrophysiological recordings from the neurons in the FC-treated slices (Stone et al., ibid).

To extend these findings to the brain in vivo we made use of a microdialysis procedure for detecting extracellular cAMP that we had developed

in the first year of the AFOSR grant. This microdialysis procedure enabled us both to stimulate brain beta adrenoceptors with catecholamines and to detect the efflux of cAMP in response to this stimulation (Egawa et al., 1988 (4); Stone & John, 1990 (8)). We found that infusion of FC virtually abolished the in vivo cAMP response just as it had the response in brain slices suggesting that the glia are the main sites of the cAMP in vivo as well as in brain slices (Stone & John (9)).

We have tentatively concluded from these indirect biochemical experiments that the glia represent a major target cell group of the noradrenergic system (Stone & John, ibid). We plan to obtain more direct evidence on this question with the use of immunohistochemical methods in the proposed studies.

b) neuronal-IEG cells

We next attempted to determine the cellular location of the IEGs responses to beta receptor activation and whether this was the same as or different from the cAMP response. In order to do this we employed an immunohistochemical procedure for the c-fos and zif-268 proteins in rats treated with yohimbine to release brain NE. It was found that yohimbine administration caused cells in many brain regions to stain positively for c-fos-like immunoreactivity and in preliminary experiments, for zif-268 immunoreactivity as well (Bing et al., submitted (2). Preliminary experiments have also indicated that this labeling occurs after administration of another alpha-2 receptor blocker, atipamezole. The immunoreactive cells have a broad distribution — spinal cord, medulla, pons, hypothalamus, thalamus, paleocortex and neocortex -- which parallels the widespread distribution of noradrenergic terminals in the brain.

Surprisingly, the cell type showing a positive reaction in the cerebral cortex appears to be neuronal rather than glial. The stained cells have a typical neuronal morphology (large rounded nucleus) and in double labeling experiments do not stain positively for the astrocytic protein, glial fibrillary acidic protein (GFAP). This appears to indicate that the IEG response to beta adrenoceptor stimulation occurs in a different cell type than the cAMP response with the former occurring in neurons and the latter occurring in glial cells. We currently hypothesize that the glial cell may represent the initial noradrenergic target cell which then transmits some signal, possibly cAMP, to neighboring neurons to activate IEGs. This hypothesis is based on previous findings in the literature of the ready release of cAMP from cultured astrocytes (7) and the apparent predominant localization of cAMP dependent protein kinase in neuronal cells (3). This hypothesis will be tested in future research using immunohistochemical methods for IEGs, cAMP and neuronal and astrocyte markers and a combined immunohistochemical-autoradiographic method for the localization of beta adrenoceptor binding sites. It will also be tested by determining if agents that inhibit glial function block the IEG responses of the neurons.

3) Methodological advances in the study of noradrenergic neurotransmission

In parallel with the above projects we have also pursued methodological studies aimed at developing new ways to measure noradrenergic neurotransmission at beta adrenoceptors in awake behaving rats. This methodology is needed to facilitate studies of the functional role of the noradrenergic system in adaptation to stress.

One technique that we have been working on is based on the use of microdialysis to both stimulate brain beta adrenoceptors in vivo with catecholamines and detect the cAMP released in response to this stimulation. We have made a number of advances with this technique.

One advance concerns the extension of the technique to unanesthetized rats. Over the past two years we have carried out extensive development of the procedure in awake rats. We have conducted NE-cAMP dose response curves, time course analyses and various pharmacological studies (Stone & John, 1990 (8)). These findings have indicated that the technique will be useful in studying various behavioral phenomena related to stress.

A second advance concerns the extension of the technique to the problem of IEG activation by noradrenergic stimuli. We have shown that infusion of NE through a microdialysis probe implanted in the prefrontal cortex produces a marked increase in c-fos-like immunoreactivity of cells in the vicinity of the probe (Stone et al., 1991 (12)). This increase could be blocked by prior infusion of the beta receptor blocker, timolol). Probes perfused with buffer alone showed almost no c-fos staining. These results indicate that the technique will enable us to examine the pharmacology of brain IEGs responses with a method that bypasses the blood brain barrier and subjects animals to very little stress.

The third advance concerns the application of the technique to the measurement of endogenous noradrenergic neurotransmission in the brain in vivo. In previous work we used only exogenous NE to stimulate cAMP formation. This past year, we have had preliminary success in stimulating cAMP release with a NE releasing drug, d-amphetamine (Stone & John, (10)). These results suggest that the technique is sensitive enough to detect endogenous neurotransmission in the brain and that it may be useful in various neuropharmacological and behavioral studies of this neuronal system.

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