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The work described here is part of an ongoing set of studies aimed at characterizing the physiological actions and anatomical organization of the monoaminergic projection systems to the rat cerebral cortex, cerebellum and hypothalamus. The underlying theme of this work is that the endogenous monoamines, norepinephrine (NE) and serotonin (5-HT), serve to modulate central neuronal responsiveness to afferent synaptic inputs and by so doing participate in the cognitive process of selective attention. Individual studies conducted during the past year have investigated: 1.) the adrenergic and amino acid receptor specificity of NE-induced facilitation of glutamate efficacy, 2.) transmembrane effects of NE on morphologically characterized neocortical neurons and 3.) the pharmacological specificity of cocaine actions on single cells in central neuronal circuits. Overall, the data provide further support for the contention that the diffusely distributed monoamine systems of the mammalian brain may enhance the performance of target neuronal circuits as a function of changing behavioral conditions.

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ANNUAL TECHNICAL REPORT

AFOSR-87-0138
"The Role of Central Monoaminergic Systems
in Arousal and Selective Attention."
4-1-88 to 3-31-89

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1. Summary

The major goal of ongoing studies has been to evaluate the role of the endogenous monoamines, norepinephrine (NE) and serotonin (5-HT), in the transfer of sensory information through neuronal networks of mammalian brain. Previously demonstrated modulatory actions of NE and 5-HT on single cell responses to synaptic inputs in the rat cerebral cortex, cerebellum and hypothalamus have suggested that these monoaminergic systems might enhance neuronal circuit function and participate in the cognitive process of selective attention. Individual studies conducted during the past year have focused on identifying the receptor-linked second messenger systems responsible for mediating noradrenergic modulatory effects. A recently initiated series of studies has started to examine the effects of NE on membrane properties of cortical neurons as determined from intracellular recordings. In these experiments individually recorded cells have been injected with Lucifer yellow and subsequently identified as layer II-VI pyramidal or stellate cells according to morphological criteria. Other studies have continued to characterize the effects of cocaine, a psychostimulant drug which elevates central synaptic levels of NE, on neurons in areas receiving noradrenergic projections. Overall, the studies conducted during this phase of the project have further elucidated the mechanisms through which synaptically released NE may enhance the performance of noradrenergic target neuronal circuits.

2. Research Objectives

The primary focus of the project has not deviated from the originally stated aims listed below; with the exception that considerable effort is now being directed toward identifying the transmembrane effects of NE on cortical neurons via intracellular recording studies. The goal of these latter studies is to elucidate the mechanisms through which NE may express its neuromodulatory actions on sensory signal processing.

Aim 1. Investigate the basic physiological actions of the noradrenergic and serotonergic projection systems in primary sensory areas of the mammalian brain. Initial studies will employ iontophoretic methods of drug application to characterize the elemental effects of NE and 5-HT on somatosensory or visual cortical neuronal responses to peripheral stimulation of afferent synaptic pathways or to microtiontophoresis of putative transmitter substances. In other studies, stimulation of the locus coeruleus or dorsal raphe nucleus will be employed to cause release of endogenous monoamines at anatomically relevant sites in target neuronal circuits and confirm results observed with iontophoretic application of NE and 5-HT. Similar studies will be carried out in other regions of the brain that relay sensory information and receive monoaminergic projections, e.g. lateral

geniculate nucleus, superior colliculus. The goal here will be to further develop the concept that NE and 5-HT operate in a neuromodulatory mode as part of a signal "gating" or "filtering" mechanism in primary sensory areas of the neocortex and other sensory information relay circuits of the brain. While these initial studies will be carried out in anesthetized rats, a major effort will be mounted to examine these issues in awake, behaving animals using recently developed techniques for chronic unit recording.

Aim 2. Analysis of the physiological actions of amphetamine and cocaine at the synaptic level in cerebrocortical and cerebellar circuits. The primary issues to be investigated here are whether these psychostimulant agents can mimic the facilitating actions of NE on neuronal responsiveness to synaptic inputs and putative transmitter substances and whether or not such effects correlate with the overt behavioral responses which have been reported for these drugs. The proposed experiments will employ the electrophysilogical assays developed previously for study of the NE system to determine the effects of these drugs on synaptic mechanisms. The merit of this approach derives from the fact that a common set of experimental neurophysiological tests can be used to examine the action of compounds which are known to interact with this system.

Aim 3. Examine the anatomical organization of monoamine-containing projection neurons with respect to sensory-specific target regions of the CNS. These investigations will employ single and double retrograde tracer techniques to study the distribution of monoamine-containing projection neurons with respect to sensory-modality specific target regions in the CNS. Initial studies using retrograde transport of HRP suggest that the monoamine nuclei have an internal organization such that activity in subsets of dorsal raphe and locus coeruleus cells may independently influence separate populations of neurons within serotonergic and noradrenergic terminal fields of the neocortex. Moreover, double-labeling protocols have revealed single dorsal raphe neurons which project to both rat visual cortex and cerebellar paraflocculus, areas which are known to receive visual information. The emphasis of the proposed studies will be to explore the possibility that central monoaminergic projections are organizaed according to the sensory function of target neuronal circuits and whether such an organization would be consistent with a postulated role of these systems in attentional mechanisms.

3. Status of Research

A brief description of individual studies (see also Publications Supported) conducted during the past year is provided below.

In Vitro Effects of NE on Glutamate Evoked Discharges of Cortical Neurons: A preliminary in vitro study in cerebrocortical tissue slices has demonstrated that NE can potentiate responses of individual neurons to threshold level iontophoretic doses of glutamate. This effect was routinely mimicked by phenylephrine and blocked by phentolamine suggesting that it was mediated through activation of an alpha type adrenoceptor. The beta agonist isoproterenol was ineffective in potentiating glutamate responses. In a few cases where NE was interacted with otherwise subthreshold iontophoretic doses of glutamate, robust excitatory discharges were revealed. Further study of interactions between NE and NMDA-evoked excitatory discharges have revealed modulatory effects similar to those observed with NE and glutamate.

In summary, these experiments have begun to characterize NE interactions with cortical neuronal responses to a specific cerebrocortical excitatory transmitter and its analogues. In addition, these experiments have revealed "gating" effects on unit responses to subthreshold iontophoretic doses of the same agent.

Mediation of NE Modulatory Effects by Second Messenger Systems: Several investigations aimed at identifying cellular mechanisms associated with noradrenergic modulatory actions are in progress. One in vivo study has demonstrated that a membrane permeant analog of 3',5' cyclic AMP can mimic the previously observed facilitating action of NE on GABA responses of Purkinje neurons. Other experiments conducted in cerebellar tissue slice preparations have confirmed this result and also shown that agents which increase intracellular levels of cyclic AMP, i.e. forskolin - (direct adenyl cyclase activation) and IBMX (phosphodiesterase inhibition), can also augment Purkinje cell responses to GABA. These results provide further evidence that NE augmentation of GABA efficacy in neuronal circuits may be mediated by activation of a beta type adrenergic receptor and subsequently increased levels of intracellular cyclic AMP.

Additional in vitro studies using cerebrocortical tissue slices have demonstrated that phorbol ester can mimic the facilitating effects of NE on glutamate evoked excitatory discharges and thus suggest that the phosphotide inositol/protein kinase C second messenger system may be involved in mediating noradrenergically-induced modulation of excitatory synaptic responses in the neocortex. One of the experimental strategies to be employed in ongoing studies is to interact agents which elevate intracellular levels of cAMP or activate protein kinase C with subthreshold and threshold level stimulation of afferent cortical pathways. These types of investigations are particularly exciting since they hold the promise of identifying specific signal transduction mechanism(s) which are capable of mediating the previously observed effects of NE on cortical neuronal responsiveness.

Psychostimulant Drug Studies: A number of studies were initiated previously to investigate the actions of the psychostimulant compound, cocaine, which is known to increase synaptic levels of NE. Essentially, the same electrophysiological assays used to define monoamine function in local neuronal circuits were employed to evaluate cocaine actions at central synapses. Using the cerebellar Purkinje cell as a model, we have observed that systemically and locally applied cocaine can augment inhibitory neuronal responses to microiontophoretically applied GABA. Cocaine has also been observed to enhance synaptically-evoked and glutamate-induced excitatory discharges in cerebrocortical and cerebellar neurons.

In the past year further specificity of these cocaine actions has been demonstrated. In animals pretreated with reserpine and alpha methyl-para-tyrosine to reduce endogenous stores of NE, cocaine was found to be ineffective in potentiating glutamate evoked discharges across a population of cerebellar Purkinje neurons. Thus, cocaine's facilitating effects on Purkinje cell responses to glutamate appear to be dependent upon endogenous NE. Overall, these results help to further characterize the electrophysiological actions of cocaine and suggest a link between this compound's psychostimulant effects and the increased sensory awareness and cognitive function which accompany the behavioral state of arousal and selective attention.

Transmembrane Effects of NE: Studies using intracellular recording procedures are now underway. The goal of these experiments is to determine which of the many possible membrane actions of NE correlates with noradrenergic modulatory effects on cerebrocortical neuronal responsiveness. Furthermore, cells recorded intracellularly will be injected with Lucifer yellow and identified by morphologic criteria. This approach will ultimately provide physiological and pharmacological data on an identified population of neocortical cells. Such information is needed in order to begin understanding the potential influence of the noradrenergic system on ensembles of functionally related cells in central neuronal circuits.

Comments on Progress: During the past year several previously initiated studies have been completed and manuscripts detailing the results of these experiments are being prepared for publication. For the coming year studies employing intracellular recording procedures in cortical tissue slice preparations will continue as well as investigations where the extracellular activity of one or more single somatosensory cortical units is recorded from awake, behaving animals while activating coeruleo-cortical and cutaneous cortical afferent inputs. These latter studies are ready to proceed following a period of development of experimental procedures. Studies investigating the effects of NE and 5HT on receptive field properties of visual cortical neurons will also be resuming following the development during the past year of a sophisticated set of computer programs for characterizing the response properties of visual neurons.

4. Publications Supported by AFOSR-87-0138, 1988-89.

Papers:

1. Sessler, F.M., Mouradian, R.D., Cheng, J-T., Liu, W., Yeh, H.H. and Waterhouse, B.D. 1989. Noradrenergic potentiation of cerebellar Purkinje cell responses to GABA: Evidence for mediation through the beta adrenoceptor-coupled cyclic AMP system. Brain Res. (In Press)

Abstracts:

1. Liu, W., Sessler, F.M., Lin, C.-S. and Waterhouse, B.D. 1989. Intracellular study of the effect of norepinephrine on somatosensory cortical neuronal response to GABA. Soc. Neurosci. Abst.

2. Mouradian, R.D., Sessler, F.M. and Waterhouse, B.D. 1989. Noradrenergic modulation of cortical neuronal excitability: pharmacological characterization in terms of adrenergic and amino acid receptor subtypes. Soc. Neurosci. Abst.

Manuscripts:

1. Waterhouse, B.D., Azizi, S.A., Burne, R.A. and Woodward, D.J. Modulation of rat cortical area 17 neuronal responses to moving visual stimuli during norepinephrine and serotonin microtiontophoresis. Brain Res. (Submitted)

2. Waterhouse, B.D., Stowe, Z.N., Jimenez-Rivera, C.A., Woodward, D.J. and Sessler, F.M. Cocaine actions in central noradrenergic circuits: enhancement of cerebellar Purkinje neuron responses to iontophoretically applied GABA. (In Preparation)

3. Jimenez-Rivera, C.A. and Waterhouse, B.D. Cocaine effects on synaptic transmission of afferent signals to rat somatosensory cortical neurons. (In Preparation).

4. Jimenez-Rivera, C.A. and Waterhouse, B.D. Cocaine actions on cerebellar neuronal responses to iontophoretically applied glutamate. (In Preparation).

5. Shin, H.-C., Jimenez-Rivera, C.A., Waterhouse, B.D. and Chapin, J.K. Cocaine-induced changes in sensory responsiveness of simultaneously recorded single neurons in the SI cortex of behaving rats. (In Preparation).

6. Waterhouse, B.D., Stowe, Z.N., Jimenez-Rivera, C.A., Sessler, F.M. and Woodward, D.J. Cocaine actions in central noradrenergic circuits: I. Enhancement of cerebellar Purkinje neuron responses to iontophoretically applied GABA.

5. Professional Personnel Directly Involved in AFOSR-87-0138

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6. Coupling Activities

N/A

7. New Discoveries, Inventions, etc.

N/A