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The overall goal of this research project is to systematically investigate a number of the possible ways through which presynaptic modulation might influence the effectiveness of local synaptic interactions at the mammalian hippocampal mossy fiber synapse. A hippocampal subcellular fraction that is highly enriched in large mossy fiber nerve endings was developed for this purpose. The morphological and metabolic properties of this synaptosomal preparation have previously been described, and both glutamate and prodynorphin derived peptides have been shown to be released from these specialized nerve endings in response to membrane depolarization by calcium-dependent mechanisms. During the first year of this research project, it was demonstrated that distinct types of voltage-gated calcium channels are required for the exocytosis of glutamate and dynorphin peptides. We were also alble to confirm that the release of glutamate from hippocampal mossy fiber terminals is regulated by a presynaptic receptor that is sensitive to (Continued)

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L(+)aminophosphonobutyric acid. The goal for the second year of this research project was to test several specific hypotheses concerning presynaptic receptors and the autoregulation of the hippocampal mossy fiber synapse. Specifically, it is hypothesized that the transmitter(s) released from the mossy fiber terminals may mediate positive or negative feedback control of the mossy fiber synaptic input, under appropriate conditions, by activating presynaptic autoreceptors. Presynaptic facilitory kainate receptors are hypothesized to enhance mossy fiber transmitter release through a mechanism that involves the activation of a guanine nucleotide-binding regulatory protein (G_s) that stimulates adenylyl cyclase and increases the activity of voltage-gated calcium channels. This presynaptic facilitation may contribute to hippocampal neurodegeneration produced by the plant-derived toxins kainate and domoate. Presynaptic inhibitory kappa opioid receptors are postulated to exert an antagonistic influence on mossy fiber transmitter release that may function to limit the overexcitation of hippocampal neurons. Moreover, we have been investigating the role of protein kinase C in the regulation of glutamate and dynorphin release from mossy fiber terminals. Overall, substantial progress has been made from the in vitro analysis of isolated hippocampal mossy fiber synaptosomes and in recent experimental work we have begun to administer intrahippocampal injections for the in vivo manipulation of the granule cell-mossy fiber system. The combination of these two methods will allow us to examine the physiological and behavioral significance of autoregulation in the mossy fiber synapse in future experiments. Finally, we have initiated a multidisciplinary investigation of the role of hippocampal dynorphin and cyclic AMP (cAMP) in the adrenal cortical stress response. A major hypothesis that will be examined in this investigation is that the dynorphin-containing mossy fiber pathway and serotonergic fibers innervating the hippocampus may work together to diminish the anxiety experience during and following stressful stimuli by limiting the formation of cAMP within CA3 pyramidal neurons.

PRESYNAPTIC MODULATION OF THE HIPPOCAMPAL MOSSY FIBER SYNAPSE

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ANNUAL TECHNICAL REPORT

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1. Summary

The overall goal of this research project is to systematically investigate a number of the possible ways through which presynaptic modulation might influence the effectiveness of local synaptic interactions at the mammalian hippocampal mossy fiber synapse. A hippocampal subcellular fraction that is highly enriched in large mossy fiber nerve endings was developed for this purpose. The morphological and metabolic properties of this synaptosomal preparation have previously been described, and both glutamate and prodynorphin-derived peptides have been shown to be released from these specialized nerve endings in response to membrane depolarization by calcium-dependent mechanisms. During the first year of this research project, it was demonstrated that distinct types of voltage-gated calcium channels are required for the exocytosis of glutamate and dynorphin peptides. We were also able to confirm that the release of glutamate from hippocampal mossy fiber terminals is regulated by a presynaptic receptor that is sensitive to L(+)aminophosphonobutyric acid. The goal for the second year of this research project was to test several specific hypotheses concerning presynaptic receptors and the autoregulation of the hippocampal mossy fiber synapse. Specifically, it is hypothesized that the transmitter(s) released from the mossy fiber terminals may mediate positive or negative feedback control of the mossy fiber synaptic input, under appropriate conditions, by activating presynaptic autoreceptors. Presynaptic facilitory kainate receptors are hypothesized to enhance mossy fiber transmitter release through a mechanism that involves the activation of a guanine nucleotide-binding regulatory protein (G_s) that stimulates adenylyl cyclase and increases the activity of voltage-gated calcium channels. This presynaptic facilitation may contribute to hippocampal neurodegeneration produced by the plant-derived toxins kainate and domoate. Presynaptic inhibitory kappa opioid receptors are postulated to exert an antagonistic influence on mossy fiber transmitter release that may function to limit the overexcitation of hippocampal neurons. Moreover, we have been investigating the role of protein kinase C in the regulation of glutamate and dynorphin release from mossy fiber terminals. Overall, substantial progress has been made from the in vitro analysis of isolated hippocampal mossy fiber synaptosomes and in recent experimental work we have begun to administer intrahippocampal injections for the in vivo manipulation of the granule cell-mossy fiber system. The combination of these two methods will allow us to examine the physiological and behavioral significance of autoregulation in the mossy fiber synapse in future experiments. Finally, we have initiated a multidisciplinary investigation of the role of hippocampal dynorphin and cyclic AMP (cAMP) in the adrenal cortical stress response. A major hypothesis that will be examined in this investigation is that the dynorphin-containing mossy fiber pathway and serotonergic fibers innervating the hippocampus may work together to diminish the anxiety experience during and following stressful stimuli by limiting the formation of cAMP within CA3 pyramidal neurons.

2. Research Objectives

The research objectives for the funding period 15 September 1990-14 September 1991 were as follows:

a) Test the hypotheses that prodynorphin-derived opioid peptides and glutamate are co-released by mossy fiber presynaptic terminals, rather than in parallel from two separate populations of mossy fibers.

b) Test the hypothesis that glutamate, through activation of a presynaptic kainate subtype of excitatory amino acid receptor, facilitates the release of both glutamate and dynorphin from isolated hippocampal mossy fiber terminals.

c) Test the hypothesis that the release of hippocampal mossy fiber transmitters is autoregulated by a bimodal, frequency-dependent, mechanism. According to this hypothesis high-frequency stimulation would result in a presynaptic inhibition of release, through activation of kappa opioid receptors, by dynorphin peptides.

d) Test the hypothesis that glutamate exocytosis requires the activation of distinct, or additional, types of voltage-gated calcium channels to those required for the release of dynorphin peptides from hippocampal mossy fiber terminals.

e) Test the hypothesis that the stimulation of protein kinase C-dependent phosphotransferase activity differentially influences the co-release of dynorphin and glutamate from hippocampal mossy fiber nerve endings.

f) Test the hypothesis that the liberation and metabolism of arachidonic acid in the presynaptic plasma membrane influences the release of endogenous glutamate from hippocampal mossy fiber terminals.

3. Status of Research

3.1 Test the hypotheses that prodynorphin-derived opioid peptides and glutamate are co-released by mossy fiber presynaptic terminals, rather than in parallel from two separate populations of mossy fibers.

Hippocampal mossy fibers (MF) arise from granule cells in the dentate gyrus and principally innervate pyramidal neurons in the CA3-4 region of Ammon's horn. Prodynorphin-derived opioid peptides are known to be exclusively localized in the MF path and are released in a calcium-dependent manner by focal stimulation of the dentate granule cells. This evidence led many investigators to suggest that dynorphin (Dyn) peptides may be the transmitter of the dentate granule cell-MF path. However, the identity of the MF transmitter has remained controversial because the opioid antagonist naloxone does not effectively block the excitatory MF synaptic input to CA3 pyramidal cells. More recently, it has been argued that glutamate may be the predominant MF transmitter and that Dyn peptides act to modulate the excitatory influence of MF stimulation. This hypothesis implies that Dyn opioids and glutamate are co-released by MF presynaptic terminals, rather than in parallel from two separate populations of MF pathways. The goal of this experiment was to test that hypothesis.

Intrahippocampal injections of colchicine were used to selectively lesion the granule cells of the right dentate gyrus in male Sprague-Dawley rats. At nine or ten days postinjection the neurotoxic effects of colchicine were examined using a Timm's stain for endogenous zinc, a commonly used marker of the MF path, and a Nissl stain. Comparison of the ipsilateral (lesioned) and contralateral (control) hippocampi confirmed that the dentate granule cells had been substantially and selectively eliminated. A further comparison of these hippocampi demonstrated that the lesion reduced the hippocampal content of Dyn B-like immunoreactivity by 57 ± 4% and virtually abolished the depolarization-induced release of this opioid from isolated hippocampal MF synaptosomes. Under superfusion conditions, the MF nerve endings prepared from control and lesioned hippocampi released Dyn B at a rate of 14.7 ± 0.4 and 1.5 ± 0.1 pg/min/mg protein, respectively. Moreover, the MF lesion reduced the amount of endogenous glutamate concomitantly released in these experiments by 52 ± 3% (8.5 ± 0.6 versus 4.2 ± 0.2 nmol of glutamate released from control and lesioned, respectively). However, MF synaptosomes isolated from control and lesioned hippocampi released glutamate at equivalent rates (263 ± 25 and 232 ± 30 pmol glutamate/min/mg protein, respectively). This work has only recently been concluded and has not yet been submitted for publication.

Based on our results, it is concluded that destruction of the hippocampal granule cell-MF pathway substantially diminishes the concomitant release of Dyn B and glutamate from isolated MF synaptosomes. These results are consistent with our conclusion that at least a subpopulation of MF terminals co-release both transmitters in response to granule cell stimulation. It is exciting to note that within the past month this conclusion has received additional support from a study in which the ultrastructural localization of Dyn immunoreactivity in the hippocampus was examined using electron microscopy. In agreement with our results, these investigators (Nitsch and Riesenberg, 1991) noted that the opioid peptide Dyn A(1-17) is only present in a subpopulation of hippocampal MF terminals. It remains to be determined how these co-transmitters interact within the MF synapse to influence the activity of pyramidal cells in the CA3 region. Indeed, this was the objective of the experiments described in sections 3.2 and 3.3 below.

3.2 Test the hypothesis that glutamate, through activation of a presynaptic kainate subtype of excitatory amino acid receptor, facilitates the release of both glutamate and dynorphin from isolated hippocampal mossy fiber terminals.

The available evidence suggests that presynaptic glutamate receptors may be unevenly distributed, providing the basis for a pathway-specific autoregulation of glutamate release. Determining where and how glutamatergic neurotransmission is subject to autoregulation is an issue of some importance, considering the central role that this excitatory amino acid plays in synaptic plasticity and in a wide range of neurological diseases. Experimental results presented over the last few years now suggest that the hippocampal MF-CA3 synapse is an ideal system for the study of presynaptic glutamate autoreceptors.

To examine this possibility, excitatory amino acid agonists and antagonists were evaluated for their ability to affect the concomitant release of glutamate and Dyn A(1-8)-like immunoreactivity from guinea pig MF synaptosomes. Previous work in this lab demonstrated that L(+)2-amino-4-phosphonobutyrate inhibits the K^+-evoked release of these endogenous transmitters from guinea pig but not rat hippocampal MF synaptosomes. Therefore, in this study we further evaluated excitatory amino acid agonists as indices to the functional properties of this L(+)2-amino-4-phosphonobutyrate-sensitive glutamatergic autoreceptor on MF terminals. Low micromolar concentrations of quisqualate, but not kainate,N-methyl-D-aspartate,norRS-alpha-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid, significantly inhibited the K^+-evoked release of both glutamate and Dyn A(1-8).

Quisqualate-induced inhibition of glutamate release from MF synaptosomes was antagonized by the non-N-methyl-D-aspartate antagonist 6-dyano-7-nitroquinoxaline-2,3-dione (CNQX). In contrast, high concentrations of kainate enhanced the K^+-evoked release of glutamate and Dyn A(1-8), and this potentiation was blocked by CNQX. Kainate (1 mM) was the only agonist that significantly enhanced the basal release of glutamate, whereas the spontaneous efflux of dynorphin A(1-8) was not affected by any of the agonists tested.

The results of this study suggest the existence of inhibitory and excitatory presynaptic glutamatergic autoreceptors that act to modulate the release of endogenous glutamate and prodynorphin-derived peptides from guinea pig hippocampal MF terminals. These inhibitory and excitatory autoreceptors, which are sensitive to quisqualate/L(+)2-amino-4-phosphonobutyrate or kainate, respectively, may play an important role in regulating synaptic activity at glutamatergic synapses throughout the central nervous system.

In an additional study, we further examined the characteristics of the kainate subtype of presynaptic receptor. This study was initiated following the discovery that domoic acid was the toxin present in contaminated oyster beds that was responsible for a recent outbreak of seafood poisoning in Canada. Domoic acid and kainate are both plant-derived excitotoxins that are known to induce an acute and non-progressive pattern of neurodegeneration that is comparable to the hippocampal sclerosis often associated with prolonged episodes of status epilepticus in humans. In this second study, we demonstrated that domoic acid exerts a presynaptic facilitory effect on hippocampal MF synaptic transmission that is similar to that exerted by kainate. Domoic acid significantly increased the K^+-evoked release of endogenous glutamate from superfused guinea pig MF synaptosomes. The presynaptic facilitation produced by domoic acid was dose-dependent and was antagonized by the prior application of CNQX. At a concentration of 30 μM, both domoic acid and kainate significantly increased the extent to which membrane depolarization augmented the availability of cytosolic free calcium in MF synaptosomes. These results provide the first direct biochemical evidence to support the suggestion that domoic acid activates a CNQX-sensitive presynaptic receptor that is located on the hippocampal MF terminals, and may account for the finding that an intact hippocampal MF pathway is required for domoic acid or kainate to maximally induce the overexcitation and subsequent degeneration of CA3 pyramidal neurons. This work has recently been published (Gannon and Terrian, 1991; Terrian et al., 1991). We hope to further examine the mechanism mediating the presynaptic facilitation of release by kainate/domoate during the next year of funding. Specifically, we intend to pursue the hypothesis that a stimulatory guanine nucleotide-binding regulatory protein, G_s, may be coupled to this presynaptic excitatory amino acid receptor.

3.3 Test the hypothesis that the release of hippocampal mossy fiber transmitters is autoregulated by a bimodal, frequency-dependent, mechanism. According to this hypothesis high-frequency stimulation would result in a presynaptic inhibition of release, through activation of kappa opioid receptors, by dynorphin peptides.

The hippocampal MF terminal field of the CA3 region forms a suprapyramidal band, the stratum lucidum, that has been shown to possess a moderate to strong immunoreactivity to Dyn and enkephalin peptides. Isolated hippocampal MF synaptosomes release both types of opioid peptides in a calcium-dependent manner when depolarized and autoradiographic studies have provided evidence for both μ and κ opioid receptor sites in the stratum lucidum of guinea pig hippocampus. These findings have raised the possibility that prodynorphin- and proenkephalin-derived peptides may be released from MF expansions and interact with multiple subtypes of opioid receptors in the immediate vicinity of their

terminus to influence the activity of hippocampal CA3 pyramidal cells. Indeed, pharmacological investigations have demonstrated that μ, κ and δ opioid receptor agonists and antagonists exert a modulatory influence on the MF-CA3 pyramidal cell synapse. However, the proposed endogenous ligands for the κ opioid receptor subtype, the prodynorphin-derived peptides, produce only weak or mixed effects on the excitability of CA3 pyramidal cells in the hippocampus. Therefore, the location and pharmacological properties of the receptors that presumably mediate these actions of endogenous opioids in the hippocampal MF synapse have remained unclear.

During the past year we have demonstrated that the κ opioid agonist U-50,488H inhibits the release of glutamate and Dyn B-like immunoreactivity Dyn B-LI from depolarized guinea pig hippocampal MF synaptosomes (Gannon and Terrian, 1991). The depressant effect of U-50,488H was the first direct biochemical evidence for an inhibitory κ opioid autoreceptor on MF terminals. However, relatively high (> 30 μM) concentrations of U-50,488H and its congeners are required to significantly depress either the electrophysiological activity of the MF-CA3 synapse in brain slices or the release of transmitters from isolated MF synaptosomes. One possible explanation for the high concentrations of both U-50,488H and U-69,593 that are required for inhibition of MF-CA3 synaptic transmission may be that a non-opioid, local anaesthetic action directly on CA3 neurons may be responsible for the observed synaptic depression, rather than an opioid receptor mediated effect. However, the synaptic depression that is produced by micromolar concentrations of U-50,488H in the MF-CA3 synapse does not mimic the effects of the local anaesthetic procaine on CA3 cells. In reviewing this evidence, we have been unable to exclude the possibility that the synaptic depressant activity of κ opioids may be mediated primarily by a presynaptic action on MF terminals, rather than a direct interaction with the CA3 pyramidal cells. In fact, a distinct class of opioid binding site, the λ site, has tentatively been proposed to function as an opioid autoreceptor on the hippocampal MFs. The λ site has a ligand specificity that distinguishes it from classical opioid receptor subtypes. The dense λ binding that is present in the stratum lucidum is substantially depleted following a colchicine-induced lesion of the dentate granule cells and their MF axons, and λ binding rapidly converts to a state of low affinity in <u>in vitro</u> preparations. The later finding may possibly account for the high agonist concentrations that are required to inhibit the release of neurotransmitters from isolated MF synaptosomes.

To address this issue, we have also tested agonists specific for μ, δ and κ opioid receptor subtypes, as well as subtype-specific and nonspecific opioid antagonists for their effects on cytosolic Ca^{2+} levels in, and transmitter release from, MF synaptosomes. The results of this analysis confirm that the depressant activity of opioids on MF terminal function is specific to agonists of the κ opioid subtype, even at high concentrations. In addition, we have obtained evidence that the inhibitory effects of κ agonists at MF synapses cannot be attributed solely to a local anaesthetic mechanism.

Opioid agonists specific for the μ, δ and κ opioid receptor subtypes were tested for their ability to modulate potassium-evoked release of L-glutamate and dynorphin B-like immunoreactivity from guinea pig hippocampal mossy fiber synaptosomes. The κ opioid agonists U-62,066E and (-)ethylketocyclazocine, but not the μ agonist [D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin (DAGO) nor the δ agonist [D-Pen^{2,5}]enkephalin (DPDE), inhibited the potassium-evoked release of L-glutamate and dynorphin B-like immunoreactivity. U-62,066E, but not DAGO or DPDE, also inhibited the potassium-evoked rise in mossy fiber synaptosomal cytosolic Ca^{2+} levels, indicating a possible mechanism for κ agonist inhibition of transmitter release. DAGO and DPDE were found to be without any effect on cytosolic Ca^{2+} levels or transmitter release in this preparation. The U-62,066E inhibition of the potassium-evoked rise in synaptosomal cytosolic Ca^{2+} levels was partially attenuated by the opioid antagonist quadazocine and insensitive to the

δ-opioid specific antagonist ICI 174,864 and the μ opioid-preferring antagonists naloxone and naltrexone. Quadazocine also reversed U-62,066E inhibition of the potassium-evoked release of L-glutamate, but not dynorphin B-like immunoreactivity. These results suggest that κ opioid agonists inhibit transmitter release from mossy fiber terminals through both κ opioid and non-κ opioid receptor mediated mechanisms. This research has been recently submitted for publication. We intend to continue our investigation of the Dyn-mediated autoregulation of MF transmitter release during the next year of funding and have recently obtained evidence which suggests that this presynaptic receptor may be coupled to adenylyl cyclase via a pertussis toxin-sensitive inhibitory G protein.

3.4 Test the hypothesis that glutamate exocytosis requires the activation of distinct, or additional, types of voltage-gated calcium channels to those required for the release of dynorphin peptides from hippocampal mossy fiber terminals.

During the previous funding period, we found that presynaptic N-type calcium channels appear to make the most substantial contribution to the calcium influx that is required for the exocytosis of Dyn A(1-8) from hippocampal MF synaptosomes. The results of this study have now been published as well as the results of an additional series of experiments in which we extended this investigation to include a characterization of the calcium channels that mediate the release of endogenous glutamate from isolated MF terminals. The results of this second study led us to conclude that no one type of presynaptic calcium channel predominately mediates calcium-dependent glutamate release from hippocampal MF terminals. Therefore, the presynaptic calcium channels mediating the concomitant release of Dyn peptides from ectopic sites and the release of glutamate from active zones on the MF terminals appear to differ in their sensitivities to calcium channel antagonists. We have suggested that glutamate release requires the opening of more than one type of calcium channel, while the activation of N-type channels may be required for the exocytosis of Dyn peptides. This work has now been published (Terrian et al., 1990).

3.5 Test the hypothesis that the stimulation of protein kinase C-dependent phosphotransferase activity differentially influences the co-release of dynorphin and glutamate from hippocampal mossy fiber nerve endings.

This has continued to be a very active field of investigation in this laboratory during the present funding period. It has been suggested that the maintenance of long-term potentiation (LTP) in the hippocampal MF synapse involves a presynaptic mechanism that does not require the activation of protein kinase C (PKC), since this enzyme appears to be absent in the MF presynaptic terminals. To evaluate this proposal, we have directly comparing the metabolic properties of hippocampal MF synaptosomes and a conventional P_2B synaptosomal preparation prepared from the same hippocampal tissue. Protein kinase C-dependent histone phosphotransferase activity was found to be comparable in MF and P_2B synaptosomes. Western blot analysis was performed using antisera prepared against four of the PKC isoforms and the results demonstrated that the α, β, and γ PKC isoforms are present in relatively equivalent amounts in these two subcellular fractions. However, the cytosolic fraction derived from the hippocampal MF synaptosomes appeared to contain a greater amount of the PKC-ε isoform when compared to the P_2B synaptosomal preparation. Four distinct endogenous substrates present in the MF synaptosomes were shown to be phosphorylated in response to PKC activation. A functional role for PKC in the hippocampal MF nerve endings seems to be indicated by the finding that 4β-phorbol 12,13-dibutyrate (PDBu) and 4β-phorbol 12,13-diacetate produce a dose-dependent potentiation of the K^+-evoked release of endogenous glutamate and dynorphin B, while the inactive 4-α-

phorbol was without effect. The PDBu-induced enhancement of transmitter release was blocked by the PKC inhibitor, staurosporine. In addition, PDBu significantly facilitated the rise in cytosolic free calcium that immediately followed depolarization of the MF synaptosomal membrane. These findings have led us to conclude that hippocampal MF presynaptic terminals possess a variety of PKC isoforms and that their activation may have an important facilitory influence on MF synaptic transmission and plasticity. This work has recently been published (Terrian et al., 1991).

At the same time that we were in the process of conducting the above study, other investigators were examining the hypothesis that LTP in the MF synapse is due to increased quantal content, rather than a change in quantal size. The results of this quantal analysis are consistent with the conclusion that an increase in transmitter release is required for MF LTP (Zalutsky and Nicoll, 1990). Considerable interest has consequently been focused on understanding the functional relationship between PKC activation and synaptic plasticity in the hippocampal MF pathway. It has been demonstrated that the activation of PKC by phorbol esters effectively increases the quanta of transmitter released from MF terminals (Yamamoto et al., 1987). In addition, the nootropic (cognitive enhancing) drug bifemaline has been shown to increase the magnitude of MF LTP (Satoh et al., 1988), stimulate the translocation of cytosolic PKC to membranes in the region of the MF synapse (Fujii et al., 1990), and enhance the release of endogenous glutamate from isolated MF nerve endings (Kuraishi et al., 1991). Therefore, the available evidence argues in favor of the conclusion that LTP of the MF synapse is maintained by a mechanism that involves the activation of PKC and enhancement of glutamate release. However, the relationship between PKC activation and glutamate release appears to be complex and must be clarified before any such conclusion may be accepted.

Both the activation and down-modulation of PKC by either an acute or prolonged exposure to phorbol esters, respectively, are reported to potentiate the release of this excitatory amino acid (Diaz-Guerra et al., 1988). A possible explanation for this discrepancy is suggested by the finding that prolonged exposure to phorbol esters not only promotes the proteolysis and down-modulation of PKC but also alters the substrate specificity of this enzyme (Ways et al., 1991). Previous studies with phorbol ester-induced down-modulation of PKC have failed to take this complexity into account because only PKC-dependent histone phosphotransferase was measured. Therefore, the enhancement of glutamate release observed by Diaz-Guerra et al. (1988) following prolonged exposure to a phorbol ester may have resulted from a decrease in the affinity of PKC for histone rather than the down-modulation of PKC-dependent phosphotransferase activity. Therefore, we have conducted a study that was designed to test the hypothesis that PKC down-modulation suppresses the release of glutamate from hippocampal mossy fiber terminals. Specifically, we have identified the isoforms of PKC that are present in isolated guinea pig hippocampal MF synaptosomes and western blot analyses with antipeptide antisera raised against these isoforms (α, β, γ, ϵ, and ζ). Using these techniques, it was possible to directly examine the time course of phorbol ester-induced PKC down-modulation, by measuring changes in the content of specific PKC isoforms, and the effect of diminished PKC on glutamate exocytosis.

Guinea pigs were used in this study because we had determined that the PKC-β isoform is absent in the central nervous system of this species. This result was confirmed using antipeptide antisera that are directed towards two different domains of the PKC molecule. Given the finding that PDBu is equally effective in facilitating the release of glutamate and Dyn B from the MF synaptosomes isolated from either rat or guinea pig hippocampi, we have concluded that the activation of PKC-β is not required for this presynaptic facilitation. Other findings of this study are: 1) Prolonged exposure (180 min) to PDBu (10 μM) reduces the content of all A-series PKC isoforms (α and γ, β not present) by more than 95%. However, as much as 25% of the endogenous PKC-ϵ and ζ remains resistant to this down-

regulation. 2) Down-regulation of the A-series of PKC isoforms significantly reduces the K^+-evoked release of Dyn B, suggesting that the activation of PKC-α or PKC-γ may be required for PDBu to facilitate the release of this opioid peptide. 3) Down-regulation of the A-series of PKC isoforms significantly increased the K^+-evoked release of endogenous glutamate, suggesting that the activation of PKC differentially influences the ectopic release of neuropeptides and the exocytosis of amino acids from the active sites along the presynaptic plasma membrane. These results are the first biochemical evidence for a differential effect of PKC activation on the release of these two distinct classes of neurotransmitters and provide further support for the suggestion that the biochemical pathways that mediate their release may be interdependent but separate. We hope to pursue this idea further during the next year of funding.

3.6 Test the hypothesis that the liberation and metabolism of arachidonic acid in the presynaptic plasma membrane influences the release of endogenous glutamate from hippocampal mossy fiber terminals.

In collaboration with Dr. Robert V. Dorman, we have continued examine the mechanisms by which arachidonate and its metabolites affect the release of glutamate from hippocampal MF nerve endings. As discussed in the previous annual technical report, we have demonstrated that the activation of phospholipase A_2 and the addition of unesterified arachidonic acid stimulated the release of endogenous glutamate from hippocampal MF synaptosomes. Moreover, both the arachidonate and K^+-evoked release of glutamate were potentiated when lipoxygenase activities were inhibited with nordihydroguaiaretic acid (NDGA). These results have now been published (Freeman et al., 1990) and led us to propose that lipoxygenase metabolites derived from arachidonate may constitute an endogenous feedback system for the modulation of neurotransmitter release. The results of our more recent experiments are in agreement with this hypothesis. We have now demonstrated that membrane depolarization evokes the release of glutamate from hippocampal MF synaptosomes, as well as the accumulation of intraterminal calcium. The presence of 12-lipoxygenase products attenuated both the induced release of glutamate and the increase in calcium content, whereas 5- or 15-lipoxygenase metabolites were ineffective. A role for lipoxygenase products in the negative modulation of MF secretion processes was further indicated by the observations that low concentrations of the lipoxygenase inhibitor NDGA potentiated the glutamate release and calcium accumulation induced by membrane depolarization. Therefore, we have suggested that the 12-lipoxygenase metabolites provide a presynaptic inhibitory signal that limits neurotransmitter release from hippocampal MF terminals. Further investigations are required to assess this proposal and the possibility that arachidonate and 12(S)-HETE are acting at the same site to modulate synaptic transmission at the MF-CA3 synapse. The results of this study have recently been published (Freeman et al., 1991).

4. Publications

4.1 Full papers and review articles

1. Freeman, E, TERRIAN, DM, Dorman, RV: Presynaptic facilitation of glutamate release from isolated hippocampal mossy fiber nerve endings by arachidonic acid. Neurochem. Res. 1990; 15: 749-756.

2. TERRIAN, DM, Dorman, RV, Gannon, RL: Characterization of the presynaptic calcium channels involved in glutamate exocytosis from rat hippocampal mossy fiber synaptosomes. Neurosci. Lett., 1990; 119: 211-214.

3. TERRIAN, DM, Dorman, RV, Damron, DS, Gannon, RL: Displacement of endogenous glutamate with D-aspartate: an effective strategy for reducing the calcium-independent component of glutamate release from synaptosomes. Neurochem. Res., 1991; 16: 35-41.

4. Gannon, RL, TERRIAN, DM: Presynaptic modulation of glutamate and dynorphin release by excitatory amino acids in the guinea pig hippocampus. Neuroscience, 1991; 41: 401-410.

5. Freeman, EJ, Damron, DM, TERRIAN, DM, Dorman, RV: 12-Lipoxygenase products attenuate the glutamate release and calcium accumulation evoked by depolarization of hippocampal mossy fiber nerve endings. J. Neurochem., 1991; 56: 1079-1082.

6. TERRIAN, DM, Ways, DK, Gannon, RL: A presynaptic role for protein kinase C in hippocampal mossy fiber synaptic transmission. Hippocampus, 1991; 1: 303-314.

7. Gannon, RL, TERRIAN, DM: U-50,488H inhibits dynorphin and glutamate release from guinea pig hippocampal mossy fiber terminals. Brain Res., 1991; 548: 242-247.

8. TERRIAN, DM, Privette, TH, Conner-Kerr, T, Gannon, RL: Domoic acid enhances the K^+-evoked release of glutamate from guinea pig hippocampal mossy fiber synaptosomes. Brain Res., 1991; 551: 303-307.

9. Gannon, RL, TERRIAN, DM: Kappa opioid agonists inhibit transmitter release from guinea pig hippocampal mossy fiber synaptosomes. Neurochem. Res., Submitted.

10. Simpson, J., Gannon, RL, McGinty, JF, TERRIAN, DM: Kainic acid depresses the ex vivo release of dynorphin B and glutamate from rat hippocampal mossy fiber synaptosomes. Neurosci. Lett., Submitted.

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4.2 Abstracts

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5. Professional Personnel Associated With the Research Project

David M. Terrian, Ph.D. - Principal Investigator
Robert L. Gannon, Ph.D. - Co-Investigator
Debbie A. Zetts, B.S. - Research Technician III
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Teresa A. Conner-Kerr - Graduate Student
Thomas H. Privette - Graduate Student
Jeffrey N. Simpson - Graduate Student
Duncan R. Simmons - Medical Student

6. Interactions

9/18/90	NASA Space Biomedical Peer Review Panel, Chairman
10/15/90-10/17/90	NYAS Symposium, "Calcium Entry and Action at the Presynaptic Nerve Terminal," Baltimore, Maryland
10/28/90-11/2/90	Society for Neuroscience, St. Louis, Missouri
11/21/90	Seminar for the Department of Pharmacology, ECU School of Medicine, "Multiple mechanisms underlying hippocampal long-term potentiation: a re-evaluation of the biochemical evidence.
3/6/91-3/9/91	Robert V. Dorman, Ph.D. consultation and collaborative research
3/10/91-3/15/91	American Society for Neurochemistry, Charleston, South Carolina
4/9/91	NASA Space Biomedical Peer Review Panel
5/22/91	Neuroscience Day at Duke, North Carolina Society for Neuroscience
8/1/91	NASA Space Physiology and Countermeasures Program Review

7. New Discoveries, Inventions, or Patent Applications

None.

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