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ANNUAL TECHNICAL REPORT

TITLE: INVOLVEMENT OF LIPID METABOLISM IN CHEMICAL
TRANSMISSION PROCESSES AT MOSSY FIBER SYNAPSES.

GRANT #: AFOSR-89-0245

PRINCIPAL INVESTIGATOR: Robert V. Dorman

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SUMMARY

In 1991 we continued our investigations on the involvement of membrane lipid metabolism in the presynaptic processes related to the evoked release of the neurotransmitter glutamate. In particular, we observed that the phospholipase A₂-dependent release of arachidonic acid from mossy fiber membrane phospholipids may modulate transmitter secretion through interactions with protein kinase C (PKC). The activation of PKC may explain the previously observed facilitation of depolarization-evoked Ca²⁺ accumulation and glutamate release induced by exogenous arachidonate. These facilitory effects may be related to the induction of long-term synaptic potentiation, which is an accepted correlate of learning and memory. In addition, we obtained evidence that presynaptic receptor activation stimulates the synthesis of arachidonate-derived prostaglandins. Thus, the metabolism of arachidonic acid may play a central role in presynaptic plasticity.

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RESEARCH OBJECTIVES

The general objective of the past year's work was to continue our investigations on the role of membrane lipids in the mechanisms of stimulus-secretion coupling at the hippocampal mossy fiber nerve terminal. Isolated mossy fiber synaptosomes were employed, since they release glutamate in a physiological fashion and have been shown to express long-term potentiation.

Specific Objectives

1. Correlate the activation and inhibition of mossy fiber PKC with the depolarization-induced accumulation of intraterminal free Ca^{2+} ($[\text{Ca}^{2+}]_i$)
2. Determine the effects of altering membrane lipid metabolism on the PKC-dependent changes in $[\text{Ca}^{2+}]_i$
3. Assess the effects of receptor activation on the production of prostaglandin F_{2a} (PGF_{2a})

STATUS OF THE RESEARCH

The investigations performed during the past year were designed to fit within the specific objectives listed above. Many of the aims have been met, while work is continuing in some areas. A summary of the completed research is given below.

1. Correlate the activation and inhibition of mossy fiber PKC with the depolarization-induced accumulation of $[\text{Ca}^{2+}]_i$

Depolarization of the mossy fiber synaptosomes stimulated an increase in $[\text{Ca}^{2+}]_i$. This increase was potentiated in the presence of the PKC activator 12,13-phorbol dibutyrate (PDBu). It was attenuated with the PKC inhibitor staurosporine (STAU). Both the potentiation with PDBu and the inhibition with STAU were dose-dependent and were observed with any depolarizing concentration of KCl (15-45 mM).

2. Determine the effects of altering membrane lipid metabolism on the PKC-dependent changes in $[\text{Ca}^{2+}]_i$

We observed that the inhibition of phospholipase A_2 with bromophenacyl bromide blocked the PDBu-dependent facilitation of depolarization-evoked Ca^{2+} accumulation. In addition, the



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PDBu effect was attenuated by voltage-sensitive Ca^{2+} channel blockers, STAU and 12-HETE. Also, the arachidonic acid (AA) dependent potentiation of Ca^{2+} accumulation was attenuated by STAU, suggesting that AA may modulate presynaptic events by activating PKC. This suggestion was supported by the observation that STAU also inhibited the enhanced Ca^{2+} accumulation induced with the phospholipase A_2 activator melittin.

That AA may stimulate an increase in presynaptic $[\text{Ca}^{2+}]_i$ by activating PKC was also indicated by the effects of STAU. Low concentrations of AA or diglyceride had no effect on the K^+ -induced accumulation of Ca^{2+} . However, when both treatments were present, the Ca^{2+} accumulation induced by 25 mM KCl was significantly increased. This effect appeared to depend on the activation of PKC, since STAU completely eliminated the potentiated response. We suggest, based on these results, that the synaptic facilitation related to the induction of long-term potentiation depends on the phospholipase A_2 -dependent release of AA and the subsequent activation of PKC.

3. Assess the effects of receptor activation on the production of $\text{PGF}_{2\alpha}$

Prostaglandins have been implicated in the modulation of stimulus-secretion coupling in a variety of tissues. However, we previously observed no direct effects of prostanoids on mossy fiber $[\text{Ca}^{2+}]_i$ or the release of glutamate. Therefore, we examined the relationship between receptor activation on the production of the major mossy fiber prostaglandin. We observed that postsynaptic receptor activation with glutamate, or any of its agonists, had no effect on $\text{PGF}_{2\alpha}$ synthesis. However, transmitters which have been proposed to modulate mossy fiber activity via presynaptic receptors did alter $\text{PGF}_{2\alpha}$ production. We found that norepinephrine, serotonin and dopamine stimulated the synthesis of this prostaglandin. In addition, these effects were inhibited with bromophenacyl bromide, voltage-sensitive Ca^{2+} channel blockers and the K^+ channel activator diazoxide. From these results, we suggest that the coupling of presynaptic receptors with $\text{PGF}_{2\alpha}$ synthesis depends on phospholipase A_2 , Ca^{2+} influx and K^+ channel activity. Further work will be necessary to determine if $\text{PGF}_{2\alpha}$ is able to modulate synaptic activity.

SUMMARY OF THE RESULTS

The above results are consistent with a role for arachidonic acid and its oxidized derivatives in the modulation of presynaptic secretion processes at the mossy fiber terminal. The effects of AA appear to be due to the activation of PKC and may be related to the synaptic potentiation that is involved in the induction of long-term

potentiation. It also appears that transmitter-dependent modulation of synaptic activity via presynaptic receptors may be reflected by the stimulation of the arachidonate cascade and the subsequent production of PGF_{2a}.

PUBLICATIONS

1. Dorman, R.V. (1991) PGF_{2a} synthesis in isolated cerebellar glomeruli: effects of membrane depolarization, calcium availability and phospholipase activity. Prostaglandins, Leukotrienes and Essential Fatty Acids 42:233-240.
2. Freeman, E.J., Damron, D.S., Terrian, D.M. and Dorman, R.V. (1991) 12-Lipoxygenase products attenuate the glutamate release and Ca²⁺ accumulation evoked by depolarization of hippocampal mossy fiber nerve endings. Journal of Neurochemistry 56:1079-1082.
3. Terrian, D.M., Dorman, R.V., Damron, D.S. and Gannon, R.L. (1991) Displacement of endogenous glutamate with D-aspartate: an effective strategy for reducing the calcium-independent component of glutamate release from synaptosomes. Neurochemical Research 16:35-41.
4. Dorman, R.V., Hamm, T.F.R., Damron, D.S. and Freeman, E.J. (1992) Modulation of glutamate release from hippocampal mossy fiber nerve endings by arachidonic acid and eicosanoids. In "Neurobiology of Essential Fatty Acids", N.G. Bazan, ed., Plenum Press, NY, (in press)

ABSTRACTS

1. Privette, T.H., Terrian, D.M., Zetts, D.A., Dorman, R.V. and Gannon, R.L. (1991) Kappa opioid autoregulation of the guinea pig hippocampal mossy fiber synapse. Transactions of the American Society for Neurochemistry 22:221.
2. Dorman, R.V., Damron, D.S., Freeman, E.J. and Terrian, D.M. (1991) Modulation of glutamate release from hippocampal mossy fiber nerve endings by arachidonic acid and eicosanoids. "Neurobiology of Essential Fatty Acids", Satellite to 13th International Society for Neurochemistry meeting, Cairns, Australia, July 10-12, p 17.

PROFESSIONAL PERSONNEL

1. Nancy Edgehouse
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2. Graduate Students receiving full or partial support
in 1991:
 - Thomas Hamm
 - Ernest Freeman
 - Derek Damron
 - Duska Separovic
 - Alex Bailey
 - Eric Aguerro
 - Lian Zhang
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INTERACTIONS

1. Continued collaboration with David M. Terrian. He is currently at East Caroline Medical School and his research is supported by AFOSR.
2. Peer reviewer for Ohio Affiliate of the American Heart Association; 1989 - present.
3. Member, Scientific Advisory Committee for the meeting: "Neurobiology of Essential Fatty Acids", to be held in Cairns, Australia, July, 1991.