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13. ABSTRACT (Maximum 200 words)

Isolated hippocampal mossy fiber synaptosomes were used to assess the relationships between membrane lipid metabolism and the evoked release of the excitatory amino acid neurotransmitter glutamate. A variety of metabolic parameters were investigated in order to develop a comprehensive model for the mechanisms of transmitter release. Mossy fiber terminals were radiolabeled with [3H] arachidonic acid and the effects of membrane depolarization and calcium influx on the labeling of the component lipid pools were determined. It was observed that depolarization and Ca<sup>2+</sup> influx stimulated the accumulation of unesterified arachidonate. This effect was correlated with increased production of prostaglandins, in particular PGF<sub>2a</sub> and could be blocked with lipase inhibitors and Ca<sup>2+</sup> channel blockers. Prostaglandin production was also blocked by cyclooxygenase and lipoxygenase inhibitors. We also observed that exogenous arachidonate stimulates the release of endogenous glutamate from mossy fiber terminals, as well as the mobilization of intraterminal free Ca<sup>2+</sup>. In addition, we identified two naturally occurring products which act synergistically to activate protein kinase C and facilitate the evoked release of glutamate.

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FINAL TECHNICAL REPORT

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

GRANT #: AFOSR-89-0245

PRINCIPAL INVESTIGATOR: Robert V. Dorman

DATE: MAY 24, 1993

SUMMARY

Isolated hippocampal mossy fiber synaptosomes were used to assess the relationships between membrane lipid metabolism and the evoked release of the excitatory amino acid neurotransmitter glutamate. A variety of metabolic parameters were investigated, in order to develop a comprehensive model for the mechanisms of transmitter release. Mossy fiber terminals were radiolabeled with [<sup>3</sup>H]arachidonic acid and the effects of membrane depolarization and calcium influx on the labeling of the component lipid pools were determined. It was observed that depolarization and Ca<sup>2+</sup> influx stimulated the accumulation of unesterified arachidonate. This effect was correlated with increased production of prostaglandins, in particular PGF<sub>2a</sub> and could be blocked with lipase inhibitors and Ca<sup>2+</sup> channel blockers. Prostaglandin production was also blocked by cyclooxygenase and lipoxygenase inhibitors. We also observed that exogenous arachidonate stimulates the release of endogenous glutamate from mossy fiber terminals, as well as the mobilization of intraterminal free Ca<sup>2+</sup>. In addition, we identified two naturally occurring products which act synergistically to activate protein kinase C and facilitate the evoked release of glutamate.

## RESEARCH OBJECTIVES

The general objective of this research was to examine the role that membrane lipids play in the mechanisms related to neurotransmitter release from a central synapse. Mossy fiber synaptosomes were isolated from rat hippocampus, because they play a role in learning and memory. In order to investigate the relationships between lipid metabolism and synaptic functions, some specific objectives were outlined.

### Specific Objectives

1. Use [<sup>3</sup>H]arachidonic acid to radiolabel nerve terminal lipids.
2. Determine the effects of membrane depolarization on arachidonate metabolism.
3. Assess the importance of external calcium on resting and depolarization-induced changes in arachidonate metabolism.
4. Investigate the possible mechanisms of arachidonate accumulation in response to depolarizing conditions.
5. Measure prostaglandin accumulation in response to membrane depolarization: assess effects of calcium antagonists and metabolic inhibitors.
6. Correlate the observed alterations in arachidonate and prostaglandin metabolism with the actual release of neurotransmitters and the accumulation of presynaptic calcium.

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## STATUS OF THE RESEARCH

The experimental work carried-out was designed to fit within the specific objectives listed above. A summary of the research follows.

### 1. Radiolabeling of glomerular lipids with [<sup>3</sup>H]arachidonate:

Attempts were made to isotopically label hippocampal mossy fiber terminals in vitro. We found that arachidonate was incorporated into complex lipids and the uptake was linear with time. This method was then used for the studies of arachidonate metabolism.

### 2. Effects of membrane depolarization on arachidonate metabolism in the isolated mossy fiber synaptosomes:

Nerve terminal lipids were radiolabeled in vitro as described above. The labeled membranes were then used to assess the effects of membrane depolarization on the metabolic flux of arachidonate. We found that depolarization of the membranes with KCl, veratridine or 4-aminopyridine stimulated the accumulation of unesterified arachidonate. We suggest that the accumulation of unesterified arachidonate is an important response of nerve terminal membranes to depolarization and that such an effect is related to stimulus-secretion coupling.

### 3. Role of calcium in depolarization-induced arachidonate accumulation:

It is accepted dogma that the release of neurotransmitters requires the presence of external calcium. Therefore, we investigated the involvement of calcium in the evoked release of arachidonate. We found substantial inhibition of arachidonate accumulation in depolarized nerve terminals when Ca<sup>2+</sup> was omitted from the incubation medium. We also found that verapamil, a Ca<sup>2+</sup> channel blocker, inhibited the evoked release of arachidonate. The importance of external calcium for arachidonate accumulation was substantiated when the calcium ionophore A23187 was used to mimic the depolarization-induced effects on arachidonate metabolism when only the ionophore and external calcium were present. We interpret these results to show that the depolarization-induced accumulation of arachidonate is a calcium dependent process.

### 4. Prostaglandin production by hippocampal mossy fiber synaptosomes:

We previously found that there is an accumulation of arachidonate in stimulated terminals. So, we examined the relationship between arachidonate availability and the production of one of its major metabolites PGF<sub>2α</sub>. We observed that membrane depolarization enhanced PGF synthesis and this effect was blocked by calcium channel blockers and phospholipase inhibitors. In addition, we found that receptor activation with certain neurotransmitter agonists stimulated PG accumulation. Although we found no direct link between PG production and the secretion of glutamate from mossy fiber nerve endings, we have suggested that PG may modulate

transmitter release.

5. Correlations between observed alterations in lipid metabolism and the evoked release of neurotransmitters:

We were able to correlate the observed changes in membrane metabolism with the ultimate effect, the actual evoked release of neurotransmitters. This has provided us with the ability to make rapid advances in our understanding of release mechanisms. A summary of some of the correlative studies follow.

First, we showed that membrane depolarization evokes the release of glutamate from mossy fiber nerve terminals, as well as the accumulation of unesterified arachidonate and presynaptic calcium. Second, we found that the presence of exogenous arachidonate causes a dose-dependent release of neurotransmitter and this process is calcium-independent. The fact that the arachidonate-stimulated neurotransmitter release is calcium-independent has led us to suggest that the calcium requirement for transmitter release may be related to the accumulation of arachidonate. Third, we found that inhibition of the conversion of free arachidonate to prostaglandins with ibuprofen enhanced the depolarization- and arachidonate-evoked release of glutamate. Fourth, we observed that an oxidized derivative of arachidonate 12-HETE inhibited depolarization-induced glutamate release. We used the above information to propose that arachidonate provides an excitatory, presynaptic signal to stimulate glutamate release and that this effect is opposed by its 12-lipoxygenase products.

6. Mechanism of arachidonate modulation of evoked glutamate release.

We reported that exogenous arachidonate enhanced the amount of glutamate release from mossy fiber terminals induced by depolarization with KCl. More recently, we found that arachidonate and the diacylglycerol analog oleoyl-acetyl-glycerol acted synergistically to enhance the evoked release of glutamate. We found the same synergism with regards to the evoked accumulation of presynaptic calcium.

Both these effects were blocked by the protein kinase C inhibitor staurosporine. Therefore, we suggested that the two lipid metabolites work in concert to activate protein kinase C and potentiate the presynaptic responses to membrane depolarization.

PUBLICATIONS SUPPORTED BY AFOSR 89-0245:

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1. Freeman, E.J., Terrian, D.M. and Dorman, R.V. (1989) Arachidonate evoked release of glutamate from mossy fiber terminals. *Transactions of the American Society for Neurochemistry* 20:267.
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4. Damron, D.S., Terrian, D.M. and Dorman, R.V. (1989) Calcium mobilization in hippocampal mossy fiber synaptosomes: phospholipase A<sub>2</sub> modulation. *Neuroscience Abstracts* 15:474.
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