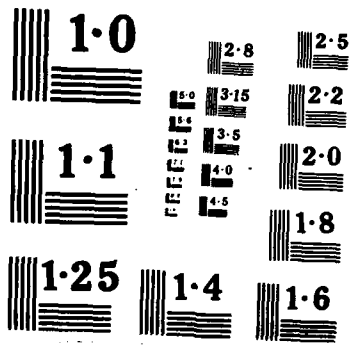


AD-A170 065 AMINE NEUROTRANSMITTER REGULATION OF LONG-TERM SYNAPTIC 1/1
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AMINE NEUROTRANSMITTER REGULATION OF
LONG-TERM SYNAPTIC PLASTICITY IN HIPPOCAMPUS

AFOSR 85-0178

Annual Technical Report

1. Summary.

The overall goal of the research project is to investigate the mechanisms of long-term synaptic potentiation (LTP) in hippocampus, with particular emphasis on the modulation of LTP by amine neurotransmitters. During the first year of the grant, several studies were completed in which a number of hypotheses were tested for the mechanisms of LTP. It was shown that LTP of the mossy fiber synapse in hippocampus is associated with an increase in the excitatory synaptic conductance with no change in reversal potential or membrane properties of the postsynaptic neuron. Because a decrease in the inhibitory response could result in an apparent increase in the excitatory response, the hypothesis that LTP was due to a long-term decrease in inhibitory conductance was also tested. The results suggested that no long-term change in the inhibitory synaptic conductance was associated with LTP at the mossy fibers. In other work, various hypotheses associated with the previously observed modulation of LTP by norepinephrine (NE) were tested. It was found that cyclic AMP could mimic the action of NE and that NE could enhance LTP in the absence of synaptic inhibition. The cellular effects of NE were explored in an isolated hippocampal neuron system in which patch-clamp techniques were utilized. It was found that NE produces an enhancement in the voltage-dependent calcium current. In other preliminary work, the feasibility of a preparation of hippocampal mossy fiber synaptosomes was explored, and progress continued towards the development of a computer model that simulates the behavior of single hippocampal neurons.

2. Research Objectives.

The research objectives for the funding period 1 April 1985 - 31 March 1986 were as follows:

- a. Test the following four hypotheses for mechanisms of LTP at the mossy fiber synapse:
 1. LTP is associated with an increase in synaptic conductance
 2. LTP is associated with a change in the reversal potential of the excitatory synaptic conductance change.
 3. LTP is associated with changes in the passive membrane properties of the postsynaptic neuron.
 4. LTP is associated with a long-term decrease in the inhibitory synaptic conductance.

- b. Test the following three hypotheses associated with the noradrenergic modulation of LTP:
 1. NE enhancement of LTP is mediated through cyclic AMP.
 2. Inhibition plays a role in the NE modulation of LTP.
 3. NE modulation of LTP takes place postsynaptically.

- c. Test the hypothesis that NE modulates voltage-dependent calcium conductances.
- d. Initiate a feasibility study for the electrophysiological investigation of mossy fiber synaptosomes.
- e. Develop single cell computer models.

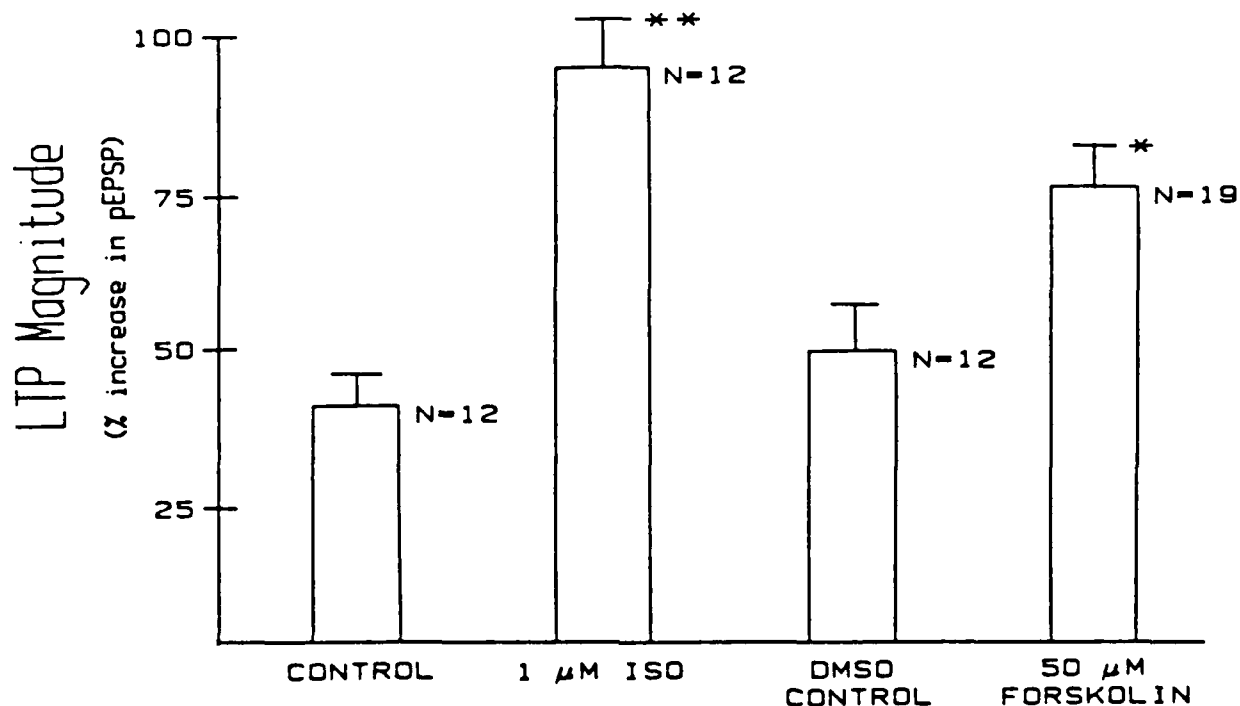
3. Status of Research.

- a. Test four hypotheses for mechanisms of LTP at the mossy fiber synapse. This represents work that began before the initial funding year of the Air Force grant but was completed during the first year of funding. This work represents a joint effort of the laboratories of Dr. Tom Brown at the City of Hope Research Institute and our laboratory at Baylor College of Medicine. Two publications have resulted from this work, and they are attached to the Annual Report. A summary of the findings follows.

Using voltage-clamp techniques, we studied the excitatory and inhibitory conductance change measured in CA3 pyramidal neurons in response to mossy fiber stimulation. We measured the conductance change before and after eliciting LTP. We found that the excitatory conductance change increased approximately 50% during LTP. We further found that there was no change in the reversal potential of the excitatory conductance, no change in passive membrane properties of the postsynaptic cell, and no change in the recurrent inhibitory synaptic conductance. This work represented the testing of four distinct and well-formulated hypotheses for mechanisms of LTP. From the results of these studies, our conclusion is that an increase in the excitatory synaptic conductance represents a mechanism for LTP. However, changes in reversal potential, postsynaptic membrane properties, or inhibitory conductances do not appear to play a role in LTP.

- b. Test three hypotheses associated with the noradrenergic modulation of LTP. In previous work, we have shown that NE increases the magnitude, duration, and probability of induction of LTP at the mossy fiber synapse. We also have shown that the beta-adrenergic receptor appears to mediate the NE effects. During the current funding period, we tested the hypothesis that the NE enhancement of LTP is mediated through the activation of adenylate cyclase leading to increases in cyclic AMP. This hypothesis was tested in two ways. The first was to exogenously apply 8-bromo cyclic AMP to the bath during the high frequency stimulus train that is used to elicit LTP. This is the identical paradigm used when adding NE. In preliminary experiments, we found that 8-bromo cyclic AMP added to the bath would indeed mimic the actions of NE in enhancing the magnitude of LTP. A second, more direct test of the hypothesis involved the use of forskolin, a specific activator of adenylate cyclase. We found that forskolin added to the bath enhanced the magnitude of LTP in a similar manner as NE. Results of our experiments are shown in Fig. 1.

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- * significantly greater than DMSO control. $p < .025$
- ** significantly greater than control. $p < .005$

Fig. 1. Enhancement of LTP by forskolin. The magnitude of LTP (mean + standard deviation) is shown for several different experimental conditions. Isoproterenol, a specific beta-adrenergic agonist, when added to the bath during the high frequency conditioning train, enhances the magnitude of LTP over that of control saline. Forskolin, a specific activator of adenylate cyclase, when added to the bath in a similar manner, produces a significant enhancement of LTP magnitude over control. Results of these experiments suggest that the beta-adrenergic enhancement of LTP is mediated through activation of adenylate cyclase, leading to increases in cyclic AMP.

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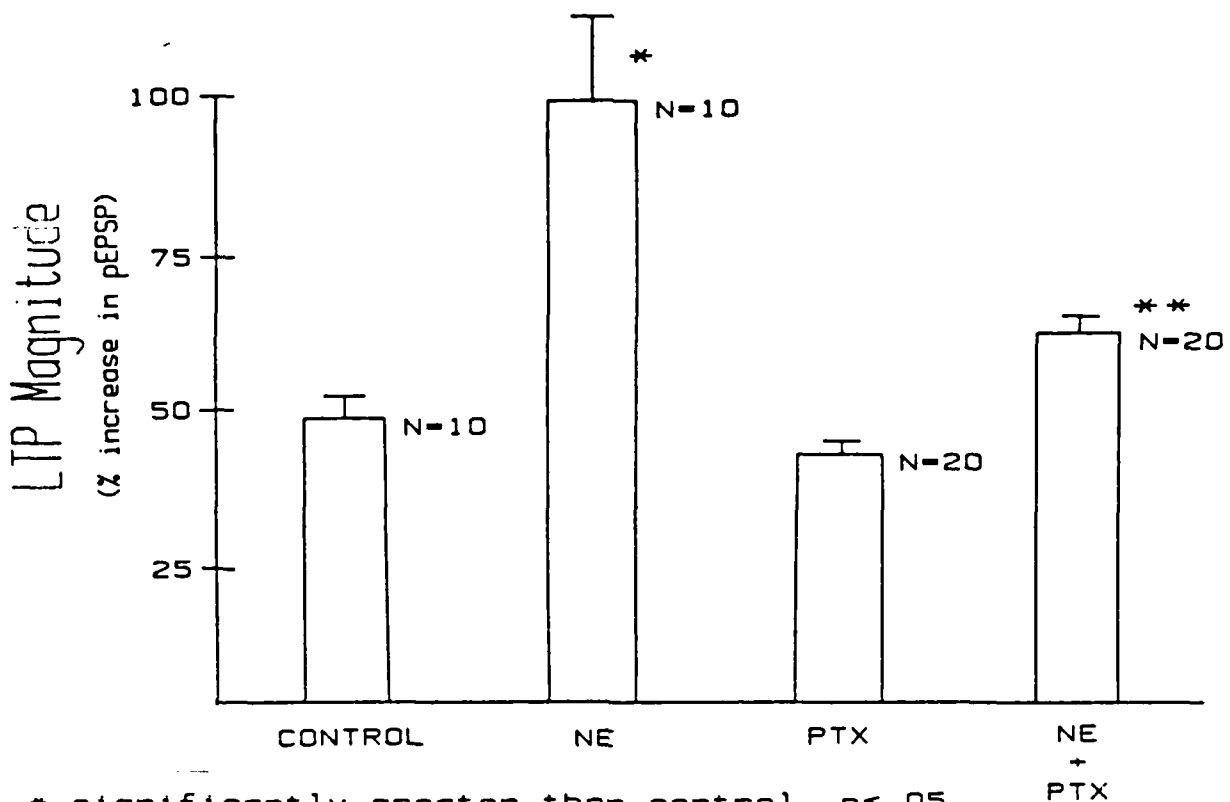
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The second hypothesis we tested was that inhibition somehow plays a role in the NE modulation of LTP. This hypothesis was tested by adding picrotoxin (PTX), which blocks recurrent inhibition, to the bath and testing for effects of NE on LTP in the presence of PTX. We found that both NE as well as a specific beta agonist, isoproterenol, still enhanced the magnitude of LTP (see Fig. 2). However, we found that propranolol, a beta receptor antagonist, although still blocking the NE enhancement of LTP, had no effect by itself on LTP (see Fig. 3). These results suggest that intact inhibition is necessary for the modulation of LTP by the endogenous release of NE by noradrenergic fibers. However, inhibition is unnecessary for NE modulation of LTP by exogenously applied NE.

The third hypothesis we wish to test is that NE modulation of LTP takes place postsynaptically. Experiments associated with the testing of this hypothesis are in progress. They consist of intracellular recordings from pyramidal cells before and during LTP. Two sets of experiments are being conducted, one with potassium acetate in the pipette, and the second with potassium acetate plus 8-bromo cyclic AMP in the pipette. If the intracellular injection of 8-bromo cyclic AMP to the postsynaptic neuron enhances LTP, then this would suggest that the NE enhancement of LTP is taking place postsynaptically. The experiments, of course, would not rule out an additional presynaptic effect, but would be strong evidence for a component of the effect taking place postsynaptically. No conclusive results have yet been obtained, as these experiments have only recently begun.

- c. Test hypothesis that NE modulates voltage-dependent calcium conductance. These experiments involve the use of the newly developed acutely exposed hippocampal neuron preparation. This preparation allows for the use of patch-clamp techniques for recording of whole-cell currents as well as currents through single ion channels. We have focused initially on studying calcium currents, using the whole-cell patch configuration. Using sodium and potassium channel blockers both inside and outside the cell, we have been able to isolate a pure voltage-dependent calcium current. We have studied this current before and after applying NE to the outside of the cell. We found that NE produces an increase in the voltage-dependent calcium current (Fig. 4). This effect is similar when using the beta-adrenergic agonist isoproterenol or 8-bromo cyclic AMP, suggesting that beta receptors and cyclic AMP are involved. We have additionally shown that alpha agonists do not increase the calcium current, whereas forskolin mimics the effect of NE, isoproterenol, and cyclic AMP (see Fig. 5). On the basis of these results, we hypothesize that the NE modulation of LTP is through enhanced calcium influx.
- d. Feasibility study for the electrophysiological investigation of mossy fiber synaptosomes. In collaboration with Dr. David Terrian of the AFSAM in San Antonio, we determined the feasibility of studying the electrophysiological properties of mossy fiber synaptosomes. We found that an enriched fraction of mossy fiber



* significantly greater than control, $p < .05$
 ** significantly greater than PTX, $p < .025$

Fig. 2. Noradrenergic enhancement of LTP in the absence of synaptic inhibition. The magnitude of LTP obtained under a variety of conditions is shown. Picrotoxin (PTX) was present where indicated. NE produced a significant enhancement in the magnitude of LTP in the presence of PTX, and therefore, in the absence of synaptic inhibition. These results suggest that synaptic inhibition is not required for noradrenergic modulation of LTP.

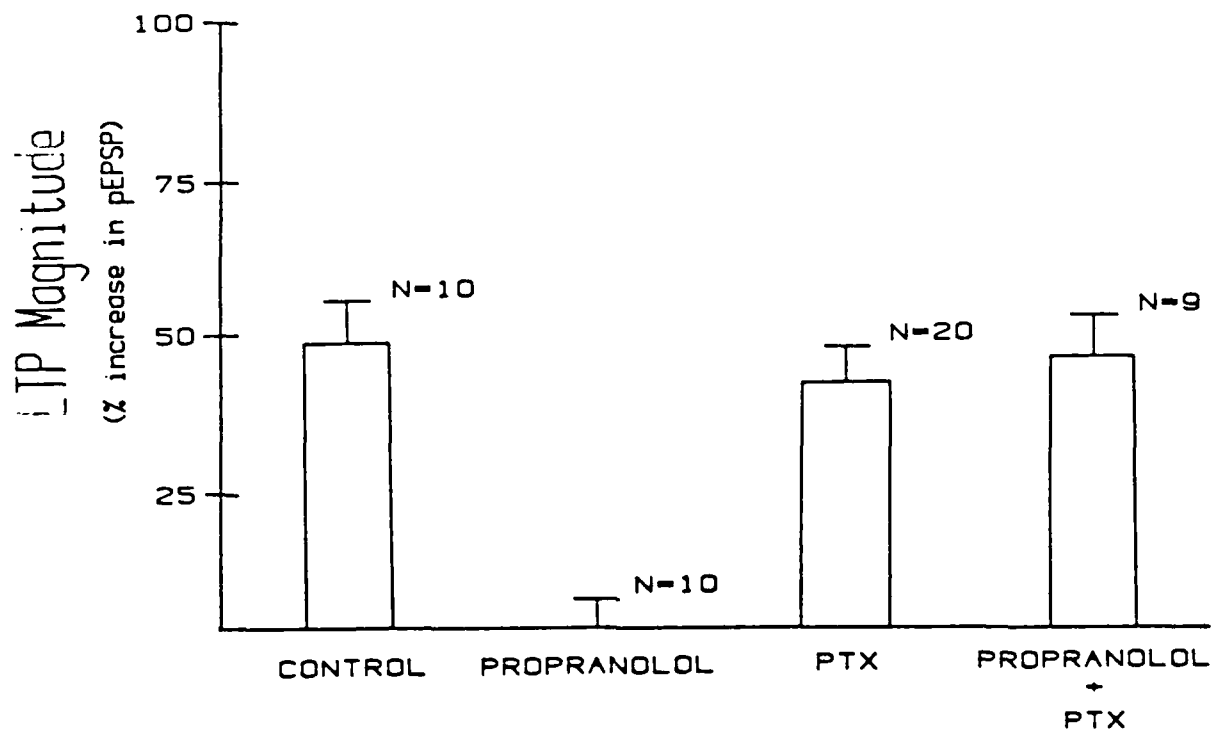


Fig. 3. Effects of propranolol on LTP. Propranolol, a specific beta-adrenergic antagonist, prevents the induction of LTP at the mossy fiber synapses. However, when picrotoxin is added to the bath to block synaptic inhibition, propranolol is shown to have no effect on LTP. These results suggest that the presence of synaptic inhibition is necessary for the modulation of LTP by endogenous NE.

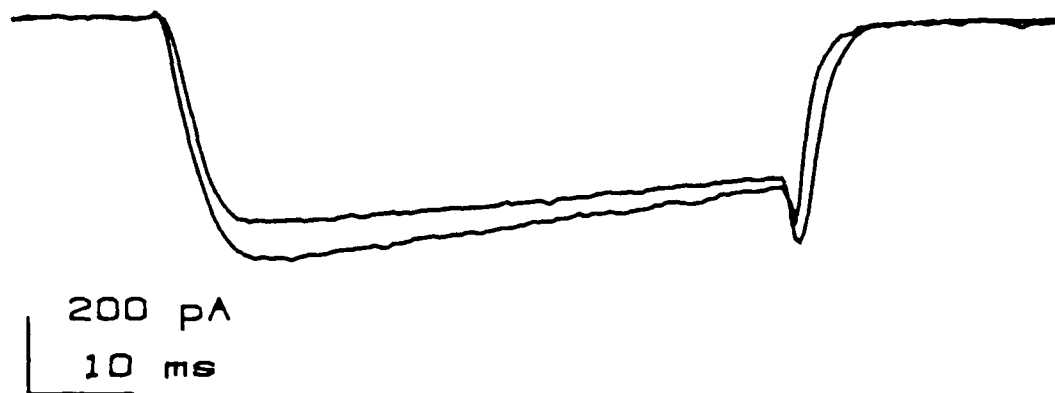
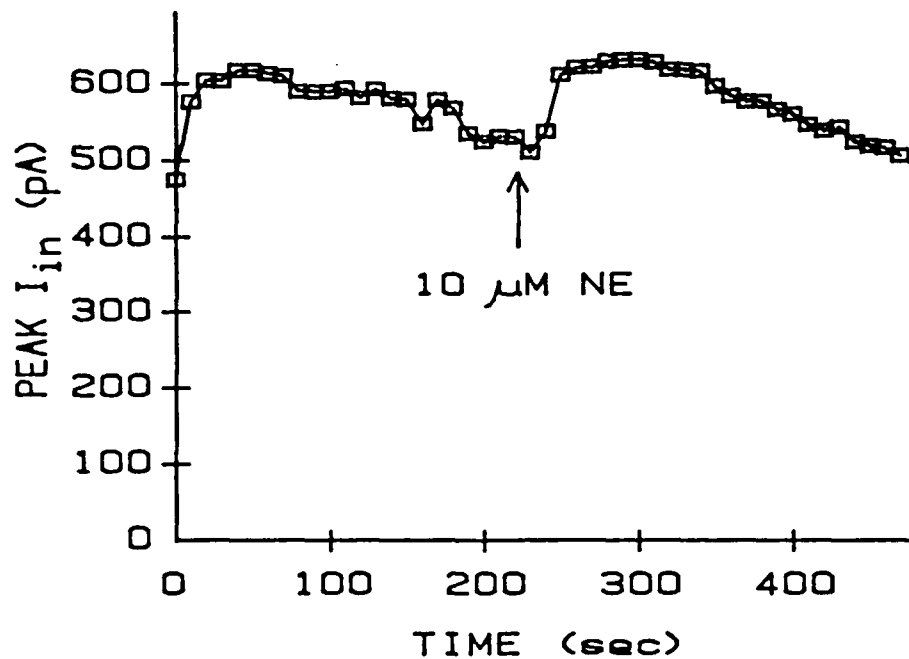


Fig. 4. Noradrenergic enhancement of calcium currents. The results shown are from a whole-cell patch recording of a granule cell from an acutely-exposed hippocampal slice preparation. The top graph displays peak calcium current as a function of time. NE is added to the outside of the cell at the arrow, and an increase in the calcium current is observed following this addition of NE. The bottom half of the figure shows two representative traces before and after applying NE. A clear increase in the peak amplitude of the calcium current can be observed following the addition of NE.

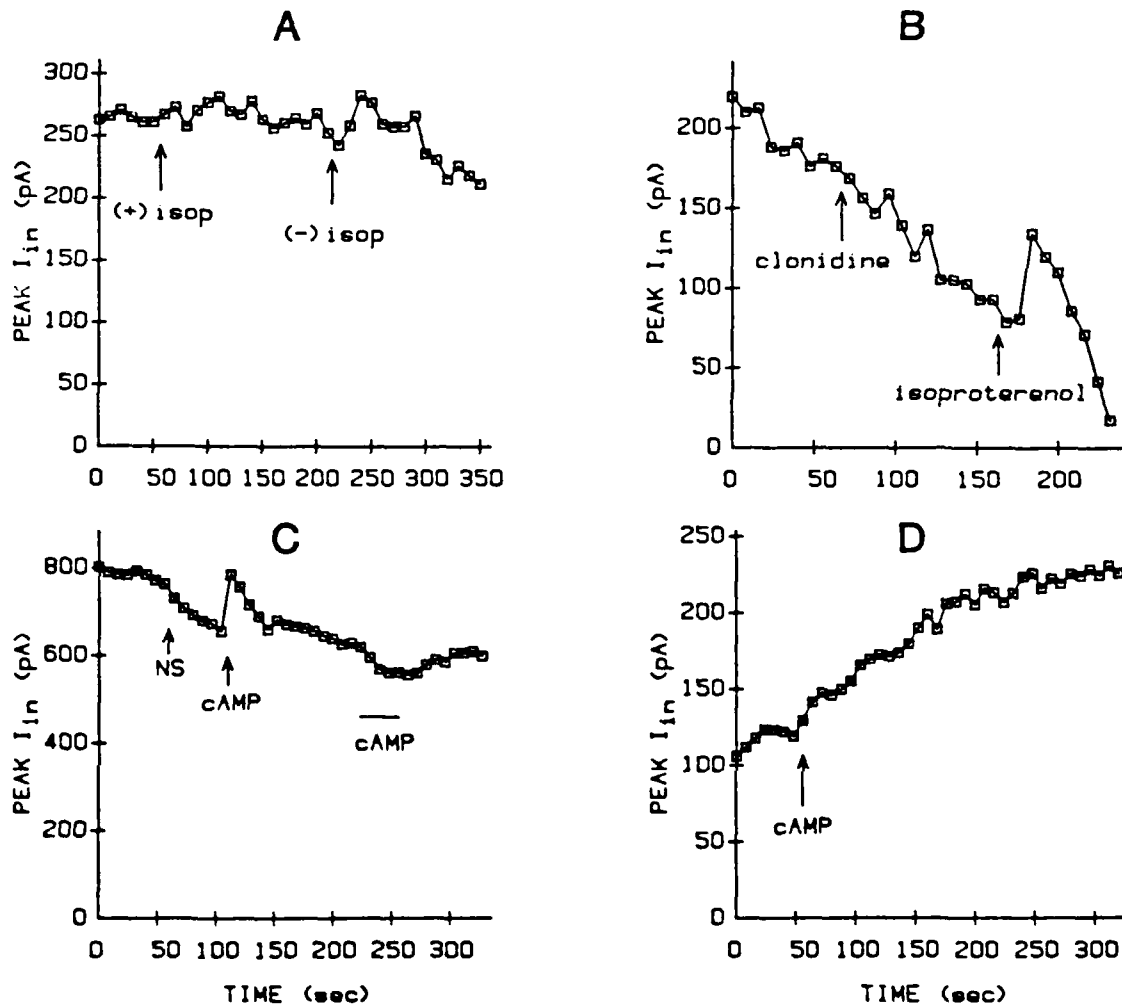


Fig. 5. Pharmacology of the NE enhancement of calcium current. Peak calcium current is plotted as a function of time in four different experiments. A) The inactive (+) and active (-) forms of the beta agonist isoproterenol were applied to the cell. Only the active form appeared to increase the calcium current. B) The alpha agonist clonidine and the beta agonist isoproterenol were applied to the cell at the arrows. Clonidine had no effect, while isoproterenol produced a clear increase in the calcium current. C) Normal saline (NS) was applied at the arrow. Cyclic AMP was applied at the the arrow and for a longer duration at the bar. Normal saline had no effect, while cyclic AMP produced a clear increase in the calcium current. D) A similar experiment as C. Cyclic AMP produced a pronounced increase in the calcium current.

synaptosomes can be isolated from the rat hippocampus and that the synaptosomes are on the order of 5 μ m in diameter. We have tried patch-clamp techniques on these synaptosomes, and have shown that gigohm seals can be formed and single ion channels measured. We are currently in the process of refining the procedures for the isolation of the synaptosomes to enhance the viability of the preparation. It is an extremely exciting and important area of research, as we believe we are the first to actually measure single ion channels from presynaptic elements. Additional equipment must be obtained, however, before this line of research can be pursued on a routine basis.

- e. Development of single-cell computer models. We are continuing the development of a set of computer programs that simulate the electrophysiological characteristics of hippocampal pyramidal cells. We have reached the stage where we have a single-cell model that allows us to incorporate the realistic electrotonic structure and certain active membrane currents (Hodgkin and Huxley sodium current, Hodgkin and Huxley potassium current, and voltage-dependent calcium current) of hippocampal neurons. The quantitative details of the various currents are not known because of the lack of experimental data. As further data are obtained, however, they can now be incorporated into our computer model. Further development of the model will consist of incorporation of realistic synaptic parameters and the networking together of groups of neurons.

4. Publications.

Hopkins, W.F. and Johnston, D. Noradrenergic modulation of synaptic plasticity in the hippocampus. In: Developmental Neurophysiology, Kellaway, P. and Purpura, D.P., (eds.), Johns Hopkins Univ. Press: Baltimore, (in press)

Gray, R. and Johnston, D. Macroscopic calcium currents in acutely-exposed neurons from adult hippocampal slices. Biophys. J., 47:66a, 1985.

Gray, R. and Johnston, D. Macroscopic calcium currents in acutely-exposed granule cells from adult hippocampus. Soc. Neurosci. Abstr. 11:792, 1985.

Griffith, W.H., Brown, T.H., and Johnston, D. Voltage-clamp analysis of synaptic inhibition during long-term potentiation in hippocampus. J. Neurophysiol. 55:xxx-xxx, 1986.

Barrionuevo, G., Kelso, S.R., Johnston, D., and Brown, T.H. Voltage-clamp analysis of long-term potentiation in monosynaptic and isolated synaptic inputs to hippocampus. J. Neurophysiol. 55:540-550, 1986.

Gray, R. and Johnston, D. Multiple types of calcium channels in acutely-exposed neurons from adult hippocampus. Biophys. J. 49:432a, 1986.

Gray, R. and Johnston, D. Norepinephrine increases voltage-dependent calcium current in hippocampal neurons. Nature (in preparation).

Hopkins, W.F. and Johnston, D. Noradrenergic modulation of long-term potentiation in hippocampus. J. Neurophysiol. (in preparation).

5. Professional Personnel Associated With Research Project.

Daniel Johnston, Ph.D.

Frank J. Lebeda, Ph.D.

Richard A. Gray (Ph.D. expected summer 1986)

William F. Hopkins (Ph.D. expected summer 1986)

Stan O. Barber, M.S.

Judy Walker, B.S.

6. Interactions.

5/17/85 Society for Computer Simulation, University of Houston at Clear Lake, Clear Lake City, Texas. Presented lecture on neural modeling of single hippocampal neurons.

9/4/85 Conference on Excitatory Amino Acids and Epilepsy, France. Presented paper on the cellular basis of epilepsy.

10/85 Travelled to Air Force School of Aerospace Medicine, San Antonio, Texas, to visit and collaborate with Dr. David Terrian on project related to mossy fiber synaptosomes.

11/85 Dr. David Terrian from the US Air Force School of Aerospace Medicine, San Antonio, Texas, travelled to Houston to collaborate on a project related to mossy fiber synaptosomes.

11/85 Neuroscience Society Annual Meeting, Dallas, Texas. Rick Gray and Dan Johnston presented paper on noradrenergic enhancement of calcium current in hippocampal neurons.

11/12/85 Fall Task Review, Wright-Patterson Air Force Base, Dayton, Ohio. Presented talk on noradrenergic modulation of LTP.

11/25/85 Rice University Math Science Department, Rice University, Houston, Texas. Presented lecture on neural modeling and networks of hippocampal neurons.

1/6/86 McGill University, Department of Neuroscience, Montreal, Quebec. Presented lecture on cellular mechanisms of epileptogenesis.

1/86 Winter Conference on Brain Research, Keystone, Colorado. Discussed our work on mechanisms of LTP.

1/86 Dr. David Terrian from the US Air Force School of Aerospace Medicine, San Antonio, Texas, travelled to Houston to collaborate on a project related to mossy fiber synaptosomes.

2/86 Biophysical Society Meeting, San Francisco, California. Rick Gray and Dan Johnston presented paper on multiple types of calcium channels in hippocampal neurons.

3/31/86 Department of Pharmacology, Texas A&M University, College Station, Texas. Presented lecture on noradrenergic effects in hippocampus.

3/86 Travelled to Air Force School of Aerospace Medicine, San Antonio, Texas, to visit and collaborate with Dr. David Terrian on project related to mossy fiber synaptosomes.

7. New Discoveries, Inventions, or Patent Applications.

None.

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