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**13. ABSTRACT** *(Maximum 200 words)*

The effects of protein kinase C activators and inhibitors on two behavioral models that probe memory functions have been studied: imprinting in the one day old chick and radial arm maze performance in the adult albino rat. The main conclusion to be drawn is that PKC is necessary but not sufficient for the enhanced durability of memory. In combination with a neural signal, however, PKC demonstrates a profound synergism. This signal can be modulated by a glial-derived factor, S-100.

Our most recent behavioral studies have involved the effects of novelty stress and food deprivation stress on yet another role of PKC: regulation of transcription factor funciton. These studies and the initial results suggest that stress can impact on gene regulation.

**14. SUBJECT TERMS**
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Annual Technical Report

1 March 1991 - 28 February 1992

# Phosphoprotein Regulation of Synaptic Reactivity

Annual Technical Report for AFOSR 90-0240

Submitted to:
Directorate of Life Sciences
Air Force Office of Scientific Research

Submitted by:
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## Annual Technical/Scientific Report

1. Project Period

The project period includes March 1, 1991 to February 28, 1992. The present report is being filed in April, 1992.

2. Summary

A major focus of our work has been on the behavioral importance of the protein kinase C mechanism for developmentally-relevant behaviors. Previously, the regulation of synaptic reactivity by protein kinase C (PKC) and its substrate proteins have been studied in this laboratory using a model of learning and memory, the long-term potentiation paradigm (LTP), biochemical analysis of purified enzymes and cellular analysis of ionic currents.

Two major projects have been recently completed. The first project involves imprinting in 1 day old chicks. The second involves spatial learning in adult rats. In both cases we have demonstrated that protein kinase C (PKC) plays a signficant role. During imprinting, the basic component (pH = 5.0) of a PKC substrate identified in 2-D gels as the MARCKS (myristoylated alanine rich C kinase substrate) is increased in its phosphorylation in relation to filial behavior. In maze learning, a PKC inhibitor has a selective effect on memory for recently learned rules and procedures. This result suggests a new categorization of memory processes.

3. Statement of Work

The research objectives were:

A - Complete the study of imprinting and protein phosphorylation in specific brain regions of the chick

B - Continue to investigate the effect of PKC inhibitors injected into hippocampus on spatial memory processes.

C - Study the effect of novelty stress on transcription factor function.

Our most recent behavioral studies have involved the effects of novelty stress and food deprivation stress on yet another role of PKC: regulation of transcription factor function. These studies and the initial results suggest that stress can impact on gene regulation.

4. Status of research

Significant accomplishments made during this period were:

A. Imprinting in the Chick

In the study of imprinting, chicks were exposed to a significant stimulus (a rotating red light) while a control group was kept in the dark. The trained group was also studied with respect to the activity levels it showed in the presence of the imprinting stimulus and with respect to its preference for that stimulus vis a vis another stimulus to which it was not imprinted.

Dissected brain was analyzed for phosphorylation. Regions previously implicated in the process of imprinting were dissected from regions previously excluded from involvement. Specifically, lesions of the visual Wulst does not appear to disrupt imprinting while the intermediate region of the hyperstiratum ventrale (IMHV) has been implicated (Horn, 1988). Interestingly, only the left IMHV has been given an important functional role, so in the present study we compared the role of the two hemispheres.

We have found that there is a significant increase in the phosphorylation of the MARCKS protein in the left IMHV in animals that have been imprinted vs. animals that have been reared in the dark (Sheu et al., 1991; Sheu et al., 1992). Interestingly, there was no effect of training on protein F1 phosphorylation. Nor were alterations detected in any other brain region studied or in any other protein studied. Thus, a selective unilateral alteration in a PKC substrate, the MARCKS protein, has been observed. It is particularly interesting that like the mammal the alteration detected is in a PKC substrate. But unlike the mammal, the bird shows a selective change in one substrate (MARCKS) but not another (F1/GAP43).

A particularly interesting feature of the 2-dimensional gel analysis was the finding that the alteration in phosphorylation was related to the basic component of the MARCKS protein - the 5.0 spot - while the acidic component (pH = 4.0) was unaffected. This is especially interesting because the 5.0 MARCKS is attached to the membrane. Thus, we speculate that PKC may be translocated to the membrane after imprinting and phosphorylate MARCKS 5.0.

B. Role of PKC in spatial memory

Because PKC inhibitors block LTP and LTP is considered a model of learning, it is reasonable to ask whether PKC inhbitors will have an effect on learning itself. For this purpose we have studied performance in the 8-arm radial maze after PKC inhibition in the hippocampus. We and others have shown that performance in this maze is highly sensitive to manipulation of the hippocampus.

Animals are trained to go back to the same location to find the food again. Note that rats are foragers and thus go to those locations that they have not visited previously. The type of behavioral pattern we impose is opposed to this tendency. Thus, the animal has to overcome natural tendencies in order to master the new task.

We have found that PMXB has a profound effect on the ability of the animal to remember the location of the food on the second trial (Cutting et al., submitted). What makes this important is that there is no effect on the ability of the rat to search for the food, the foraging behavior.

Moreover, the effect is not permanent as the animal appears to be normal the next day.

It is reasonable to ask whether the impairment is in one or the other strategy. One can also view the first type of memory as closely related to the initial enhancement induced by an LTP-like process. The second type of memory is more closely related to a long-term storage process. If the first is the case, then we would imagine that it is acting postsynaptically, since we have recently shown that PKC post-synaptically is important in the earliest stages (0-15 min) of the development of LTP (Huang, Colley and Routtenberg, 1992).

C. Stress and Gene Expression: Effect of Novelty on Transcription Factor Function

What do we learn from a stressful experience? How long do we retain the memory of that experience? What is the mechanism for that retention? We have recently begun to study the effects of brief novel experiences on the regulation of gene expression of precisely those proteins, PKC and protein F1, which we have identified as critical for short-term storage of information.

We are proposing two ideas: first, PKC activation occurs both at the synapse and at the cell body and that the former mediates the initial changes in synaptic efficacy and the latter the subsequent long-term changes. In the initial stages PKC phosphorylates proteins present at the synapse; in the second stage PKC phosphorylates transcription factors that regulate gene expression.

We mildly stress our animals by placing them in a strange environment, one they have not seen before. In this situation they explore the maze for the first 7-10 min and then they typically remain motionless until removed from the environment. We have found that under the circumstance when the animal is removed from the maze after 4 min, a significant increase in the binding of protein from hippocampus to the DNA recognition elements that regulate transcription is observed (Kinney and Routtenberg, submitted). However, when the animal is allowed to stay in the maze for 15 min and then removed, no alteration is observed.

These results strongly suggest that mild novelty stress alters the binding of protein to DNA Moreover, this process would appear to be under post-translational control. We suggest the following scenario. While the animal is exploring its environment, neuronal activity in the hippocampus is generated and this in turn activates PKC. This alters the phosphorylation state of nuclear proteins (transcription factors) which increases their binding to DNA. In the circumstance when the animal is removed from the maze at 15 min, the PKC activation is finished, the transcription factor is dephosphorylated and binding to DNA is reduced.

5.Articles published, accepted for publication and submitted.

Published

1. Florez, J.C., Nelson, R.B. and Routtenberg, A. Contrasting patterns of protein phosphorylation in human normal and Alzheimer brain: Focus on protein kinase C and protein F1/GAP-43. Experimental Neurology, 1991, 112, 264-272.

2. Routtenberg, A. A tale of two contingent protein kinase C activators: Both neutral and acidic lipids regulate synaptic plasticity and information storage. Progress in Brain Research, W. Gispen and A. Routtenberg (Eds.), Vol. 89, 1991, 249-261.

3. Gispen, W.H. and Routtenberg, A. (Eds.) Protein Kinase C and its Brain Substrates: Role in Neuronal Growth and Plasticity, Progress in Brain Research, Vol. 89, 1991, Elsevier Publishers.

4. Meberg, P.J. and Routtenberg, A. Selective expression of F1/GAP43 mRNA in pyramidal but not granule cells of the hippocampus. Neuroscience, 1991, 45:3, 721-731.

5. Routtenberg, A. Trans-synaptophobia revisited. Long-Term Potentiation: A Debate of Current Issues, M. Baudry and J.L. Davis (Eds.), MIT Press, 1991, 155-167.

6. Cain, S.T. and Routtenberg, A. Phosphorylation of pyruvate dehydrogenase in the hippocampal slice: time course of response to cellular depolarization. Neuroscience Letters, 1991, 130, 65-68.

7. Cain, S.T., Akers, R.F., and Routtenberg, A. Functional regulation of brain pyruvate dehydrogenase: Postnatal development, anesthesia and food-deprivation. Neurochem Int., 1991, 19, No. 4, 549-558.

In press

1. Huang, Y.Y., Colley, P.A. and Routtenberg, A. Postsynaptic then presynaptic protein kinase C activity may be necessary for long-term potentiation. Neuroscience, 1992, in press.

2. Routtenberg, A. Resisting memory storage by activating endogenous protein kinase C inhibitors that regulate signal transduction. Phosphclipids and Signal Transmission, L.A. Horrocks (Ed.), Springer Verlag, Berlin, 1992, in press.

Submitted

1. Sheu, F.-S., McCabe, B.J., Horn, G. and Routtenberg, A. Learning selectively increases protein kinase C substrate phosphorylation in specific regions of the chick brain, submitted.

2. Kinney, W. and Routtenberg, A. Brief exposure to a novel environment enhances binding of hippocampal transcription factors to DNA. Submitted.

3. Colley, P.A. and Routtenberg, A. Long-term potentiation as synaptic dialogue. Submitted.

4. Meberg, P.J., Barnes, C.A., McNaughton, B.L. and Routtenberg, A. Selective alterations in hippocampal gene expression after synaptic enhancement. Submitted.

5. Farley, J. and Routtenberg, A. Potassium channel activity in hippocampal synaptosomes is reduced during LTP. Submitted.

6. Sheu, F.-S., Azmitia, E.C., Marshak, D.R., Parker, P.J. and Routtenberg, A. Glial-derived S100b protein selectively inhibits recombinant beta protein kinase C phosphorylation of neuron-specific protein F1/GAP43: implications for a glial-neuronal interaction. Submitted.

6. Personnel

| Name | Title | Dates of Service | % Effort |
|---|---|---|---|
| A. Routtenberg | Professor/PI | 9/83-present | 25% |
| P. Meberg | Grad. Res. Asst. | 9/87-present | 50% |
| W. Kinney | Sr. Technician | 4/91-present | 50% |
| Y. Chen | Res. Assoc. | 7/91-present | 50% |
| S. Grishayev | Tech. Programmer | 1/92-present | 50% |

7. Coupling Activities (Meetings, Seminars)

1. Routtenberg, A. Invited speaker. Hitachi Chemical Research Center, Inc., Irvine, California, May 5-7, 1991. (Dr. Gabriel Eilon)

2. Routtenberg, A. Invited speaker. Department of Cell, Molecular, and Structural Biology, Northwestern University, "Membranes, Molecules, Memories, Modules and the Mind."

3. Routtenberg, A. Invited speaker. American Association of Anatomists 1991 annual meeting, "Cellular Mechanisms of Behavioral Plasticity." Chicago, Illinois, April 20, 1991.

4. Farley, J. and Routtenberg, A. LTP reduces K+ Channel activity in hippocampal synaptosomes. Soc. Neurosci., 1991, 17:1

5. Meberg, P.J., McCabe, B.J., Horn, G., Rosenfeld, J.P. and Routtenberg, A. Differential mRNA distribution in chick brain of two protein kinase C (PKC) substrates, F1/GAP43 and Marcks. Soc. Neurosci. 1991, 17:140.

6. McCabe, B.J., Sheu, F.-S., Horn, G. and Routtenberg, A. Memory alters protein kinase C substrate (Marcks) phosphorylation. Soc. Neurosci. 1991, 17: 140.

7. Sheu, F.-S., Azmitia, E.C., Marshak, D.R., Parker, P.J. and Routtenberg, A. Glial-derived S-100 protein selectively inhibits the neuron-specific protein F1/GAP-43 phosphorylation by beta 1 recombinant protein kinase C: Implications for a glial-neuronal interaction. Soc. Neurosci. 1991, 17: 140.

8. Routtenberg, A. Co-organizer and speaker. "Nuts and bolts of memory." Winter conference on the Neurobiology of Learning and Memory, Park City, Utah, January 9-13, 1992.

9. Routtenberg, A. Invited speaker. The American Psychological Society 1992 Annual Meeting, San Diego, California, June 20-22, 1992. (Dr. Jim McGaugh)

10. Routtenberg, A. Invited speaker. Limerick, Ireland, August, 1992.

11. Routtenberg, A. Invited speaker. 32nd International Congress of Physiological Sciences, Glasgow, Scotland, August 1-6, 1993. (Dr. Gabriel Horn)

8. New Directions/Discoveries

Since presynaptic terminal potassium ion channels are involved in the regulation of neurotransmitter release at many synapses, a persistent reduction in their activity induced by tetanizing stimulation might be expected to contribute to LTP. We have examined this possibility by incorporating hippocampal synaptosomal vesicle membranes, from animals in which LTP was induced into planar lipid bilayers (Farley and Routtenberg, submitted). We observed a near-elimination of K+ channel activity in these membranes, as compared to that of sham and low-frequency stimulation controls. The possibility that this effect was mediated by protein kinase C was considered.

We plan to study the effect of novelty stress on channel activity and determine whether alterations observed are mediated by PKC.