PHOSPHOPROTEIN REGULATION OF SYNAPTIC REACTIVITY: ENHANCEMENT OF A MOLECULE. (U) NORTHWESTERN UNIV. EVANSTON IL COLL. OF ARTS AND SCIENCES. A ROUTTENBERG. 15 AUG 07.

UNCLASSIFIED AFOSR-TR-07-1499 AFOSR-07-0042 F/G 6/1 ML.
Phosphoprotein Regulation of Synaptic Reactivity: Enhancement of a Molecular Gating Mechanism

Aryeh Routtenberg

12. PERSONAL AUTHORS

13a. TYPE OF REPORT
Annual Technical Progress

13b. TIME COVERED
FROM 10/1/86 TO 9/30/87

August 15, 1987

14. DATE OF REPORT (Yr., Mo., Day)

15. PAGE COUNT

16. SUPPLEMENTARY NOTATION

17. COSATI CODES

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

Synapse; plasticity; reactivity; phosphorylation; protein kinase C; protein Fl; fatty acids

10. ABSTRACT (Continue on reverse if necessary and identify by block number)

This research is focussed on the role of molecular switches in brain which regulate synaptic reactivity. We have identified a 47,000 mol wt phosphoprotein and its kinase (c) which play a pivotal role in this regulation. We have enhanced communication among nerve cells studied electrophysiologically by activation of this kinase. In the proposed research we specifically manipulate this brain phosphoprotein with novel kinase activating agents. In a new initiative, both the kinase and the Fl substrate will be studied with the goal of enhancing synaptic reactivity by regulating the activity of proteins that play a pivotal role in this process.

UNCLASSIFIED
1. Project Period

The project period includes October 1, 1986 to September 30, 1987. The present report is being filed in August, 1987.

2. Summary

The regulation of synaptic reactivity by protein kinase C and its substrate proteins has been studied using the long-term potentiation paradigm (LTP). In the past year we have studied the effects of protein kinase C activators and inhibitors on durability of synaptic reactivity. The main conclusion to be drawn is that protein kinase C is necessary but not sufficient for the enhanced durability. In combination with a neural signal, however, PKC demonstrates a profound synergism. Synergism is also observed in the analysis of metal ion regulation of protein kinase C activity. Calcium and zinc interact in their effect on the enzyme in a bidirectional manner (see below).

3. Statement of Work

The research objectives during this period were to:

a. Study effects of protein kinase C inhibitors

b. Study activators of PKC in the synaptic zone
c. Study metal ion regulation of PKC

4. Status of Research

Significant accomplishments made during this period were:

a. Effect of inhibitors

We used three separate inhibitors of protein kinase C activity,
polymyxin B, mellitin and H-7, each with a different mechanism of action. Application was made by micro-pressure ejection into the molecular layer of the dentate gyrus before or after LTP. The major result of this study was that inhibitors had no effect on the initiation of LTP but completely eliminated the enhanced response 10-15 min after its initiation. This provides strong support for our view that PKC plays a critical role in the maintenance but not the initiation of LTP.

b. Study of PKC activators (PDBu and oleate)

A crucial question in the analysis of the role of PKC in synaptic reactivity is the site of action of the compound. Indirect evidence suggested a synaptic site since PKC is found in high concentration there. To assess this view directly we compared application dosages required to facilitate synaptic reactivity duration in the dentate hilus, a nearby site, and the molecular layer of the dentate, 100 micra from the granule cells, precisely the point where perforant path terminals synapse. We have found that only 10-16% of the dosage is required when the application, iontophoretic or micro-pressure, is made at the synaptic zone. This provides strong support for the synaptic site of action of these protein kinase C activators.

c. Metal ion regulation of protein kinase C activity

Recent evidence describing the primary structure of protein kinase C by several laboratories indicates several different motifs: ATP-binding, Ca-binding, kinase domain, zinc "fingers". This suggested the possibility that both zinc as well as calcium might regulate protein kinase C activity.
Since we have recently discovered that protein kinase C can be activated in the absence of calcium it was now feasible to study the effects of zinc both in the presence and the absence of calcium. A novel mechanism for regulating protein kinase C activity was discovered in which zinc ions, found in highest concentration in the hippocampus, enhance protein kinase C activity at low calcium levels. At higher levels of calcium, zinc inhibits. We propose a model of protein kinase C with a low calcium affinity binding sites and a distinct zinc binding site.

5. Articles published, accepted for publication and submitted.


Linden, D., Murakami, K., and Routtenberg, A. A newly discovered protein kinase C activator (oleic acid) preserves synaptic plasticity and promotes the growth of long-term potentiation. Brain Research, 1986, 379, 358-363.


Nelson, R.B., Friedman, D.P., O'Neill, J.B., Mishkin, M., and Routtenberg, A. Gradients of PKC substrate phosphorylation along the primate visual
cortical processing pathway: Highest levels are in visual memory areas. Brain Research, 1987, in press.

Akers, R.F. and Routtenberg, A. Calcium-promoted translocation of protein kinase C to synaptic membranes: relation to the phosphorylation of an endogenous substrate (Protein F1) involved in synaptic plasticity. J. Neurosci., 1987, in press.


Linden, D., Shew, F.-S., Murakami, K., and Routtenberg, A. Cis fatty acid regulation of synaptic potentiation: Relation to phospholipase A2 and protein kinase C activation. J. Neurosci., 1987, in press.


Benowitz, L.I. and Routtenberg, A. A membrane phosphoprotein associated with neural development, axonal regeneration, phospholipid metabolism, and synaptic plasticity. Submitted.

Lovinger, D.M. and Routtenberg, A. Synapse specific protein kinase C activation enhances maintenance of long-term potentiation in rat hippocampus. Submitted.

Nelson, R.B., Linden, D.J., and Routtenberg, A. Two protein kinase C substrates and two vesicle-associated phosphoproteins are directly
correlated with persistence of long-term potentiation: A quantitative analysis of two-dimensional gels. Submitted.

Nelson, R.B., Hyman, C., Pfenninger, K.H., and Routtenberg, A. Two protein kinase C substrates directly correlated with persistence of long-term potentiation in adult rat brain are the major phosphoproteins found in nerve growth cones. Submitted.

Chan, S.Y., Nelson, R.B., Murakami, K., and Routtenberg, A. Protein kinase C substrate (F1) phosphorylation: Phospholipid-independent, Ca2+-enhanced CIS-fatty acid activation. Submitted.


Nelson, R. and Routtenberg, A. The protein F1/protein kinase C module and neurite growth: Potential utility in facilitating brain

6. Personnel

<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
<th>Dates of Service</th>
<th>% Effort</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Routtenberg</td>
<td>Professor/PI</td>
<td>9/83-present</td>
<td>25%</td>
</tr>
<tr>
<td>S. Chan</td>
<td>Res. Neurobiologist</td>
<td>2/84-present</td>
<td>25%</td>
</tr>
<tr>
<td>K. Murakami</td>
<td>Res. Neurobiologist</td>
<td>4/84-present**</td>
<td>25%</td>
</tr>
<tr>
<td>P. Colley</td>
<td>Grad. Res. Asst.</td>
<td>7/83-present</td>
<td>50%</td>
</tr>
<tr>
<td>D. Linden</td>
<td>Grad. Res. Asst.</td>
<td>9/84-present</td>
<td>50%</td>
</tr>
<tr>
<td>D. Lovinger</td>
<td>Grad. Res. Asst.</td>
<td>7/83-present*</td>
<td>50%</td>
</tr>
<tr>
<td>R. Nelson</td>
<td>Grad. Res. Asst.</td>
<td>7/83-present*</td>
<td>50%</td>
</tr>
<tr>
<td>F. Sheu</td>
<td>Grad. Res. Asst.</td>
<td>9/85-present</td>
<td>50%</td>
</tr>
<tr>
<td>K. Wong</td>
<td>Grad. Res. Asst.</td>
<td>4/86-present</td>
<td>50%</td>
</tr>
</tbody>
</table>

* - Ph.D. awarded 6/87
** - Assistant Professor, University of Buffalo, September, 1987.

7. Coupling Activities

Colley, P. and Routtenberg, A. Hypothesis: Protein kinase C activation acts synergistically with a calcium-mediated event to induce long-lasting synaptic changes in the hippocampus. *Soc. Neurosci.*, 1986, 12, 1168.

Lovinger, D., Barnes, C.A., Mizumori, S.J.Y., Chan, S.Y.; Linden, D., Murakami, K., Sheu, F.-S., and Routtenberg, A. Protein F1, previously related to synaptic plasticity, exhibits decreased phosphorylation in...

Nelson, R.B., Friedman, D.P., Mishkin, M., and Routtenberg, A. Protein kinase C substrate phosphorylation in primate cerebral cortex (E.G. Protein F1) is increased in those stages of the occipitotemporal visual processing pathway important for information storage. Soc. Neurosci., 1986, 12, 1168.


Murakami, K., Chan, S.Y., and Routtenberg, A. Protein kinase C (PKC) activation by cis-fatty acid is distinct from previously described phospholipid and Ca2+ activation. Soc. Neurosci., 1986, 12, 1169.


Routtenberg, A. Phospholipid and fatty acid regulation of signal transduction at synapses: Potential role for protein kinase C in information storage. Invited Lecture: Fourth meeting of the International Study Group on The Pharmacology of Memory Disorders Associated with Aging. Hotel Zurich, Zurich, Switzerland, January

END
Feb.
1988
Dtic