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# FEEDBACK CONTROL OF THE INOSITOL PHOSPHOLIPID RESPONSE IN RAT BRAIN IS SENSITIVE TO ACTH

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Activation of receptors known to mobilize intracellular calcium results in the specific hydrolysis of inositol phospholipids, yielding diacylglycerol (DG) and inositol phosphates. At least one of the inositol trisphosphate ($IP_3$) isomers and inositol phosphates containing more phosphate groups have been implicated in calcium mobilization, whereas DG stimulates protein kinase C (for references see Majerus et al., 1985; Michell, 1986). In brain synaptic plasma membranes the protein B-50 ($M_r$ 48 kDa, IEP 4.5) is a major substrate for protein kinase C. As enhanced phosphorylation of B-50 is accompanied by a decrease in the activity of phosphatidylinositol 4-phosphate (PIP) kinase, it has been proposed that the sequence DG – protein kinase C activation – B-50 phosphorylation – PIP kinase inhibition may represent a negative feedback control in the receptor-mediated hydrolysis of inositol phospholipid (Gispen, 1986). Several implications of this model are evident.

For instance, direct modulation of protein kinase C activity prior to receptor activation would lead to altered amounts of phosphatidylinositol 4,5-bisphosphate ($PIP_2$) available for receptor-activated hydrolysis. In fact, several authors have recently shown that direct activation of protein kinase C by means of phorbol esters reduces the production of inositol phosphates in response to muscarinic receptor activation in brain (see e.g. Labarca et al., 1984). We have shown previously that protein kinase C can be inhibited by ACTH and congeners and that such a mechanism may account for part of the effects of these peptides in the nervous system (Gispen, 1986). In the present study we report for the first time that in hippocampal slices, ACTH may modulate signal transduction through the above suggested sequence of events.

Transverse rat hippocampal slices were cut (400 $\mu$m thick), incubated (3 per tube) in Krebs Ringer buffer (KRB; 124 mM NaCl, 5 mM KCl, 1.2 mM $NaH_2PO_4$, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, 10 mM glucose and 2 mM $CaCl_2$; pH 7.4, gassed with 95% $O_2$-5% $CO_2$). After preincubation for 30 min, ACTH-(1-16)$NH_2$ ($5 \times 10^{-5}$ M; Organon Int. BV, Oss, NL) was added by replacing the KRB (t = 30 min). At t = 50 min, phorbol-12,13-dibutyrate (PDB, 1 $\mu$M) was added and at t = 60 min the mixture was replaced by fresh KRB, containing 2 mM carbamylcholine (Sigma, St. Louis, U.S.A.), 10 mM LiCl, 2 $\mu$Ci myo-2-[$^3$H]inositol (16.9 Ci/mmol, Amersham, U.K.) and ACTH-(1-16)$NH_2$ and PDB when indicated. Incubations were terminated at 150 min by washing the slices twice with KRB after which the lipids were extracted according to the method of Hunter et al. (1985). Inositol-1-phosphate (IP) formation was determined by batch absorption of 300 $\mu$l of the methanol/water phase to 0.25 ml Dowex (1 × 8, 100-200 mesh, formate form). Non-incorporated label was removed with 4 washes (2.5 ml) of 5 mM myo-inositol in distilled water. Subsequently, the glycerophosphoinositides were eluted with 5 × 2.5

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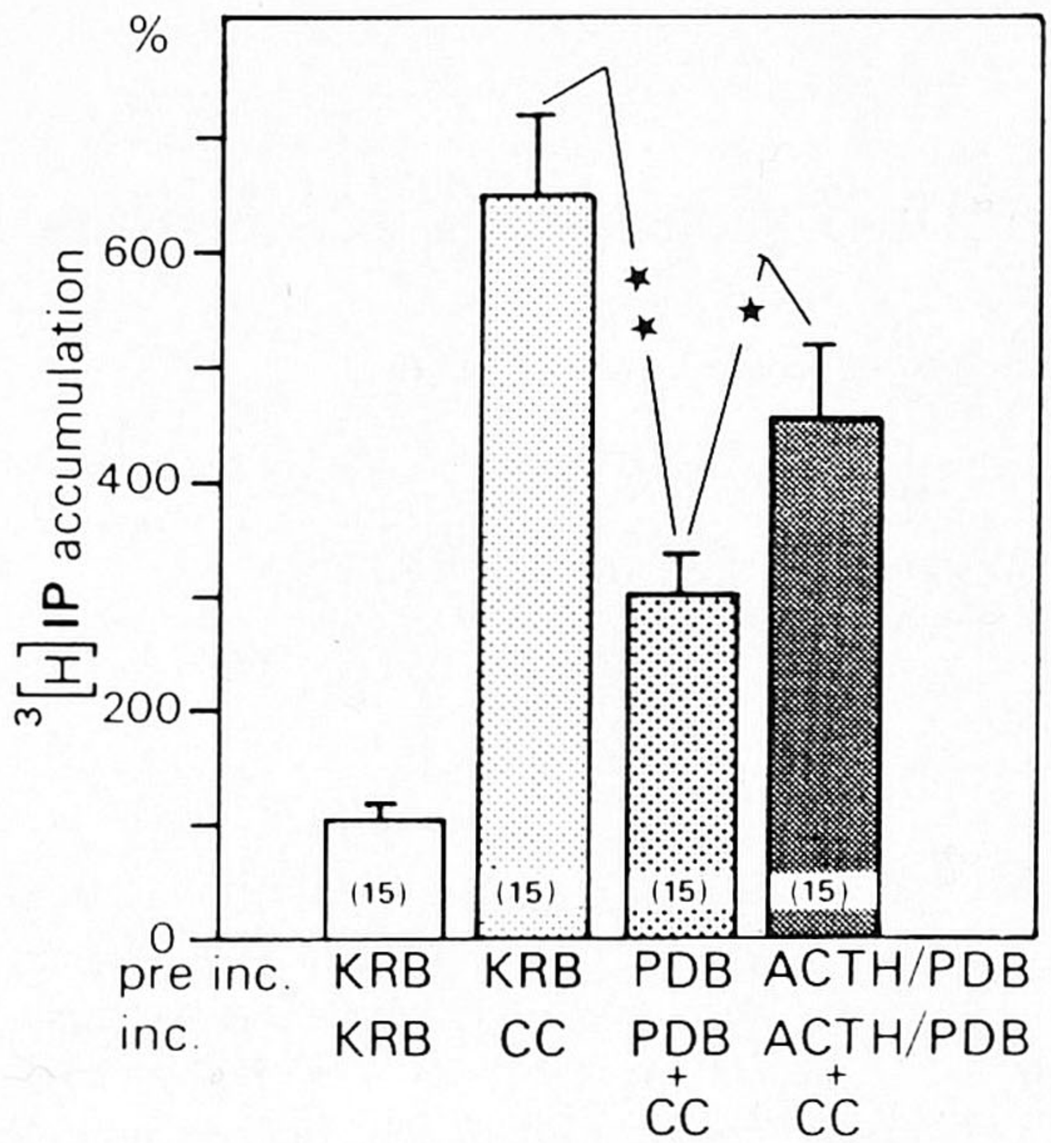


Fig. 1. The effect of ACTH-(1-16)$NH_2$ on the inhibition of carbamylcholine (CC)-stimulated [$^3$H]inositol 1-phosphate ([$^3$H]IP) accumulation by phorbol-12,13-dibutyrate (PDB) in rat hippocampal slices. Pre inc., preincubation; inc., incubation; KRB, Krebs Ringer buffer; CC, $2\times10^{-3}$ M; PDB, $10^{-6}$ M; ACTH-(1-16)$NH_2$, $5\times10^{-5}$ M. Data are from 3 independent experiments (n = 5 each). The mean $^3$H-incorporation into IP in the controls (n = 15) was 440 dpm/mg dry weight. The data are expressed as percentage of the control ± S.E.M. ** Significant difference ($P < 0.001$); * significant difference ($P < 0.05$; ANOVA, followed by supplemental t-test, two-tailed).

ml 5 mM Na-tetraborate, 60 mM Na-formate. IP was eluted with 0.5 ml 100 mM formic acid and 200 mM ammonium formate and the radioactivity was determined in an aliquot of 0.4 ml by liquid scintillation counting (efficiency 25%). The incorporation was expressed as dpm [$^3$H]IP/mg dry weight (Hunter et al., 1985). Carbamylcholine stimulated the formation of IP 6.5-fold whereas preincubation with 1 $\mu$M PDB inhibited the carbamylcholine stimulation by half (fig. 1). These data are in agreement with those reported by Labarca et al. (1984) who used similar incubation conditions. Incubation of slices with either ACTH or PDB alone did not affect IP labelling. Furthermore, ACTH did not affect the carbamylcholine-induced stimulation of IP (data not shown). However, pretreatment of slices with ACTH-(1-16)$NH_2$ reduced the effect of PDB on carbamylcholine-stimulated IP labelling.

Experiments of this nature do not reveal which of the inositol phospholipids is hydrolyzed. In view of the long incubation time, it is likely that more than one inositol phospholipid may have contributed to the IP accumulation, as current research by others using a variety of systems seems to indicate (Majerus et al., 1985). However, hydrolysis of inositol phospholipids results in all instances in the production of DG, thus activating the proposed feedback loop. The present data suggest that peptide modulation of signal transduction is apparent after sustained receptor-mediated kinase C activation. In summary, these data support the proposed mechanism of action of ACTH whereby the peptide may influence chemical neurotransmission in certain synapses by modulating a feedback control in receptor-activated inositol phospholipid hydrolysis (Gispen, 1986).

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## References

Gispen, W.H., 1986, Phosphoprotein B-50 and phosphoinositides in brain synaptic plasma membranes: a possible feedback relationship, Trans. Biochem. Soc. U.K. 14, 163.

Hunter, J.C., M. Goedert and R.D. Pinnock, 1985, Mammalian tachykinin-induced hydrolysis of inositol phospholipids in rat brain slices, Biochem. Biophys. Res. Commun. 127, 616.

Labarca, R., A. Janowsky, J. Patel and S.M. Paul, 1984, Phorbol esters inhibit agonist-induced [$^3$H]inositol 1-phosphate accumulation in rat hippocampal slices, Biochem. Biophys. Res. Commun. 123, 703.

Majerus, P.W., D.B. Wilson, Th.M. Connolly, T.E. Bross and E.J. Neufeld, 1985, Phosphoinositide turnover provides a link in stimulus-response coupling, TIBS 10, 168.

Michell, R.H., 1986, Inositol phosphates; profusion and confusion, Nature 319, 176.