

# Modulation of brain polyphosphoinositide metabolism by acth and beta-endorphin

Citation for published version (APA):

Jolles, J., Bar, P. R., & Gispen, W. H. (1981). Modulation of brain polyphosphoinositide metabolism by acth and beta-endorphin: structure-activity studies. *Brain Research*, 224(2), 315-326.  
[https://doi.org/10.1016/0006-8993\(81\)90862-3](https://doi.org/10.1016/0006-8993(81)90862-3)

## Document status and date:

Published: 01/01/1981

## DOI:

[10.1016/0006-8993\(81\)90862-3](https://doi.org/10.1016/0006-8993(81)90862-3)

## Document Version:

Publisher's PDF, also known as Version of record

## Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

## General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

[www.umlib.nl/taverne-license](http://www.umlib.nl/taverne-license)

## Take down policy

If you believe that this document breaches copyright please contact us at:

[repository@maastrichtuniversity.nl](mailto:repository@maastrichtuniversity.nl)

providing details and we will investigate your claim.

## MODULATION OF BRAIN POLYPHOSPHOINOSITIDE METABOLISM BY ACTH AND $\beta$ -ENDORPHIN: STRUCTURE-ACTIVITY STUDIES

J. JOLLES, P. R. BÄR and W. H. GISPEN\*

*Division of Molecular Neurobiology, Rudolf Magnus Institute for Pharmacology and Laboratory of Physiological Chemistry, Medical Faculty, Institute of Molecular Biology, State University of Utrecht (The Netherlands)*

(Accepted March 19th, 1981)

**Key words:** ACTH — endorphins — polyphosphoinositides — structure-activity studies — phosphatidylinositol — grooming behavior — phosphorylation

---

### SUMMARY

This study describes effects of ACTH<sub>1-24</sub> and  $\beta$ -endorphin on brain polyphosphoinositide metabolism *in vitro*. The interconversion of these polyanionic phospholipids was studied by incubation of a lysed synaptosomal fraction with [ $\gamma$ -<sup>32</sup>P]ATP. Of the membrane phospholipids only PA, DPI and TPI became labeled. The reference peptide ACTH<sub>1-24</sub> stimulated the formation of TPI and inhibited the production of PA. For effects on TPI formation both the sequences ACTH<sub>5-7</sub> and ACTH<sub>10-16</sub> were needed. Effects on PA formation required the sequences ACTH<sub>7-10</sub> and ACTH<sub>10-16</sub>. The basic amino acids in ACTH<sub>10-16</sub> seemed to be of crucial importance for the peptide effects. A stimulatory effect on DPI was visible when ACTH was shortened from the N-terminus, and the essential information was in ACTH<sub>7-10</sub>.  $\beta$ -endorphin inhibited PA formation and this effect was abolished by C-terminal shortening to  $\gamma$ -endorphin. Other fragments of the C-terminus of  $\beta$ -LPH, including the enkephalins, were ineffective. It is concluded that the structure-activity relationship on TPI/PA formation correlates with a similar relationship obtained on excessive grooming behavior *in vivo*. A possible correlation between the effects on polyPI metabolism and opiate-like effects, and effects on extinction of active avoidance behavior *in vivo* is discussed.

---

\* To whom correspondence should be sent at: Division of Molecular Neurobiology, Institute of Molecular Biology and Rudolf Magnus Institute for Pharmacology, University of Utrecht, Padualaan 8, 3508 TB Utrecht, The Netherlands.

## INTRODUCTION

Peptides, which are structurally related to ACTH, MSH and  $\beta$ -LPH, are important modulators of both animal and human behavior<sup>5</sup>. Among the reported behavioral effects are those on avoidance behavior<sup>14,15</sup> and on induction of excessive grooming behavior in the rat<sup>10,12</sup>. These effects are a consequence of a direct action of these peptides on specific areas of the central nervous system<sup>4,5</sup>. In *in vitro* studies, effects of ACTH-like peptides on brain membrane processes have been obtained. In these membranes, structure-dependent effects on adenylate-cyclase<sup>35</sup> and protein phosphorylation<sup>37</sup> were found that correlated with the structure-activity relationship observed for the induction of excessive grooming behavior *in vivo*<sup>12,13</sup>. In addition, ACTH<sub>1-24</sub> was found to affect the metabolism of a special class of brain membrane phospholipids, the polyphosphoinositides<sup>20,21</sup> and a causal relationship between polyPI metabolism and membrane phosphorylation was demonstrated<sup>20</sup>. Adrenal DPI and TPI were also affected, both after *in vivo* and *in vitro* administration of ACTH<sup>6-8</sup>.

In the present study fragments of ACTH and of C-terminal  $\beta$ -LPH were tested for their capacity to modulate the polyPI metabolism. The structure-activity relationship is compared to other brain-ACTH interactions (active avoidance conditioning, excessive grooming, counteraction of morphine-induced analgesia, opiate receptor affinity, adenylatecyclase and protein phosphorylation).

## MATERIALS AND METHODS

*Animals and brain dissection*

Male rats (150 g) of an inbred Wistar strain were used (TNO, Zeist, The Netherlands). After decapitation the brain was rapidly removed. All subsequent operations were performed at 0-4 °C. Limbic structures (hippocampus, septum, basal ganglia, pyriform cortex, diencephalon, mesencephalon) were dissected as described before<sup>9</sup>.

*Subcellular fractionation*

The preparation of the enzyme fraction was performed essentially as described previously<sup>21</sup>. The dissected material from one rat brain (0.3 g) was homogenized in 3 ml homogenization medium (0.32 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4). The crude mitochondrial/synaptosomal pellet (P2) was obtained and subjected to osmotic lysis: the P2 was suspended in 10 vols. aqua bidest and stirred for 10 min at 4 °C. This suspension was centrifuged for 20 min at 10,000 *g* and the supernatant of the lysed P2 (further referred to as membrane-cytosol fraction) taken as the enzyme fraction.

*Phosphorylation assay*

Endogenous lipid phosphorylating activity was assayed as described previously<sup>21</sup>. Briefly, the incubation was performed under the following conditions: 7.5  $\mu$ M

ATP, 3  $\mu\text{Ci}$  [ $\gamma$ - $^{32}\text{P}$ ]ATP (approx. 3000 Ci/mmol, Amersham, U.K.), 50 mM sodium acetate, 10 mM magnesium acetate, pH 6.5 in a final volume of 25  $\mu\text{l}$ , containing 10–15  $\mu\text{g}$  protein) was preincubated for 5 min and the incubation started by the addition of the ATP. Aliquots of the incubation buffer (5  $\mu\text{l}$ ) plus or minus peptides were added 15 s prior to the ATP. The incubations were terminated after 10 s by the addition of 2 ml ice-cold chloroform/methanol/13 N HCl (200:100:0.75 by volume see ref. 21). Protein determination was according to Lowry et al.<sup>25</sup>.

#### *Preparation of lipid/B-50 kinase fraction, and lipid kinase assay*

The ACTH-sensitive B-50 protein kinase was purified according to Zwiers et al.<sup>38,39</sup>. In short, a crude synaptosomal plasma membrane fraction was prepared from rat brain. The membrane-bound kinase activity was solubilized with 0.5% Triton-X-100 with 75 mM KCl in 6 mM Tris, 0.1 mM dithiothreitol (pH 8.1) and the solubilized proteins separated by DEAE cellulose chromatography. The proteins were eluted with a salt gradient in 10 mM Tris, 1 mM  $\text{CaCl}_2$ , 0.1 mM dithiothreitol, pH 7.4. The fractions containing the peak of the ACTH-sensitive lipid kinase activity (eluting at 170–205 mM salt), were collected and pre-incubated for 5 min at 30 °C in the presence of exogenous DPI (40  $\mu\text{M}$ ), followed by incubation with [ $\gamma$ - $^{32}\text{P}$ ]ATP (7.5  $\mu\text{M}$ ) for 15 min. The addition of peptide was 15 s before ATP was added.

#### *Lipid extraction and thin layer chromatography*

After termination of the phosphorylation reaction, carrier polyPI (10  $\mu\text{g}$  P) was added and extraction of the labeled phospholipids was performed as described<sup>21</sup>. Briefly, by adding 0.375 ml 0.6 N HCl a biphasic system was obtained. The upper phase was removed and the lower phase washed two times with chloroform/methanol/0.6 N HCl (3:48:47 by volume). The resulting lower phase containing the polyphosphoinositides was dried under  $\text{N}_2$  and re-dissolved in chloroform/methanol/water (75:25:2 by volume). Separation of labeled phospholipids was performed by one-dimensional High Performance Thin Layer Chromatography (HPTLC Merck; layer thickness 25  $\mu\text{m}$ ). The plates were impregnated with potassium oxalate and activated for 15 min at 110 °C<sup>21</sup>. The solvent was chloroform/acetone/methanol/glacial acetic acid/water (40:15:13:12:8 by volume). The lipids were visualized with iodine vapour and  $^{32}\text{P}$ -labeled spots were detected by autoradiography on Kodak Royal X-Omat film (10–20 h). The spots were scraped from the plates and radioactivity was determined by liquid scintillation counting<sup>21</sup>.

#### *Statistics*

Differences between groups were tested with the Student *t*-test<sup>36</sup>, and assigned to be significant for  $P < 0.05$ , two-tailed.

#### *Materials*

The following synthetic peptides were used: ACTH<sub>1–24</sub>, ACTH<sub>1–16</sub>, ACTH<sub>1–13</sub>, ACTH<sub>1–10</sub>, (D-Phe<sup>7</sup>)ACTH<sub>1–10</sub>, ACTH<sub>11–24</sub>, ACTH<sub>5–18</sub>, ACTH<sub>7–16</sub>, ACTH<sub>4–10</sub>, (D-Phe<sup>7</sup>)ACTH<sub>4–10</sub>, (Met(O)<sup>4</sup>,D-Lys<sup>8</sup>,Phe<sup>9</sup>)ACTH<sub>4–9</sub>, (Met(O)<sup>4</sup>,D-Lys<sup>8</sup>,Phe<sup>9</sup>,D-Lys<sup>11</sup>)

TABLE I

*ACTH-fragments and polyphosphoinositide metabolism*

A membrane-cytosol fraction was prepared as described (see Methods). The membrane samples were preincubated for 5 min at 30°C and the incubation was started by the addition of ATP plus [ $\gamma$ - $^{32}$ P]ATP. Buffer solution plus or minus peptide (100  $\mu$ M) was added 15 s before ATP. Incubation time was 10 s. The results (mean  $\pm$  S.E.M.; n = 4) are expressed as fmol P incorporated per  $\mu$ g protein. Numbers between parentheses refer to the percentage difference ( $P < 0.05$ ) to control incubations.

<i>ACTH sequence</i>	<i>Incorporation (fmol P per <math>\mu</math>g protein)</i>		
	<i>TPI</i>	<i>DPI</i>	<i>PA</i>
Control	1.02 $\pm$ 0.11	2.40 $\pm$ 0.15	5.44 $\pm$ 0.24
(1-24)	2.63 $\pm$ 0.13 (+158)	2.05 $\pm$ 0.16	2.46 $\pm$ 0.21 (-55)
(1-16)	1.54 $\pm$ 0.04 (+51)	2.30 $\pm$ 0.17	3.12 $\pm$ 0.17 (-43)
(1-13)	1.23 $\pm$ 0.03 (+21)	2.38 $\pm$ 0.18	4.17 $\pm$ 0.23 (-23)
(Ac-Ser <sup>1</sup> ) (1-13) NH <sub>2</sub>	1.15 $\pm$ 0.02	2.34 $\pm$ 0.15	5.20 $\pm$ 0.25
(1-10)	1.07 $\pm$ 0.11	2.46 $\pm$ 0.20	4.97 $\pm$ 0.30
(D-Phe <sup>7</sup> ) (1-10)	0.70 $\pm$ 0.06 (-31)	3.63 $\pm$ 0.10 (+51)	5.40 $\pm$ 0.23
(11-24)	0.90 $\pm$ 0.09	2.78 $\pm$ 0.19	5.98 $\pm$ 0.53
(1-10)+ (11-24)	1.01 $\pm$ 0.08	2.79 $\pm$ 0.20	6.08 $\pm$ 0.44
(5-18)	2.37 $\pm$ 0.17 (+132)	5.54 $\pm$ 0.49 (+130)	2.76 $\pm$ 0.17 (-49)
(7-16)	0.87 $\pm$ 0.10	4.23 $\pm$ 0.09 (+76)	2.20 $\pm$ 0.11 (-60)
(5-14)	0.48 $\pm$ 0.05 (-53)	3.19 $\pm$ 0.13 (+33)	5.56 $\pm$ 0.36
(4-10)	1.12 $\pm$ 0.07	2.87 $\pm$ 0.06 (+19)	5.88 $\pm$ 0.09
(D-Phe <sup>7</sup> ) (4-10)	0.97 $\pm$ 0.04	2.96 $\pm$ 0.10 (+23)	6.04 $\pm$ 0.26

ACTH<sub>4-16</sub>,  $\beta$ -LPH<sub>61-91</sub>,  $\beta$ -LPH<sub>61-77</sub>,  $\beta$ -LPH<sub>62-77</sub>,  $\beta$ -LPH<sub>61-76</sub>,  $\beta$ -LPH<sub>62-76</sub>, (Met<sup>65</sup>) $\beta$ -LPH<sub>61-65</sub>, (Leu<sup>65</sup>) $\beta$ -LPH<sub>61-65</sub>,  $\alpha$ -MSH. They were obtained from Organon Int. B.V. (Oss, The Netherlands).

## RESULTS

*Peptide-modulated polyPI metabolism: structure-activity relationship*

In a series of experiments the effects of ACTH fragments on polyPI metabolism was studied. A membrane-cytosol fraction was obtained as described (see Methods) and incubated for 10 s in the presence of peptide and [ $\gamma$ - $^{32}$ P]ATP. Under the conditions

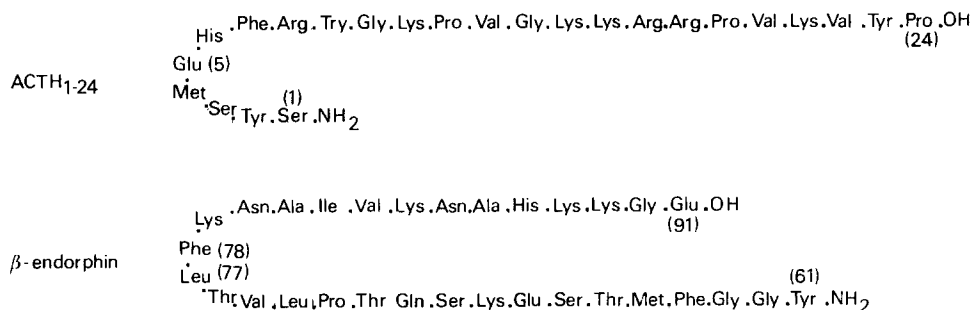


Fig. 1. Amino acid sequence of ACTH<sub>1-24</sub> and  $\beta$ -endorphin ( $\beta$ LPH<sub>61-91</sub>).

used, only TPI, DPI and PA became labeled<sup>21</sup>. At the dose level tested (100  $\mu$ M) a structure-activity relationship could be established for the ACTH fragments (Table I; see Fig. 1 for the amino acid sequence of ACTH<sub>1-24</sub> and  $\beta$ -endorphin). ACTH<sub>1-24</sub> stimulated the formation of TPI and at the same time inhibited the formation of PA (Table I and ref. 10). Identical effects were obtained with some of the other N-terminal fragments of ACTH. The order of decreasing potency was ACTH<sub>1-24</sub> > ACTH<sub>5-18</sub> > ACTH<sub>1-16</sub> > ACTH<sub>1-13</sub>. Interestingly, shortening the ACTH sequence from the N-terminal side dissociated the effects on TPI from those on PA, since ACTH<sub>7-16</sub> had no effect on TPI formation but fully inhibited PA production: ACTH fragments that were shortened at the N-terminal side exhibited a reduced effect on TPI. Instead the formation of DPI was stimulated by these peptides (in order of decreasing potency on DPI formation): ACTH<sub>5-18</sub> > ACTH<sub>7-16</sub> > ACTH<sub>5-14</sub> (Table I). A stimulation of DPI formation was also found for (D-Phe<sup>7</sup>)ACTH<sub>4-10</sub>, ACTH<sub>4-10</sub>, ACTH<sub>5-14</sub> and (D-Phe<sup>7</sup>)ACTH<sub>1-10</sub>. ACTH<sub>1-10</sub> and ACTH<sub>11-24</sub> and their combination were completely inactive in every respect.

A possible correlation between effects on polyPI metabolism and conditioned avoidance behavior was explored by testing some ACTH analogues which are extremely potent in inhibiting extinction of a conditioned avoidance response but fail to induce excessive grooming<sup>15</sup>. As shown in Table II, the potentiated sequences (4-9) and (4-16) did not affect TPI or PA formation, but both peptides stimulated the formation of DPI.

We then tested the effects of C-terminal fragments of  $\beta$ -LPH, some of which also display profound influences on extinction of conditioned avoidance behavior and on induction of excessive grooming behavior<sup>12</sup>.  $\beta$ -LPH<sub>61-91</sub> had no effect on DPI or TPI formation but inhibited the formation of PA in a dose-dependent manner (Fig. 2) As shown in Table III, fragments of  $\beta$ -endorphin, including the enkephalins were without any detectable effect.

TABLE II

*ACTH-analogs and polyphosphoinositide metabolism*

Incubations were performed as described in the legend to Table I. Peptides (100  $\mu$ M) were added 15 s before ATP. The results (mean  $\pm$  S.E.M.) are expressed in fmol P per  $\mu$ g protein. Numbers between parentheses refer to the percentage difference ( $P < 0.05$ ) to control incubations.

Peptide	Incorporation (fmol per $\mu$ g protein)		
	TPI	DPI	PA
Control	0.99 $\pm$ 0.10	2.38 $\pm$ 0.11	5.46 $\pm$ 0.20
ACTH <sub>1-24</sub>	2.75 $\pm$ 0.18 (+178)	2.15 $\pm$ 0.16	2.68 $\pm$ 0.19 (-51)
ACTH <sub>4-9</sub> *	0.84 $\pm$ 0.03	2.74 $\pm$ 0.05 (+15)	5.11 $\pm$ 0.11
ACTH <sub>4-16</sub> **	0.89 $\pm$ 0.05	2.85 $\pm$ 0.11 (+20)	5.52 $\pm$ 0.46

\* (Met(O)<sup>4</sup>, D-Lys<sup>8</sup>, Phe<sup>9</sup>) ACTH<sub>4-9</sub>, \*\* (Met(O)<sup>4</sup>, D-Lys<sup>11</sup>) ACTH<sub>4-16</sub>.

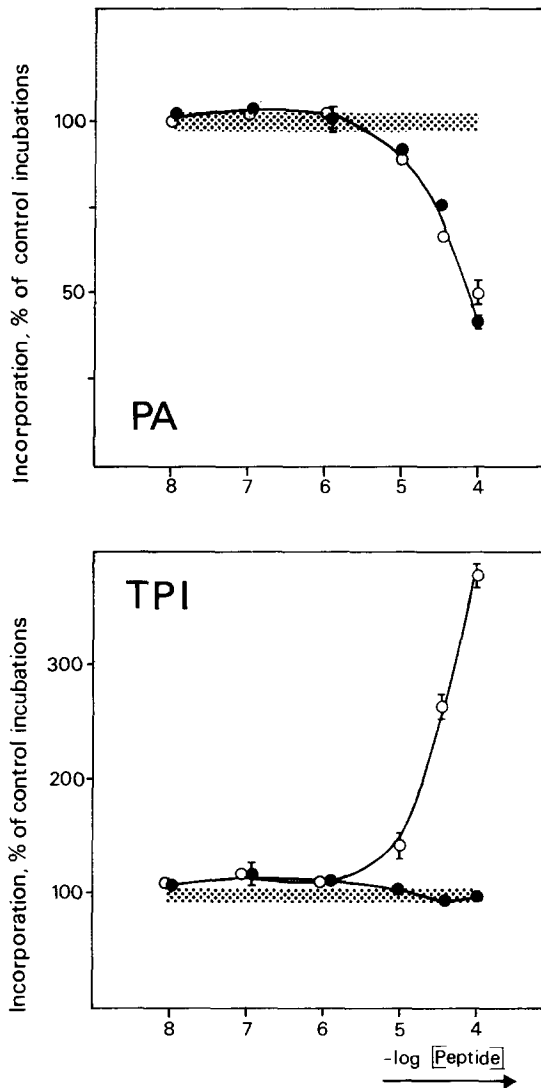


Fig. 2. Dose response relation of  $ACTH_{1-24}$  and  $\beta$ -endorphin on polyphosphoinositide metabolism in the membrane-cytosol fraction. Incubations were performed as described in the legend to Table I. Peptides (0.01–100  $\mu$ M) were added 15 s before ATP. The results (mean  $\pm$  S.E.M.,  $n = 6$ ) are expressed as percentage of control incubations.  $\circ$ , ACTH;  $\bullet$ ,  $\beta$ -endorphin.

*Effects of  $ACTH_{1-24}$  and  $\beta$ -endorphin on polyPI metabolism in a partially purified enzyme preparation*

The possibility that ACTH and  $\beta$ -endorphin act on the same membrane components, was studied by comparing the effects on the polyPI metabolism in a partially purified enzyme preparation. This preparation contained ACTH-sensitive protein kinase activity<sup>20,38</sup> and lipid kinase activity<sup>20,22</sup>. It appeared (Table IV) that both  $ACTH_{1-24}$  and  $\beta$ -endorphin dose-dependently inhibited the formation of PA.

TABLE III

*Fragments of  $\beta$ LPH<sub>61-91</sub> ( $\beta$ -endorphin) and polyphosphoinositide metabolism*

Incubations were performed as described in the legend to Table I. Peptides (100  $\mu$ M) were added 15 s before ATP. The results (mean  $\pm$  S.E.M.; n = 4) are expressed in fmol per  $\mu$ g protein. Numbers between parentheses refer to the percentage difference ( $P < 0.05$ ) to control incubations.

$\beta$ -LPH sequence	Incorporation (fmol P per $\mu$ g protein)		
	TPI	DPI	PA
Control	0.99 $\pm$ 0.10	2.38 $\pm$ 0.11	5.46 $\pm$ 0.20
(61-91) ( $\beta$ -endorphin)	0.97 $\pm$ 0.05	2.48 $\pm$ 0.14	1.97 $\pm$ 0.22 (-64)
(61-77) ( $\gamma$ -endorphin)	0.82 $\pm$ 0.07	2.35 $\pm$ 0.09	5.41 $\pm$ 0.44
(62-77) (des-Tyr- $\gamma$ -endorphin)	0.94 $\pm$ 0.03	2.55 $\pm$ 0.01	5.03 $\pm$ 0.27
(61-76) ( $\alpha$ -endorphin)	0.96 $\pm$ 0.03	2.09 $\pm$ 0.08	5.11 $\pm$ 0.11
(62-76) (des-Tyr- $\alpha$ -endorphin)	1.15 $\pm$ 0.10	2.41 $\pm$ 0.10	5.27 $\pm$ 0.31
(61-65) (Met-enkephalin)	0.97 $\pm$ 0.01	2.61 $\pm$ 0.12	5.31 $\pm$ 0.20
(61-65) (Leu <sup>65</sup> ) (Leu-enkephalin)	0.98 $\pm$ 0.04	2.55 $\pm$ 0.14	4.99 $\pm$ 0.22

ACTH markedly stimulated the production of TPI whereas  $\beta$ -endorphin had only minor influence on this polyphosphoinositide.

## DISCUSSION

The present study describes effects of ACTH,  $\beta$ -endorphin and congeners on the metabolism of the polyphosphoinositides in rat brain. Although the peptide effects may seem complex, a logical relationship exists between them, in view of the close relation that exists between the affected lipids. As shown in Fig. 3, PI, DPI and TPI are rapidly interconverted by phosphorylation and dephosphorylation (reactions 1 and 2)<sup>26</sup>. The ionic head group, containing the inositol ring and one, two or three phosphate groups, can be cleaved by a phospholipase-C type reaction (reaction 3). The resulting 1,2-diacylglycerol is rapidly phosphorylated to PA (reaction 4). In experi-

TABLE IV

*Effect of ACTH<sub>1-24</sub> and  $\beta$ -endorphin on polyPI metabolism in a partially purified lipid kinase preparation*

The purification of the lipid kinase was as described previously (see Methods). The results (mean  $\pm$  S.E.M.; n = 4) are expressed as fmol per  $\mu$ g protein per min. Numbers between parentheses refer to the percentage difference ( $P < 0.05$ ) to control incubations.

Treatment	Incorporation (fmol P per $\mu$ g DEAE protein/min)	
	TPI	PA
No addition	234 $\pm$ 6	346 $\pm$ 18
ACTH <sub>1-24</sub> (10 $\mu$ M)	276 $\pm$ 8 (+18)	189 $\pm$ 9 (-80)
ACTH <sub>1-24</sub> (50 $\mu$ M)	397 $\pm$ 7 (+70)	72 $\pm$ 8 (-80)
$\beta$ -endorphin (10 $\mu$ M)	237 $\pm$ 20	228 $\pm$ 16 (-34)
$\beta$ -endorphin (50 $\mu$ M)	300 $\pm$ 6 (+28)	55 $\pm$ 6 (-84)



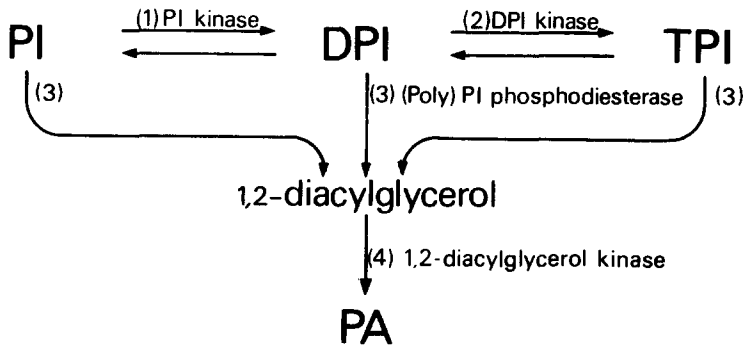


Fig. 3. Interconversion of the polyphosphoinositides.

ments with neurotransmitters<sup>1,2</sup> and the  $\text{Ca}^{2+}$  ionophore A23187<sup>16</sup> it has been found that a decreased formation of DPI and TPI is correlated to an increased production of PA. Therefore, and in view of the inverse relation between PA-formation and TPI–DPI formation that was found in the present study, the production of PA may be a manifestation of the breakdown of (poly)PI. With regard to the mechanism of action of ACTH, it has been demonstrated that the effect of the peptide is only evident in conditions where active phosphorylation can be monitored<sup>21,22</sup>. ACTH exclusively acts on the lipid kinase(s) involved (reactions 1,2,4) thus leaving the phosphomono- and diesterase activity unaffected.

#### *Structural requirements needed for stimulation of DPI and TPI formation*

The presently reported structure–activity data will be discussed in terms of multiple affinity sites within the peptide structure<sup>5</sup> (see Figs. 1 and 3). With regard to the PI kinase, both the sequences (4–10) and (7–16) stimulated DPI formation. Therefore, we assume that this property may have originated from the common sequence (7–10). With respect to the DPI kinase (reaction 2), the sequences (7–16) and (5–14) were inactive, whereas (5–18), (1–16) and (1–13) stimulated TPI formation. Therefore, it may be that both the sequence (5–7) and the basic amino acids at the C-terminus (Lys<sup>11</sup> in (1–13) or Lys<sup>15</sup>, Lys<sup>16</sup>, Arg<sup>17</sup>, Arg<sup>18</sup> in (1–16) or (1–18)) are a prerequisite for ACTH-stimulation of the DPI kinase. The PI- and the DPI-kinase are different enzymes<sup>26</sup>. The accumulation of DPI after incubation with ACTH<sub>7–16</sub> indicates that this peptide contains the structural requirements to stimulate PI kinase but not DPI kinase. N-terminal elongation to ACTH<sub>5–16</sub> then adds information that is essential for the stimulation of the DPI kinase (i.e. the sequence 5–7). This may explain that TPI accumulates after incubation with ACTH<sub>5–18</sub> and ACTH<sub>1–16</sub>.

#### *Structural requirements needed for inhibition of PA formation*

As discussed above, the formation of DPI/TPI may take place at the cost of PA. However, some fragments stimulated DPI formation without a concomitant decrease in PA formation (e.g. ACTH<sub>4–10</sub>, ACTH<sub>5–14</sub>). This suggests that effects on PA formation are a direct result of inhibition of reaction 4, probably accompanied by a

stimulatory effect on reaction 1 (and 2). As was also argued for the structure–activity relationship with respect to DPI kinase activation, the inhibition of 1,2-diacylglycerol kinase (reaction 4) requires both the presence of the (7–10) sequence and of basic amino acids at the C-terminus. The fragments (1–10), (11–24), (5–14) and (4–10) were inactive, whereas (7–16), (5–18), (1–16) and (1–13) were active (Table I). The structural requirements needed for inhibition of PA formation appear to be in the sequence (7–16). As sequences containing different amounts of basic amino acids in their C-terminal region have different potency in effects on TPI and PA, these basic amino acids seem of crucial importance for the peptide–lipid kinase interaction.

Interestingly,  $\beta$ -endorphin also had an inhibitory effect on the formation of PA (Table III). A relationship between the effects of ACTH<sub>1–24</sub> and  $\beta$ -endorphin is suggested by the finding that both peptides were similarly effective in this inhibition of PA production in a purified protein–lipid kinase preparation (Fig. 2; refs. 20 and 22).  $\beta$ -endorphin also had some effect on TPI formation but in comparison to ACTH the effect was small. C-terminal shortening to  $\gamma$ -endorphin ( $\beta$ -LPH<sub>61–77</sub>) abolished the effect on PA. None of the other endorphin fragments tested, had any effect on the polyPI metabolism. This may indicate that the basic properties of the endorphin fragment  $\beta$ -LPH<sub>78–91</sub> are responsible for the effects of  $\beta$ -LPH<sub>61–91</sub>; this C-terminal fragment shows some resemblance to ACTH<sub>5–16</sub> (Fig. 1): (1) both peptides contain a Lys-doublet at their C-terminus, (2) a third Lys-residue is present which is separated from the doublet by three amino acids (i.e. Lys<sup>84</sup> in  $\beta$ -endorphin and Lys<sup>11</sup> in ACTH) and (3) both peptides contain the combination Phe-basic amino acid residue at their N-terminus.

As the conversion of PI to PA may underly calcium-gating in the membrane<sup>26</sup>, the presently reported data on the effect of  $\beta$ -endorphin are in support of the recent demonstration that  $\beta$ -endorphin can inhibit the influx of calcium into synaptosomes, leading to a reduced release of neurotransmitters<sup>17</sup>.

#### *Correlation between peptide effects on polyPI metabolism and those obtained on other parameters*

Also in other CNS effects of ACTH the affinity sites in ACTH<sub>4–10</sub> and in ACTH<sub>10–16</sub> coded for activity, i.e. delay of extinction of avoidance behavior<sup>4,5</sup>, binding to opiate receptors<sup>31</sup>, counteraction of morphine-induced analgesia<sup>11</sup>, induction of excessive grooming behavior<sup>10</sup>, modulation of cAMP production<sup>35</sup>, and of protein synthesis in a cell-free system<sup>29</sup>.

If we now consider these ACTH–CNS structure–activity relationships obtained with ACTH, a great similarity in structural requirements appears to exist for the effects on TPI- and PA-formation, and those on the inhibition of the phosphorylation of B-50 protein (active are (1–24), (5–18), (1–16), (1–13)<sup>37</sup>). A relation was found between protein phosphorylation and TPI formation<sup>20</sup> and nearly all peptides that inhibited B-50 protein phosphorylation induced excessive grooming<sup>13,40</sup>. The mechanism by which the peptides induce this behavior may involve modulation of DPI kinase activity, presumably as a result of a reduction in the degree of phosphorylation of the B-50 protein<sup>20,22</sup>. The correspondence in structure activity studies suggests that the

effects of ACTH on B-50 phosphorylation and TPI formation on the one hand, and on excessive grooming behavior on the other, are correlated. More research should be performed to establish whether a causal relationship exists, to determine whether the neurochemical events indeed underly the physiological effects of ACTH.

Further study is also needed to account for the effects of (D-Phe<sup>7</sup>)ACTH<sub>4-10</sub> and  $\alpha$ -MSH. These peptides induce excessive grooming but lack effect on TPI/PA formation: (D-Phe<sup>7</sup>)ACTH<sub>4-10</sub> stimulated the formation of DPI, but not TPI, and  $\alpha$ -MSH did not have any effect on the brain (poly)PI. There was also a marked discrepancy in the affinity of ACTH<sub>1-13</sub> and  $\alpha$ -MSH ((Ac-Ser<sup>1</sup>)ACTH<sub>1-13</sub>-NH<sub>2</sub>)<sup>32</sup> at the brain opiate receptor<sup>11</sup>.

The structural requirements for ACTH-effects on extinction of active avoidance behavior are well characterized and seem less exacting than those obtained for excessive grooming: affinity sites were in the regions (4-7), (7-10) and (10-16)<sup>15</sup>. The same seems to hold for the stimulation of DPI formation, which could be elicited by (4-10), (7-16), (5-14), (1-10), (D-Phe<sup>7</sup>) (4-10), (D-Phe<sup>7</sup>) (1-10) and by the two potentiated analogs of the sequences (4-9) and (4-16). So it is tempting to relate the effects on DPI formation to those on extinction of active avoidance behavior *in vivo*.

Some of the CNS effects of ACTH may be caused by interaction with opiate receptors<sup>19,34</sup>. Therefore, the inhibition of PA formation which is observed after ACTH<sub>1-24</sub> and after the opioid peptide  $\beta$ -endorphin may reflect the interaction with opiate receptors. Likewise, the sequence (7-16) which has the same affinity for brain opiate receptors as ACTH<sub>1-24</sub><sup>11</sup> is also a very potent inhibitor of PA formation (Table I). Anionic lipids are reported to be important for opiate receptor binding<sup>23,24</sup>. Therefore, the effects found with  $\beta$ -endorphin, ACTH<sub>7-16</sub> and larger ACTH fragments may indicate that some parallel exists between opiate-like effects and effects on the metabolism of the polyanionic phosphoinositides. However, a close correlation between peptide effects on PA formation and opiate-like activity does not appear from the data, as the sequences (4-10), (1-10) and (5-14) have affinity for opiate receptors<sup>31</sup> but have no effect on PA formation at the dose level tested (100  $\mu$ M).

In conclusion, the presently reported findings that different sites within the ACTH molecule are important or essential for different molecular effects, are consistent with the hypothesis<sup>5</sup> that there are multiple affinity sites within the sequence ACTH<sub>1-24</sub>, accounting for the diversity of ACTH-brain cell interactions. The parallel between effects of ACTH and congeners on the metabolism of the phosphoinositides and other *in vivo* and *in vitro* parameters is of interest in view of the possible functional significance of these polyanionic membrane components (see 23). The polyphosphoinositide system seems to be involved in the gating of Ca<sup>2+</sup> through the membrane<sup>26,27</sup>. DPI and TPI bind Ca<sup>2+</sup> very avidly<sup>3,28</sup> and the enzymes involved in their metabolism are among the fastest acting known<sup>18,22</sup>. A function in the regulation of the membrane potential has been suggested<sup>33</sup>, and they seem to be involved in the steroidogenic actions of ACTH in the adrenals<sup>6,7,8</sup>.

Although the presently reported findings suggest that the polyphosphoinositides could indeed play an important role in the regulation of membrane characteristics, and thereby of the activity of the neurone, one should be cautious in asserting functional

correlates to the peptide-induced changes in polyPI metabolism. After all, the present research has only established a correlation between lipid changes measured *in vitro*, and behavior changes measured *in vivo*. Further research should make clear whether alterations in the polyPI metabolism *in vivo* could underlie the behavioral effects of the neuropeptides.

## REFERENCES

- 1 Abdel Latif, A. A., Green, K. and Smith, J. P., Sympathetic denervation and the triphosphoinositide effect in the rabbit iris smooth muscle: a biochemical method for the determination of  $\alpha$ -adrenergic receptor supersensitivity, *J. Neurochem.*, 32 (1979) 225–228.
- 2 Akhtar, R. A., and Abdel Latif, A. A., Studies on the properties of triphosphoinositide phosphomonoesterase and phosphodiesterase of rabbit iris smooth muscle, *Biochim. biophys. Acta*, 527 (1978) 159–170.
- 3 Dawson, R. M. C., 'Phosphatido-peptide'-like complexes formed by the interaction of calcium triphosphoinositide with protein, *Biochem. J.*, 97 (1965) 134–138.
- 4 De Wied, D., Effects of peptide hormones on behavior. In W. F. Ganong and L. Martini (Eds.), *Frontiers in Neuroendocrinology*, Oxford Univ. Press, New York, 1969, pp. 97–140.
- 5 De Wied, D. and Gispen, W. H., Behavioral effects of peptides. In H. Gainer (Ed.), *Peptides in Neurobiology*, Plenum Press, New York, 1977, pp. 397–448.
- 6 Farese, R. V. and Sabir, A. M., Polyphosphorylated glycerolipids mimic adrenocorticotropin-induced stimulation of mitochondrial pregnenolone synthesis, *Biochim. biophys. Acta*, 575 (1979) 299–304.
- 7 Farese, R. V., Sabir, A. M. and Vandor, S. L., Adrenocorticotropin acutely increases adrenal polyphosphoinositides, *J. biol. Chem.*, 254 (1979) 6842–6844.
- 8 Farese, R. V., Sabir, A. M., Vandor, S. L. and Larson, R. E., Are polyphosphoinositides the cycloheximide-sensitive mediator in the steroidogenic actions of adrenocorticotropin and adenosine-3',5'-monophosphate? *J. biol. Chem.*, 255 (1980) 5728–5734.
- 9 Gispen, W. H., Schotman, P. and De Kloet, E. R., Brain RNA and hypophysectomy: a topographical study, *Neuroendocrinology*, 9 (1973) 285–296.
- 10 Gispen, W. H., Wiegant, V. M., Greven, H. M. and De Wied, D., The induction of excessive grooming in the rat by intraventricular application of peptides derived from ACTH: structure activity studies, *Life Sci.*, 17 (1975) 645–652.
- 11 Gispen, W. H., Buitelaar, J., Wiegant, V. M., Terenius, L. and De Wied, D., Interaction between ACTH-fragments, brain opiate receptors and morphine analgesia, *Europ. J. Pharmacol.*, 39 (1976) 393–397.
- 12 Gispen, W. H., Wiegant, V. M., Bradbury, H. F., Hulme, E. C., Smyth, D. G., Snell, C. R. and De Wied, D., Induction of excessive grooming in the rat by fragments of lipotropin, *Nature (Lond.)*, 264 (1976) 794–795.
- 13 Gispen, W. H., Zwiers, H., Wiegant, V. M., Schotman, P. and Wilson, J. E., The behaviorally active neuropeptide as neurohormone and neuromodulator: the role of cyclic nucleotides and phosphoproteins. In Y. H. Ehrlich, J. Volavka, L. G. Davis and E. G. Brunngaber (Eds.), *Modulators, Mediators and Specifiers in Brain Function*, Plenum Press, New York, 1979, pp. 199–244.
- 14 Greven, H. M. and De Wied, D., The influence of peptides derived from corticotropin (ACTH) on performance. Structure-activity studies. In E. Zimmermann, W. H. Gispen, B. H. Marks and D. de Wied (Eds.), *Drug Effects on Neuroendocrine Regulation*, Elsevier, Amsterdam, 1973, pp. 429–442.
- 15 Greven, H. M. and De Wied, D., Influence of peptides structurally related to ACTH and MSH on active avoidance behavior in the rat: a structure-activity relationship. In F. Tilders, D. Swaab, Tj. B. Van Wimersma Greidanus (Eds.), *Frontiers of Hormone Research*, Karger, Basel, 1977, pp. 140–152.
- 16 Griffin, H. D. and Hawthorne, J. N., Calcium-activated hydrolysis of phosphatidyl-myo-inositol 4-phosphate and phosphatidyl-myo-inositol 4,5-bisphosphate in guinea pig synaptosomes, *Biochem. J.*, 176 (1978) 541–552.

- 17 Guerrero-Munoz, F., deLourdes Guerrero, M., Leong Way, E. and Li, C. H., Effect of  $\beta$ -endorphin on calcium uptake, *Science*, 206 (1979) 89–91.
- 18 Hawthorne, J. N. and Kai, M., Metabolism of phosphoinositides. In A. Lajtha (Ed.), *Handbook of Neurochemistry*, Plenum Press, New York, 1970, pp. 491–508.
- 19 Jacquet, Y. F., Opiate effects after adrenocorticotropin or  $\beta$ -endorphin injection in the periaqueductal grey matter of rats, *Science*, 201 (1978) 1032–1034.
- 20 Jolles, J., Zwiers, H., Van Dongen, C. J., Schotman, P., Wirtz, K. W. A. and Gispen, W. H., Modulation of brain polyphosphoinositide metabolism by ACTH-sensitive protein phosphorylation, *Nature (Lond.)*, 286 (1980) 623–625.
- 21 Jolles, J., Zwiers, H., Dekker, A., Wirtz, K. W. A. and Gispen, W. H., Corticotropin-(1-24)-tetracosapeptide affects protein phosphorylation and polyphosphoinositide metabolism in rat brain, *Biochem. J.*, 194 (1981) 283–291.
- 22 Jolles, J., *Neuropeptides, Brain Membrane Phosphorylation and Grooming Behavior*, Thesis, University of Utrecht, Utrecht, 1980, pp. 99–121.
- 23 Jolles, J., Aloyo, V. J. and Gispen, W. H., Molecular correlates between pituitary hormones and behavior. In I. R. Brown, (Ed.), *Molecular Approaches to Neurobiology*, Acad. Press, New York, 1981, in press.
- 24 Loh, H. H., Law, P. Y., Ostwald, T., Cho, T. M. and Leong Way, E., Possible involvement of cerebroside sulphate in opiate receptor binding, *Fed. Proc.*, 37 (1978) 147–152.
- 25 Lowry, O., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with the Folin phenol reagent, *J. biol. Chem.*, 193 (1951) 265–275.
- 26 Michell, R. H., Inositol phospholipids and cell surface receptor function, *Biochim. biophys. Acta*, 415 (1975) 81–148.
- 27 Michell, R. H., Inositol lipids in membrane function, *Trends biochem. Sci.*, 4 (1979) 282–285.
- 28 Palmer, F. B. St. C. and Dawson, R. M. C., Complex formation between triphosphoinositide and experimental allergic encephalitogenic protein, *Biochem. J.*, 111 (1969) 637–646.
- 29 Schotman, P., Van Heuven-Nolsen, D. and Gispen, W. H., Protein synthesis in a cell-free system from rat brain, sensitive to ACTH-like peptides, *J. Neurochem.*, 34 (1980) 1661–1670.
- 30 Shaikh, N. A. and Palmer, F. B. St. C., Phosphoinositide kinases in chick brain and sciatic nerve, a developmental study, *J. Neurochem.*, 28 (1977) 395–402.
- 31 Terenius, L., Gispen, W. H. and De Wied, D., ACTH-like peptides and opiate receptors in the rat brain: structure–activity studies. *Europ. J. Pharmacol.*, 33 (1975) 395–399.
- 32 Terenius, L., Somatostatin and ACTH are peptides with partial agonist-like opiate receptor selectivity, *Europ. J. Pharmacol.*, 38 (1976) 211–213.
- 33 Torda, C., Model of molecular mechanism able to generate a depolarization–hyperpolarization cycle, *Int. Rev. Neurobiol.*, 16 (1974) 1–66.
- 34 Wiegant, V. M., Gispen, W. H., Terenius, L. and De Wied, D., ACTH-like peptides and morphine: interaction at the level of the CNS, *Psychoneuroendocrinology*, 2 (1977) 63–69.
- 35 Wiegant, V. M., Dunn, A. J., Schotman, P. and Gispen, W. H., ACTH-like neurotropic peptides: possible regulators of rat brain cyclic AMP, *Brain Research*, 168 (1979) 565–584.
- 36 Winer, B. J., *Statistical Principles in Experimental Design*, McGraw-Hill, New York, 1962.
- 37 Zwiers, H., Wiegant, V. M., Schotman, P. and Gispen, W. H., ACTH-induced inhibition of endogenous rat brain protein phosphorylation in vitro: structure activity, *Neurochem. Res.*, 3 (1978) 445–463.
- 38 Zwiers, H., Tonnaer, J., Wiegant, V. M., Schotman, P. and Gispen, W. H., ACTH-sensitive protein kinase from rat brain membranes, *J. Neurochem.*, 33 (1979) 247–256.
- 39 Zwiers, H., Schotman, P. and Gispen, W. H., Purification and some characteristics of an ACTH-sensitive protein kinase and its substrate protein in rat brain membranes, *J. Neurochem.*, 34 (1980) 1689–1699.
- 40 Zwiers, H., Verhoef, J., Schotman, P. and Gispen, W. H., A new phosphorylation-inhibiting peptide (PIP) with behavioral activity from rat brain membranes. *FEBS Lett.*, 112 (1980) 168–172.