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EFFECTS OF ACTH-(1-24) ON DOPAMINE AND NORADRENALINE RELEASE, B-50 PHOSPHORYLATION AND CALMODULIN BINDING TO B-50 IN VITRO.

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Summary

ACTH-(1-24), 1 μM, enhanced the Ca^{2+}-dependent release of [^{3}H]dopamine ([^{3}H]DA) from intact septal synaptosomes by approximately 30%, but had no effect on the release of [^{3}H]noradrenaline ([^{3}H]NA) from intact cortical synaptosomes. Since a strong correlation has been reported between B-50 (phosphorylation) and [^{3}H]NA release from intact or streptolysin-O- (SL-O-) permeated cortical synaptosomes, we investigated whether the effects of ACTH-(1-24) on the release of radiolabelled transmitters are mediated by B-50. We observed that the increment in the release of [^{3}H]DA from SL-O-permeated septal synaptosomes as a result of exposure to a high Ca^{2+} concentration was much less pronounced than that of the release of [^{3}H]NA from SL-O permeated septal and cortical synaptosomes. ACTH-(1-24) concentration-dependently inhibited [^{3}H]NA release from SL-O-permeated cortical synaptosomes (IC_{50} value of approximately 10 μM) when ACTH-(1-24) was added 150 s prior to the Ca^{2+} trigger. Simultaneous addition of ACTH-(1-24), SL-O and Ca^{2+}-buffers to cortical synaptosomes did not lead to a change in [^{3}H]NA release at any of the ACTH-(1-24) concentrations tested. ACTH-(1-24) had no effect on B-50 phosphorylation in intact synaptosomes, whereas it concentration-dependently inhibited B-50 phosphorylation in permeated cortical synaptosomes (IC_{50} value of 100 μM). ACTH-(1-24) inhibited (IC_{50} value of 10 μM) B-50/calmodulin binding in vitro. We conclude that the effects of high concentrations of ACTH-(1-24) on various biochemical B-50 related parameters are not likely to represent the mechanisms underlying the action of ACTH-(1-24) on neurotransmitter release.

There is ample evidence in support for the notion that melanocortins influence arousal, attention, motivation and learning of the rat (1-3). Moreover, ACTH/MSH-like peptides have been reported to stimulate neuronal development (4) and to facilitate the functional recovery of lesions in the central nervous system (5,6) and peripheral nerves (7,8), while also changes in neurotransmitter content and neurotransmitter release in specific brain areas have been reported (9,10).

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The signal transduction pathway by which ACTH-(1-24) and its congeners modulate neurotransmitter release is unknown. However, ACTH-(1-24) has been shown to inhibit the phosphorylation of B-50 by PKC in synaptosomal plasma membranes (11,12). Direct evidence for the notion that B-50 (phosphorylation) is involved in the mechanism of neurotransmitter release was obtained by Dekker et al. (13-16). These authors observed that the degree of PKC-mediated phosphorylation of B-50 in hippocampal slices and cortical synaptosomes is correlated with neurotransmitter release. In one of their studies cortical synaptosomes permeated by exposure to streptolysin-O (SL-O), a toxin that makes pores in the synaptosomal membrane thus enabling ions and proteins to pass the membrane, were used. The release of [^{3}H]noradrenaline ([^{3}H]NA) was induced by elevation of the extracellular Ca^{2+} concentration. Application of antibodies against B-50, which inhibit phosphorylation of B-50, resulted in an inhibition of Ca^{2+}-induced [^{3}H]NA release (14), indicating the importance of B-50 (phosphorylation) in the release process. B-50 (GAP-43), a major substrate of protein kinase C (PKC), is a nervous tissue-specific protein predominantly localized at the inner plasma membrane of the presynaptic nerve terminal (17,18).

Apart from being a presynaptic PKC-substrate, B-50 has peculiar calmodulin binding properties. In the absence of Ca^{2+} dephospho-B-50 (19) and calmodulin (CaM) form complexes (20,21). Interestingly, PKC-mediated B-50 phosphorylation and CaM binding to B-50 are mutually exclusive (19). The importance of CaM in the release process in synaptosomes has been shown previously (22). It has been postulated that B-50 could serve as a local CaM store in the submembrane compartment (23). According to this hypothesis a depolarization-induced Ca^{2+}-influx would dissociate CaM from B-50. CaM could than activate Ca^{2+}/CaM dependent processes such as CaM kinase II which in turn can phosphorylate Synapsin I; a cascade implicated in the recruitment of synaptic vesicles for neurotransmitter release (24). In this context, the finding of Coggins and Zwiers (25) that ACTH-(1-24) prevents the association of B-50 and CaM in vitro might be of importance.

In the present study, we investigated whether the effects of ACTH-(1-24) on neurotransmitter release are mediated either through PKC-mediated B-50 phosphorylation or CaM binding to B-50. We used intact and SL-O permeated synaptosomes to investigate whether the reported effect of ACTH-(1-24) on B-50 phosphorylation in SPM is also observed in intact or SL-O permeated synaptosomes. The use of synaptosomal preparations permitted us to measure the degree of B-50 phosphorylation and neurotransmitter release under comparable experimental conditions. Moreover, we evaluated the inhibitory effect of ACTH-(1-24) on the formation of a B-50/CaM complex in vitro.

Methods

Materials: $^{32}P_i$ (carrier free), [γ-^{32}P]ATP, [7,8-^{3}H]dopamine and L-[7,8-^{3}H]noradrenaline were purchased from Amersham; desipramine and calmodulin were from Sigma; SL-O was obtained from the Wellcome trust (Weesp, The Netherlands); GBR 12909 was generously donated by Dr. W. Hespe, Gist-Brocades, Haarlem, The Netherlands; ACTH-(1-24) was a gift from Dr. J. Van Nispen, Organon International BV, Oss, The Netherlands; Anti-B-50 IgG was prepared as described earlier (26).

Preparation of synaptosomes: Male Wistar rats (TNO, Zeist, The Netherlands) weighing 120-200 g were killed by decapitation. The brains were excised rapidly. Subsequently, the brain structures were dissected on ice and homogenized in 10 volumes (w/v) of ice-cold 0.32 M sucrose, pH 7.4, at 4°C with a Potter-Elvehjem glass homogenizer fitted with a rotating teflon pestle (700 rpm; clearance= 50 µM). The homogenate was centrifuged for 10 min at 1000 g.

The supernatant was used for the preparation of purified synaptosomes according to Dunkley et al. (27). Cortical synaptosomes were collected from fraction 4, whereas for septal synaptosomes fraction 3 and 4 were combined.

Release of tritiated neurotransmitters from intact and SL-O-permeated synaptosomes: For the measurement of the release of tritiated neurotransmitters from intact synaptosomes, the synaptosomal preparation was diluted in carbogenated buffer A (124 mM NaCl; 5 mM KCl; 1.3 mM $MgSO_4$; 2 mM $CaCl_2$; 10 mM glucose; 26 mM $NaHCO_3$, pH 7.4) (2:3 (v/v)) and then centrifuged for 10 min at 1500 g. For the measurement of radiolabelled neurotransmitter release from SL-O-permeated synaptosomes, the synaptosomal preparation was diluted in carbogenated buffer B (123 mM NaCl; 5 mM KCl; 2 mM $MgCl_2$; 1.15 mM NaH_2PO_4; 2 mM $CaCl_2$; 5.6 mM glucose; 20 mM PIPES, pH 6.8) and then centrifuged for 10 min at 1500 g. The resulting pellet was resuspended in buffer to a concentration of 1 mg protein/ml and pre-incubated for 15 min at 37°C. Subsequently, the synaptosomal preparation was labelled with 5 µM L-[7,8-^{3}H]noradrenaline ([^{3}H]NA) or [7,8-^{3}H]dopamine ([^{3}H]DA) for 15 min. In experiments with [^{3}H]NA, the selective DA uptake inhibitor GBR 12909 (1-[2-[bis-(4-fluorphenyl) methoxy]ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride, $5x10^{-7}$ M) was also added to the medium; in those with [^{3}H]DA the selective NA uptake inhibitor desipramine ($3x10^{-6}$ M) was added. After labelling, the suspension was centrifuged four times for 10 min at 1500 g at 4°C. Between each centrifugation step, the pellet was resuspended in 8 ml ice-cold buffer A or buffer B minus $CaCl_2$ for the measurement of the release of tritiated catecholamines from intact or SL-O-permeated synaptosomes, respectively.

For neurotransmitter release from intact synaptosomes, the remaining pellet was resuspended in buffer A to a concentration of 1 µg protein/µl buffer, using a Potter-Elvehjem glass homogenizer. An aliquot corresponding with 20 µg protein was added to an Eppendorf vial containing 100 µl buffer A with or without ACTH-(1-24), which was then placed in a waterbath of 37°C for 5 min. For neurotransmitter release from permeated synaptosomes, the pellet was suspended in buffer B minus $CaCl_2$ and an aliquot corresponding with 20 µg protein was added to a reaction mixture containing buffer B, 2 mM Na_2ATP, different Ca^{2+}-/EGTA buffers, SL-O and ACTH-(1-24), unless otherwise stated. The suspensions were then centrifuged 30 s in an Eppendorf centrifuge. The supernatants were collected and counted in a Packard Model 2000 CA liquid scintillator counter.

B-50 phosphorylation: B-50 phosphorylation in the SL-O-permeated synaptosomes using [γ-^{32}P]ATP was measured as described previously (16). Briefly, synaptosomes were prepared as described above and 20 µl synaptosomes were added to the reaction mixture containing buffer B with 0.1 mM Na_2ATP, SL-O and low Ca^{2+}/EGTA buffer. At t= 150 and t= 210 s high Ca^{2+}/EGTA buffer and 4 µCi [γ-^{32}P]ATP were added, respectively. The reaction was terminated by the addition of a SDS-denaturing stop solution (2% (w/v) SDS; 10% v/v glycerol; 5% (v/v) 2-mercaptoethanol; 0.001% (w/v) bromophenolblue; 62.5 mM Tris-HCl, pH 6.8). Protein phosphorylation was analyzed by SDS-PAGE and autoradiography as described by Kristjansson et al. (28). B-50 phosphorylation in intact synaptosomes and hippocampal slices using ^{32}P-orthophosphate was measured according to Dekker et al. (15) and De Graan et al. (29), respectively.

B-50/CaM-complex formation: Complex formation was investigated as described by De Graan et al. (21) with slight modifications. Purified dephospho-B-50 (0.15 µg) was pre-incubated at 37°C for 5 min in the presence or absence of 13 U CaM (0.5 U/µl final concentration, approximately 0.2 µg CaM) in a buffer containing 20 mM Hepes, 5 mM $MgCl_2$ and either EGTA or Ca^{2+}/EGTA buffer, pH 7.4. Disuccinimidyl suberate (DSS) was added at t= 5 min

(final concentration, 1 mM; total incubation volume 26 µl). After 15 min of incubation, the reaction was terminated by adding 13 µl SDS-containing stopmix plus 1 µl 0.1 M EGTA. The samples were subjected to SDS-PAGE and the separated proteins were analyzed on Western blots as described by Van Hooff et al. (30).

TABLE I

The effect of 1 µM ACTH-(1-24) on the release of [^{3}H]DA from septal and [^{3}H]NA from cortical synaptosomes.

Brain structure	Triated transmitter	Ca^{2+}-dependent release (% of total radioactivity)	
		control	ACTH-(1-24)
Septum	[^{3}H]DA	5.4±0.2	6.9±0.3*
Cortex	[^{3}H]NA	7.3±0.5	7.0±0.2

The data are presented as percentage of total radioactivity corrected for Ca^{2+}-independent efflux of tritiated neurotransmitter (efflux measured in the presence of 1 mM EGTA) which amounted to 39.5% and 15.9% of total radioactivity in septal and cortical synaptosomes, respectively. Each value is the mean ± S.E.M. of 6-15 observations obtained in two (cortical synaptosomes) or five (septal synaptosomes) separate experiments. *P<0.05 for difference with controls (ANOVA, followed by Tukey B tests).

Results

ACTH-(1-24), 1 µM, caused an increase in the Ca^{2+}-dependent release of [^{3}H]DA from intact septal synaptosomes of approximately 30%, but it had no effect on the release of [^{3}H]NA from intact cortical synaptosomes (table I). B-50 phosphorylation in ^{32}P-labeled septal or cortical synaptosomes was not affected by ACTH-(1-24) (data not shown). Table II shows that the

TABLE II

Comparison of basal and Ca^{2+}-induced tritiated neurotransmitter release from SL-O-permeated cortical and septal synaptosomes.

Brain structure	Tritiated transmitter	Efflux of radioactivity (% of total radioactivity)			
		non-permeated 10^{-8} M Ca^{2+}	permeated 10^{-8} M Ca^{2+}	10^{-5} M Ca^{2+}	Δ
Cortex	[^{3}H]NA	22.0±0.3	32.9±0.4	41.3±0.4*	8.4±0.5
Septum	[^{3}H]NA	23.6±2.5	29.5±1.2	33.4±1.3*	4.0±0.5
Septum	[^{3}H]DA	26.8±2.2	35.5±1.2	38.4±1.3*	2.7±0.1

Synaptosomes prepared from different brain regions were permeated with 0.4 IU/ml SL-O in the presence of the indicated concentration of free Ca^{2+}. Data are mean ± S.E.M. of 8-12 observations obtained in 6 separate experiments. * P<0.05 for difference with permeated, 10^{-8} M Ca^{2+} (Student's t-test).

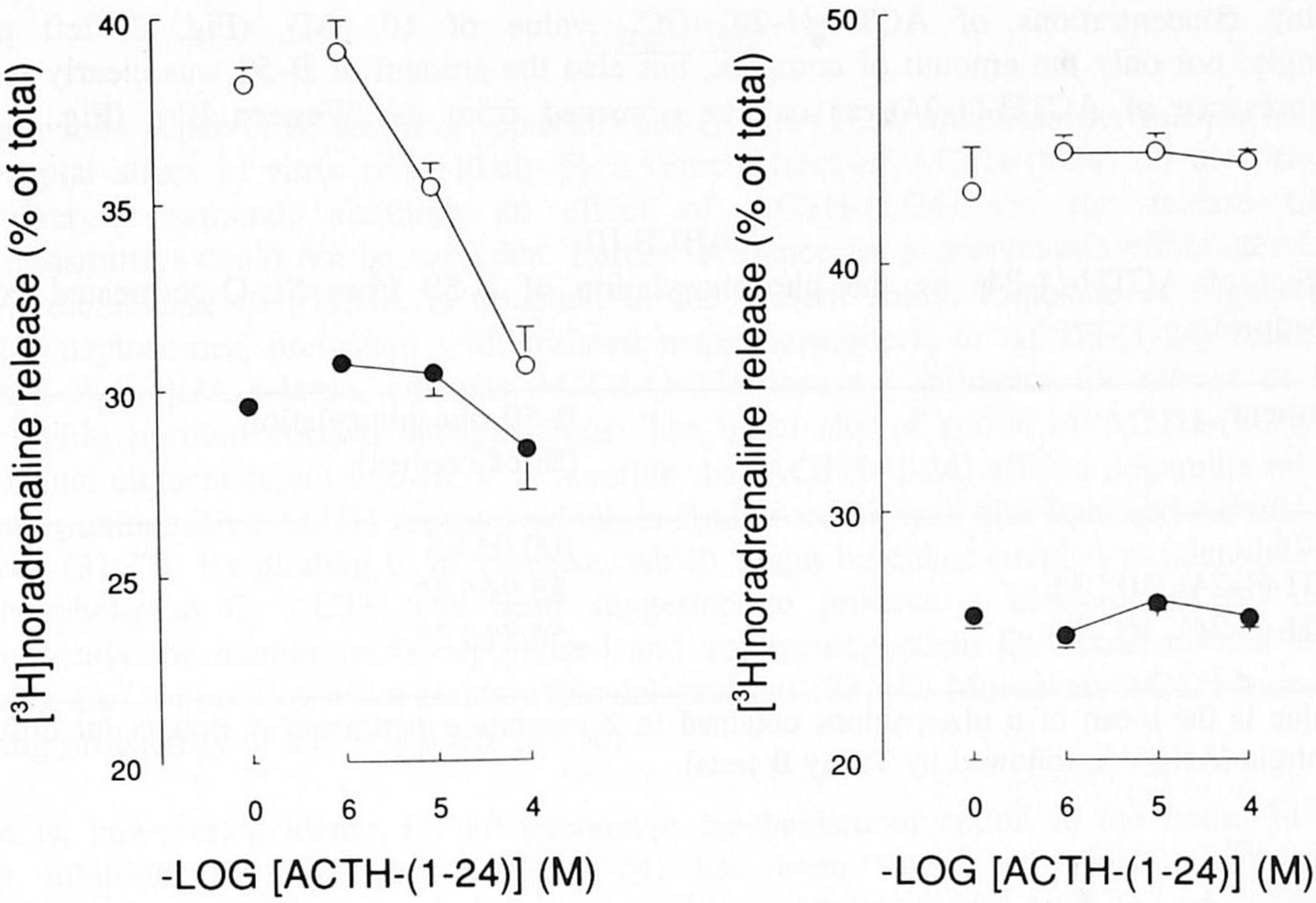

FIG. 1

The effect of ACTH-(1-24) on the release of [^{3}H]NA from SL-O-permeated cortical synaptosomes. Left panel: After a delayed addition (t= 150 s) of 10^{-8} (●) or 10^{-5} (○) M Ca^{2+} buffer to the synaptosomal preparation, ACTH-(1-24) concentration-dependently inhibits the release of [^{3}H]NA from permeated synaptosomes ($P<0.05$, ANOVA). Each value represents the mean ± S.E.M. of 12 observations obtained in 4 separate experiments. Right panel: Absence of an effect of ACTH-(1-24) on [^{3}H]NA release from permeated synaptosomes upon simultaneous addition of SL-O, ACTH-(1-24) and 10^{-8} (●) or 10^{-5} (○) M Ca^{2+} buffer to the synaptosomal preparation (ANOVA). Each value is the mean ± S.E.M. of 6 observations. The data were corrected for the efflux of [^{3}H]NA in the absence of SL-O (16.3 %). No effect of ACTH-(1-24) on unpermeated synaptosomes was observed (efflux of [^{3}H]NA in the presence of ACTH-(1-24) amounted 16.5 % of total radioactivity).

amount of [^{3}H]DA released by SL-O-permeated septal synaptosomes at a low Ca^{2+} concentration (10^{-8} M Ca^{2+} buffer) is comparable to the amount of [^{3}H]NA release by SL-O-permeated cortical synaptosomes. The Ca^{2+}-induced [^{3}H]DA release from SL-O-permeated septal synaptosomes was considerably lower than the Ca^{2+}-induced [^{3}H]NA release from SL-O-permeated cortical or septal synaptosomes.

ACTH-(1-24), present 150 s prior to the Ca^{2+} trigger, reduced the Ca^{2+}-evoked [^{3}H]NA release at different ACTH-(1-24) concentrations (IC_{50} value of approximately 10 µM) (fig. 1, left panel). In contrast, when ACTH-(1-24), SL-O and the Ca^{2+}-buffers were added simultaneously, no change in [^{3}H]NA release was observed at any of the ACTH-(1-24) concentrations tested (fig. 1, right panel). In the absence of SL-O, [^{3}H]NA release was not affected by an elevation of the extracellular Ca^{2+} concentration or by the addition of 1 µM ACTH-(1-24). Under experimental conditions similar to those used for the measurement of [^{3}H]NA release from SL-O-permeated synaptosomes, ACTH-(1-24) concentration-dependently inhibited the phosphorylation of B-50 in SL-O-permeated synaptosomes, with a 45% inhibition at 10^{-4} M (Table III). Incubation of purified dephosphorylated B-50 and CaM with the crosslinker disuccinimidyl suberate (DSS) in the presence of 10^{-8} M Ca^{2+}, resulted in the formation of a B-50/CaM-complex. The amount of complex was clearly diminished after the addition of

increasing concentrations of ACTH-(1-24) (IC_{50} value of 10 μM), (Fig. 2, left panel). Surpisingly, not only the amount of complex, but also the amount of B-50 was clearly reduced in the presence of ACTH-(1-24), as can be observed from the Western Blot (Fig. 2, right panel).

TABLE III

The effect of ACTH-(1-24) on the phosphorylation of B-50 from SL-O-permeated cortical synaptosomes.

Treatment	B-50 phosphorylation (% of control)
Control	100.0±4.5
ACTH-(1-24), 10^{-5} M	85.9±5.4*
ACTH-(1-24), 10^{-4} M	54.8±4.7*

Each value is the mean of 6 observations obtained in 2 separate experiments. * $P<0.05$ for difference with controls (ANOVA, followed by Tukey B tests).

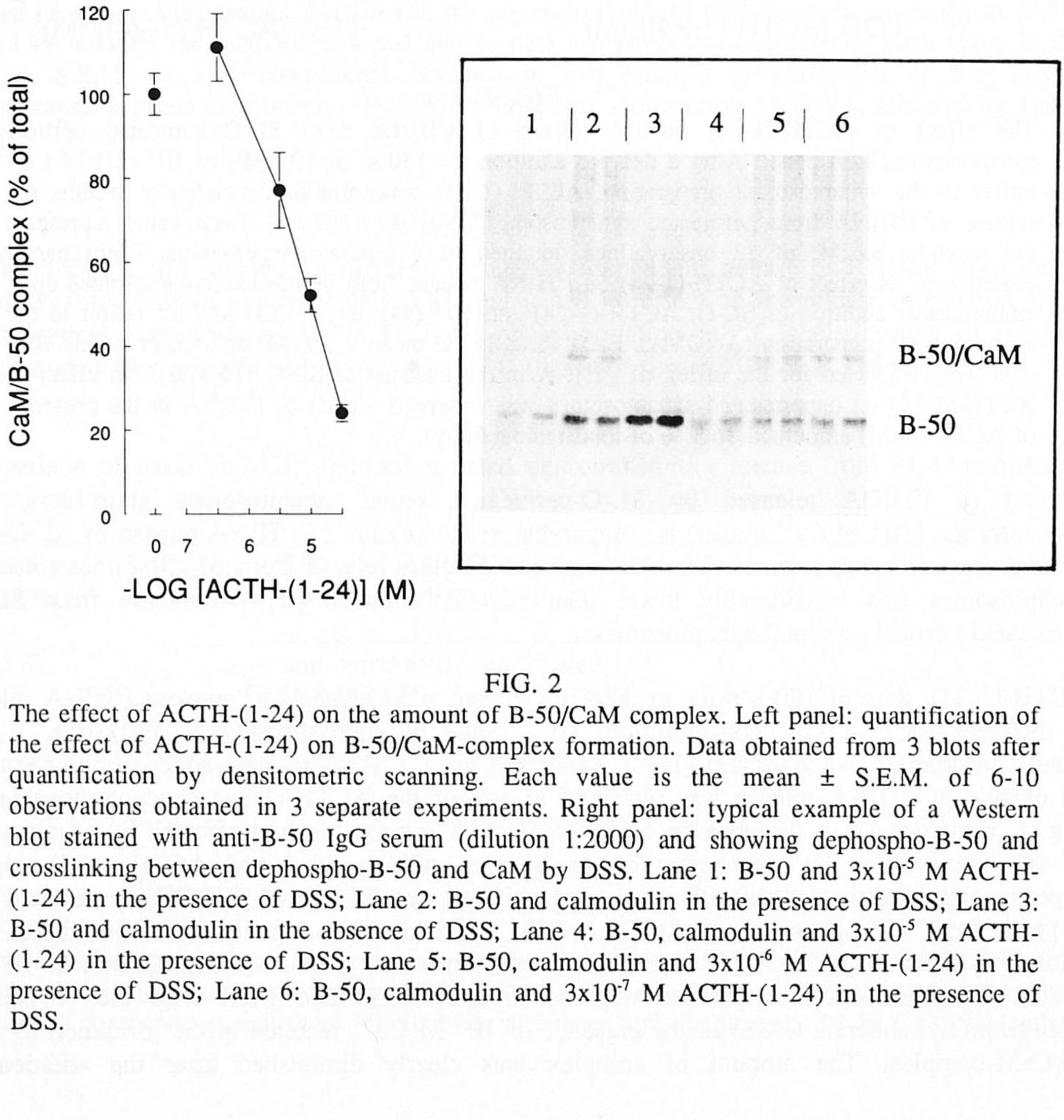

FIG. 2

The effect of ACTH-(1-24) on the amount of B-50/CaM complex. Left panel: quantification of the effect of ACTH-(1-24) on B-50/CaM-complex formation. Data obtained from 3 blots after quantification by densitometric scanning. Each value is the mean ± S.E.M. of 6-10 observations obtained in 3 separate experiments. Right panel: typical example of a Western blot stained with anti-B-50 IgG serum (dilution 1:2000) and showing dephospho-B-50 and crosslinking between dephospho-B-50 and CaM by DSS. Lane 1: B-50 and $3x10^{-5}$ M ACTH-(1-24) in the presence of DSS; Lane 2: B-50 and calmodulin in the presence of DSS; Lane 3: B-50 and calmodulin in the absence of DSS; Lane 4: B-50, calmodulin and $3x10^{-5}$ M ACTH-(1-24) in the presence of DSS; Lane 5: B-50, calmodulin and $3x10^{-6}$ M ACTH-(1-24) in the presence of DSS; Lane 6: B-50, calmodulin and $3x10^{-7}$ M ACTH-(1-24) in the presence of DSS.

Discussion

In a previous paper (10), we have reported that ACTH-(1-24) enhances the release of [^{3}H]DA from septal slices in vitro, most likely by a direct effect of ACTH-(1-24) on the presynaptic dopaminergic terminal, although an effect of ACTH-(1-24) via the release of other neurotransmitters could not be excluded. Further evidence for a presynaptic effect of ACTH-(1-24) on the release of [^{3}H]DA is obtained in the present study. Exposure of highly purified septal synaptosomes, preloaded with tritiated neurotransmitters, to ACTH-(1-24) results in an increase of [^{3}H]DA release, whereas ACTH-(1-24) does not influence the release of [^{3}H]NA from highly purified cortical synaptosomes. The exact site of action of ACTH-(1-24) on the presynaptic element is not known. It is possible that ACTH-(1-24) affects dopamine release via an as yet unidentified ACTH receptor which is similar to the well characterized adrenal ACTH-receptor (31,32). By binding to its receptor, which might be either coupled to adenylate cyclase or phospholipase C, ACTH has been suggested to produce a blockade of K^+ channels. Consequently the membrane is depolarized and voltage-dependent Ca^{2+}-channels are activated, allowing Ca^{2+} influx down the electrochemical gradient (33,34). Moreover, ACTH increases the opening probability of Ca^{2+}-channels (35,36).

There is, however, evidence for an alternative mechanism of action in the brain. In SPM, a direct inhibition of PKC by ACTH-(1-24) has been found to result in a diminished phosphorylation of the nervous-tissue specific protein B-50 and to an increase in phosphatidylinositol 4,5-bisphosphate (PIP_2) levels. Under a variety of conditions, it has been found that there is a reciprocal relationship between the degree of B-50 phosphorylation and the activity of PIP-kinase, which converts phosphatidylinositol 4-phosphate (PIP) to PIP_2 (12,37). PIP_2 is a substrate of phospholipase C, thus being a key compound in the agonist-mediated production of the second messenger molecules inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DG). As DG is the endogenous activator of PKC, it has been proposed that the sequence PIP_2 production, DG formation, PKC activation, B-50 phosphorylation, PIP-kinase inhibition represents a negative feedback system (17). Changes in polyphosphoinositide metabolism (e.g. second messenger production) and B-50 phosphorylation (see introduction) have been shown to affect neurotransmitter release (38).

Whereas ACTH-(1-24) causes inhibition of B-50 in SPM, no effect of ACTH-(1-24) on B-50 phosphorylation could be detected in intact septal synaptosomes or growth cones (39). This might be due to the fact that ACTH-(1-24) has no access to PKC because it can not cross the membrane. Activation of a putative ACTH receptor coupled to PLC or a Ca^{2+}-channel will most likely result in an increased B-50 phosphorylation, but this was not observed in our experiments. It might be, though, that the sensitivity of the phosphorylation assay used is not sufficient to detect the presumably small change in B-50 phosphorylation (as judged from the small effect of ACTH-(1-24) on [^{3}H]DA release from intact septal synaptosomes) following receptor activation.

An approach to investigate the effects of ACTH and the possible relationship between release and B-50 phosphorylation is the use of SL-O-permeated synaptosomes. The synaptosomal plasma membrane can then be passed by substances which cannot permeate the membrane, whereas the release machinery remains largely intact. This system enabled Dekker et al. (14) to introduce anti-B-50 IgGs, which were known to inhibit B-50 phosphorylation, into the synaptosome and to measure their inhibitory effect on Ca^{2+}-evoked [^{3}H]NA release. We observed that the Ca^{2+}-induced [^{3}H]DA and [^{3}H]NA release from septal SL-O-permeated synaptosomes was much less pronounced than the Ca^{2+}-induced release of [^{3}H]NA from cortical SL-O-permeated synaptosomes. The reason for such a remarkable difference in the released

amounts is not clear, though the differences may partly depend on the brain region which is chosen. For practical reasons we decided to focus on the effect of ACTH-(1-24) on [^{3}H]NA release and B-50 phosphorylation from permeated cortical synaptosomes.

ACTH-(1-24) concentration-dependently inhibited the release of [^{3}H]NA from permeated synaptosomes, but only when the 10^{-5} M Ca^{2+} buffer was added 150 s later than the other components of the reaction mixture (fig. 1, left panel vs. right panel). Apparently, ACTH-(1-24) has to be present prior to the Ca^{2+} trigger, either because it penetrates slower than Ca^{2+} ions, or because ACTH-(1-24) is easily incorporated in the lipid membrane (40) and easily sticks to proteins, due to the hydrophilic, charged part of the molecule (41). The influx of Ca^{2+}, and the rapidly increased Ca^{2+}-level in the nerve ending, induces neurotransmitter release and B-50 phosphorylation within a few seconds (38). Therefore it is feasible that ACTH-(1-24) reaches its target too late to affect the release process.

The experimental conditions for the measurement of B-50 phosphorylation were adapted from those used in the experiments on Ca^{2+}-evoked neurotransmitter release. Table III shows that ACTH-(1-24) inhibits B-50 phosphorylation in SL-O-permeated synaptosomes. The concentration needed to obtain a 50% inhibition of PKC is much higher than that found when using SPM (IC_{50} value 1-10 µM, depending on the conditions of the assay (42)). This might be due to the above mentioned sticky character of the peptide, but also to the ionic composition of the permeation buffer used. Although ACTH-(1-24) inhibits both [^{3}H]NA and B-50 phosphorylation, it is questionable whether the observed effect of ACTH-(1-24) on [^{3}H]NA release is due to a direct effect on PKC. As in intact synaptosomes only stimulatory effects were observed, it is very unlikely that the ACTH effects in vivo are mediated through the B-50/CaM pathway. In this context, a remarkable observation is that ACTH-(7-16)-NH_2, a peptide fragment which has no effect on B-50 phosphorylation (11), was more potent than ACTH-(1-24) in inhibiting [^{3}H]NA release (3 µM ACTH-(7-16)-NH_2 reduces basal and Ca^{2+}-induced release by 50 and 100%, respectively (n= 4)). Previously, we have observed that ACTH-(7-16)-NH_2 inhibits the binding of a radiolabelled ligand to the dopamine D_2 receptor more potently than does ACTH-(1-24) (41). Extensive structure-activity studies indicate the importance of the charged amino acids at the C-terminal end of the peptide, which allows ACTH-(1-24) to bind to (receptor)proteins or phospholipids (40,41,43) by electrostatic interactions.

Fig. 2 shows that the amount of B-50/CaM complex is reduced in the presence of ACTH-(1-24). However, not only the amount of B-50/CaM-complex is diminished, but the amount of B-50 as well (fig. 2b). When a blot was stained with anti-ACTH-(1-24) IgGs, a diffuse staining around the B-50 band could be observed (not shown), suggesting the crosslinking of B-50 to different ACTH-(1-24) polymers. The formation of an ACTH-(1-24)/CaM complex could not be detected, but was not expected as ACTH-(1-24)/CaM complex formation depends on the presence of Ca^{2+} (44). Therefore, an interaction of ACTH-(1-24) with B-50, rather than with CaM (45) at low Ca^{2+}-concentrations is suggested. Thus under basal Ca^{2+}-conditions, ACTH-(1-24) would prevent CaM binding to B-50. At higher Ca^{2+}-concentrations ACTH-(1-24) might bind CaM. Both mechanisms may underly the observed inhibition of [^{3}H]NA.

In conclusion, in intact septal synaptosomes an effect on [^{3}H]DA release, but not on PKC-mediated B-50 phosphorylation, could be observed. In contrast, [^{3}H]NA release and B-50 phosphorylation in SL-O-permeated synaptosomes were markedly inhibited by ACTH-(1-24), as was the formation of a complex between dephospho B-50 and CaM. However, these effects are not likely to represent the underlying mechanisms of action of ACTH-(1-24) on neurotransmitter release in vivo.

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