European Journal of Pharmacology, 88 (1983) 185–193
Elsevier Biomedical Press

SOMATOSTATIN AND ANALOGS INHIBIT ENDOGENOUS SYNAPTIC PLASMA MEMBRANE PROTEIN PHOSPHORYLATION IN VITRO

LINDA A. DOKAS *,†, HENK ZWIERS **, DAVID H. COY *** and WILLEM HENDRIK GISPEN **

** Departments of Neurosciences and Biochemistry, Medical College of Ohio, Toledo, OH 43699, U.S.A., ** Division of Molecular Neurobiology, Institute of Molecular Biology and Rudolf Magnus Institute for Pharmacology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands, and *** Department of Medicine, Tulane University, School of Medicine, 1430 Tulane Avenue, New Orleans, LA 70112, U.S.A.*

Received 19 May 1982, revised MS received 23 November 1982, accepted 21 December 1982

L.A. DOKAS, H. ZWIERS, D.H. COY and W.H. GISPEN, *Somatostatin and analogs inhibit endogenous synaptic plasma membrane protein phosphorylation in vitro,* European J. Pharmacol. 88 (1983) 185–193.

The addition of somatostatin to hippocampal synaptic plasma membrane (SPM) preparations in vitro decreased subsequent phosphorylation of specific protein bands. 10^{-4} M somatostatin inhibited the phosphorylation of protein bands with apparent molecular weights between 10 000 and 20 000 daltons and, to a lesser extent, 48 000 daltons (B-50) and 52 000. Increasingly greater degrees of inhibition were seen in response to somatostatin-28 and [D-Trp^8]somatostatin. Inhibition of B-50 protein phosphorylation in the presence of [D-Trp^8]somatostatin was most prominent in SPM preparations from the hippocampus and amygdala, with lesser degrees of inhibition seen in the cortex and hypothalamus. Addition of [D-Trp^8]somatostatin to an ammonium sulfate-precipitated fraction (ASP 55-80) from cortex only slightly inhibited endogenous B-50 phosphorylation. The injection of [D-Trp^8] somatostatin intracerebroventricularly into rats did not induce excessive grooming behavior but resulted in barrel rotation. These results suggest that somatostatin and congeners affect SPM protein phosphorylation in a manner different from that of ACTH, presumably involving membrane sites that bind somatostatin.

Somatostatin Protein phosphorylation Hippocampus Synaptic plasma membranes

1. Introduction

The phosphorylation of synaptic plasma membrane (SPM) proteins is regulated by neuropeptides. The ACTH-sensitive B-50 protein kinase system has been best characterized in this regard (Zwiers et al., 1979, 1980), but a number of other peptides are also reported to have effects on SPM protein phosphorylation. These peptides include methionine enkephalin, β-endorphin (Bär et al., 1980; Ehrlich et al., 1980) and dynorphin-(1-13) (Zwiers et al., 1981).

Somatostatin (SRIF; somatostatin-14) has been localized within the central nervous system (Perutz et al., 1977; Elde and Hökfelt, 1979). A number of biologically active analogs of somatostatin are available and their relative affinities for brain membrane binding sites have been determined (Reubi et al., 1981; Srikant and Patel, 1981a,b). The hippocampus is a potentially significant site of action for somatostatin (Rezek et al., 1976). High levels of somatostatin-like immunoreactivity have been localized in the hippocampus (Perutz et al., 1977), particularly in a dense population of nerve terminals surrounding the pyramidal cells. Somatostatin increases the turnover rate of acetylcholine in the hippocampus (Malthe-Sorenssen et al., 1978; Wood et al., 1979). The highest number of somatostatin binding sites are found on hippocampal membranes (Reubi et al., 1981). These reports suggest that somatostatin may be an

† To whom all correspondence should be addressed: Dept. of Biochemistry, Medical College of Ohio, Toledo, Ohio 43699, U.S.A.

important regulator of hippocampal synaptic physiology.

We have tested somatostatin and two somatostatin-related peptides in vitro with respect to SPM protein phosphorylation, focusing especially on interactions of these peptides with membrane preparations from the hippocampus. We report here that somatostatin inhibits the phosphorylation of distinct SPM proteins. In the case of somatostatin, somatostatin-28 and [D-Trp^8]somatostatin, the degree of inhibition seems to correlate with the affinity of the peptides for binding sites. The comparison of the effects of [D-Trp^8]somatostatin with those of ACTH on inhibition of protein phosphorylation and induction of excessive grooming behavior implied a mechanism for somatostatin and its analogs independent of the mechanism produced in response to ACTH.

2. Materials and methods

2.1. Preparation of synaptic plasma membranes (SPM) and ammonium sulfate fraction (ASP 55-80)

Male Sprague-Dawley rats were sacrificed by decapitation following the injection of sodium pentobarbital (Pentosol), 5 mg/100 g body weight. We found no qualitative difference in SPM protein phosphorylation pattern in preparations from animals sacrificed with and without pentobarbital anaesthesia. The hippocampus was dissected and tissue from 4 rats was combined for preparation of the SPM fraction. The amygdala and hypothalamus were dissected by the method of Luine et al. (1974). For preparation of cortex SPM, a piece of temporal/parietal cortex, comparable in size to the underlying hippocampus, was removed from each rat.

Brain tissue was homogenized with a Teflon-glass homogenizer in 10 vol of 0.32 M sucrose. The homogenate was spun at $1000 \times g$ for 10 min in a SS34 rotor. The resulting supernatant was spun at $10\,000 \times g$ for 20 min to prepare a crude mitochondrial-synaptosomal pellet (Whittaker et al., 1964). The pellet was lysed in 6 ml of water and homogenized in the Teflon-glass homogenizer by moving the pestle 10 strokes up and down by hand. After incubation at 4°C for 10 min, the lysate was spun at $10\,000 \times g$ for 20 min. The resulting supernatant was layered onto a discontinuous sucrose gradient consisting of 8 ml of 1.0 M sucrose overlayered with 4 ml of 0.4 M sucrose and spun for 80 min at $100\,000 \times g$ in a Beckman SW27 rotor. SPM material was collected from the interface of the 0.4 and 1.0 M sucrose layers (DeRobertis and Rodriguez de Lores Arnaiz, 1969) and diluted to a final vol of 17 ml with buffer A (10 mM sodium acetate, 10 mM magnesium acetate, 1 mM calcium acetate, pH 6.5) and spun at $100\,000 \times g$ for 30 min in the Beckman SW27 rotor. The final SPM pellet was suspended in 200 μl of buffer A. The material so obtained was highly enriched in presynaptic membranes of low buoyant density (Sorensen et al., 1981; Bär et al., 1982) and electron microscopic analysis did not show contamination with mitochondria and myelin (Burbach et al., 1981).

The protein fraction obtained from the crude mitochondrial-synaptosomal pellet of rat brain cortex was enriched in B-50 phosphorylating ability (Zwiers et al., 1980). Briefly, the crude mitochondrial-synaptosomal pellet was extracted with 0.5% Triton X-100 in 75 mM KCl. The extract was chromatographed on DEAE-cellulose using a NaCl gradient and B-50 protein kinase-containing fractions were subjected to ammonium sulfate precipitation. Proteins precipitating between 55 and 80% saturation were collected by centrifugation and termed ASP 55-80 (ammonium sulfate precipitate, fraction 55-80).

The protein content of the SPM and ASP 55-80 fractions was determined by the method of Lowry et al. (1951) with bovine serum albumin as standard.

2.2. Protein phosphorylation assays

Endogenous protein phosphorylation was measured as described previously (Zwiers et al., 1976, 1980; Wiegant et al., 1978). The assay contained 25 μg SPM or 5 μg ASP 55-80 protein, 2 μCi [γ-^{32}P]ATP at a final concentration of 7.5 μM in a total vol of 25 μl. All constituents of the assay were dissolved in buffer A. SPM or ASP 55-80 protein was preincubated at 30°C for 5 min. Where

indicated, peptides were added at 15 s before the start of the assay with labeled ATP. After addition of [γ-^{32}P]ATP, SPM protein was incubated for 20 s and ASP 55-80 protein for 60 s. Reactions were stopped by adding 12.5 μl of a solubilization mixture to bring the final concentration to 62.5 mM Tris-HCl, 2% SDS, 10% glycerol, 0.001% bromophenol blue and 5% 2-mercaptoethanol. Samples were frozen at $-20°C$ until their analysis by slab gel electrophoresis.

2.3. Slab gel electrophoresis

Proteins were separated on SDS-polyacrylamide slab gels as described previously (Zwiers et al., 1976). The acrylamide concentration of the running gel was 11% and of the stacking gel was 3%. Proteins were applied to the gel and separated at 40 mA per gel for 2 h at room temperature. Proteins were stained with Fast Green (0.1% Fast Green in 50% methanol-10% acetic acid) and destained in the same solution without Fast Green. The gels were dried down under vacuum and exposed to Kodak SB panoramic dental X-ray film (DF-85) for time periods which allowed a linear relationship between radioactivity and grain density on the film. Labeling of proteins was determined by densitometric scanning of bands on the developed film with a MacBeth Quantilog densitometer. Alternately, labeled protein bands were cut out of the gel and counted in a Beckman model LS-250 scintillation counter. Comparable results were obtained using either method. Comparisons were made only between samples on one gel. Incorporation of [^{32}P]phosphate into band B-50 under basal conditions was 1850 cpm (= 3.1 fmol P/μg total SPM protein for SPM and 13 200 cpm (= 111 fmol P/μg total ASP protein) for ASP 55-80.

Estimates of protein molecular weights were obtained by comparing their separation to the electrophoretic separation of the following proteins of known molecular weights (in daltons): phosphorylase b, 94 000; bovine serum albumin, 67 000; ovalbumin, 43 000; carbonic anhydrase, 30 000; soy bean trypsin inhibitor, 20 100; and α-lactalbumin, 14 400.

2.4. Excessive grooming test

[D-Trp^8]somatostatin or ACTH-(1-24) was administered in 3 μl of normal saline by intracerebroventricular (i.c.v.) injection of rats with an indwelling cannula (Brakkee et al., 1979). Grooming behavior was monitored over a 50 min period using the assay described by Gispen et al. (1975).

2.5. Chemicals

[γ-^{32}P]ATP (specific activity between 10–40 Ci/mmol) was obtained from New England Nuclear Corp. (Boston, MA) and adjusted to a concentration of 37.5 μM with cold ATP in buffer A. Five μl of this solution was used per assay. Somatostatin was purchased from Sigma Chemical Co. (St. Louis, MO). Somatostatin-28 and [D-Trp^8]somatostatin were synthesized stepwise by using solid phase synthesis followed by preparative partition chromatography (Meyers et al., 1978, 1980). ACTH-(1-24) was obtained from Organon Int. B.V. (Oss, The Netherlands).

3. Results

3.1. Effects of somatostatin and analogs on hippocampal SPM protein phosphorylation

The pattern of endogenous protein phosphorylation in preparations of hippocampal SPM incubated with [γ-^{32}P]ATP is shown in fig. 1. A heterogeneous pattern of phosphorylated proteins is seen. The major labeled band has a molecular weight of 48 000 daltons. Based on its molecular weight and the sensitivity of its phosphorylation to Ca^{2+} and ACTH (data not shown, see also Gispen et al., 1979), this labeled band is assumed to be the phosphoprotein B-50. The addition of 10^{-4} M somatostatin inhibited the in vitro labeling from [γ-^{32}P]ATP of some of these protein bands (fig. 1). Densitometric analysis of autoradiograms from three separate experiments has shown B-50 phosphorylation to be decreased by $-11.7 \pm 0.6\%$ in the presence of somatostatin. In addition, the phosphorylation of a protein band of molecular

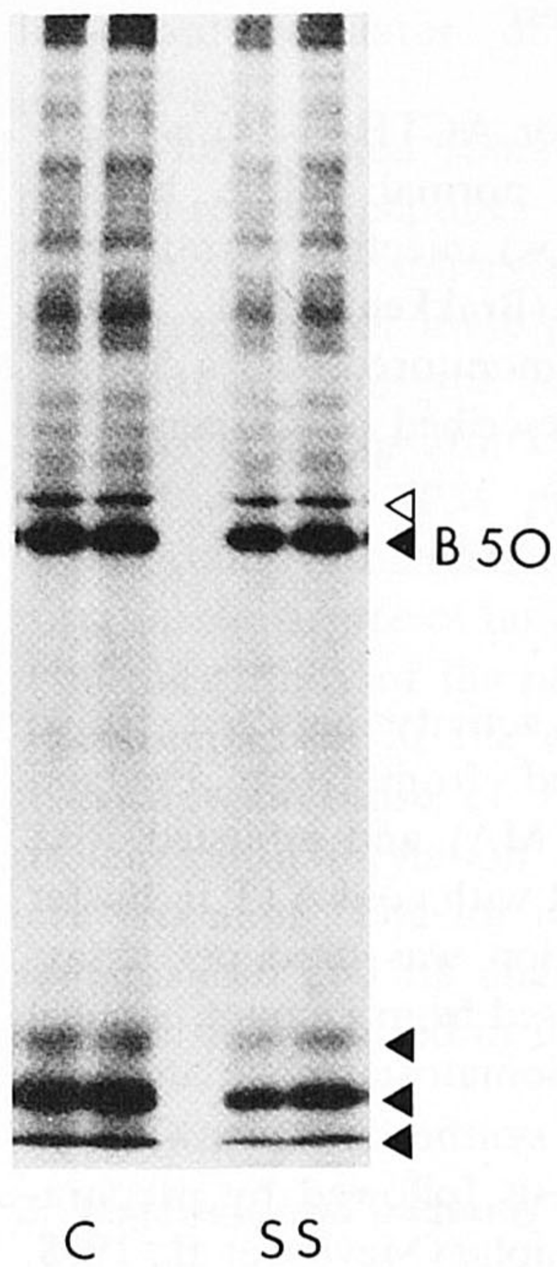

Fig. 1. Autoradiogram of a hippocamal SPM fraction incubated with [γ-^{32}P]ATP. C, control assays of endogenous protein phosphorylation; SS, assays done following a 15 s preincubation with 10^{-4} M somatostatin.

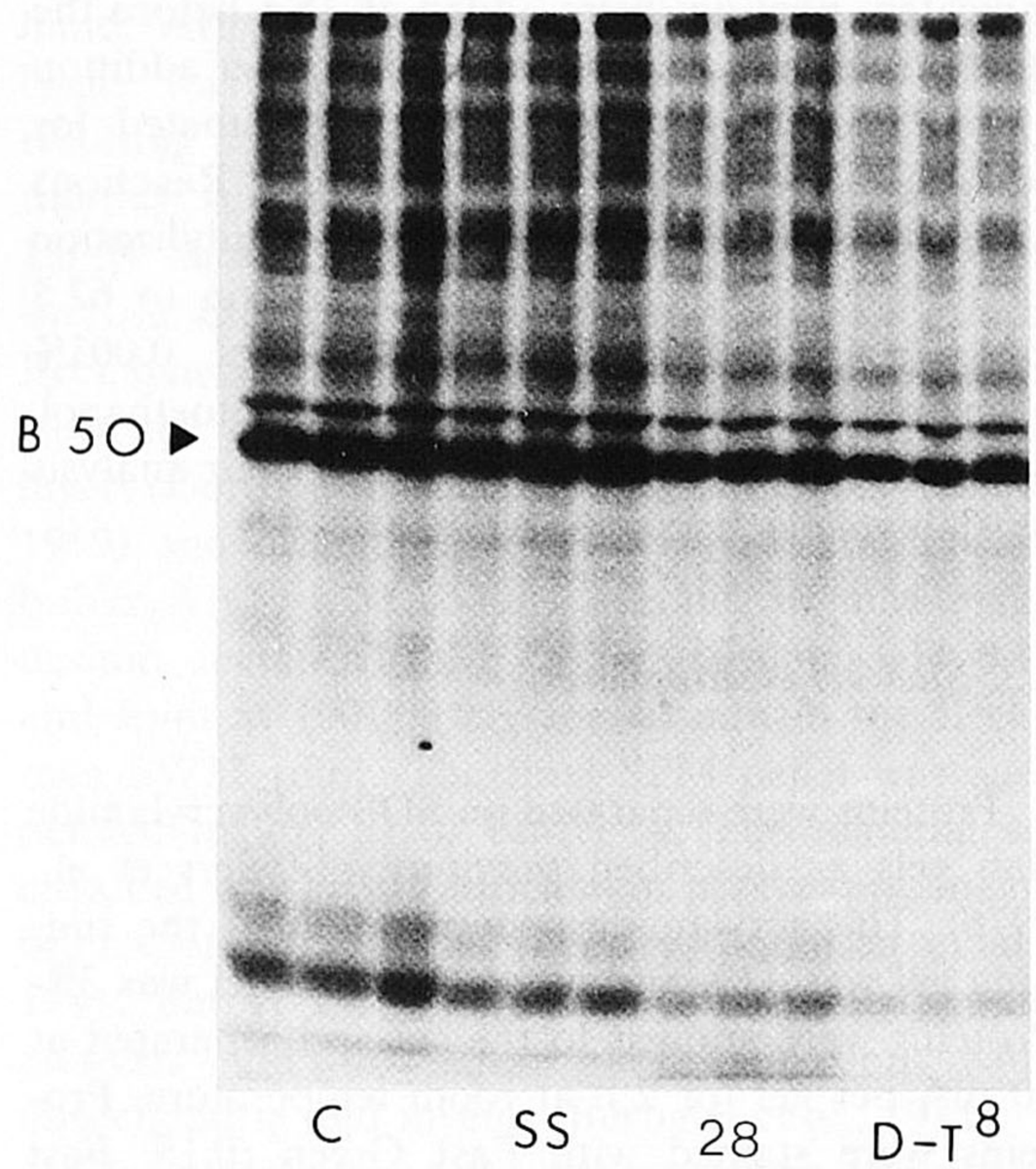

Fig. 2. Autoradiogram of a hippocampal SPM fraction showing the effect of somatostatin and analogs on endogenous protein phosphorylation. C, control assays; other assays were done following a 15 s preincubation with the following peptides at a concentration of 10^{-4} M. SS, somatostatin; 28, somatostatin-28; D-T^8, [D-Trp^8]somatostatin.

weight 52 000 daltons (open arrow, fig. 1) was inhibited by $-12.2 \pm 2.5\%$ by somatostatin. Phosphorylation of three low molecular weight bands (approximately 18 000, 17 000 and 14 000 daltons; closed arrows, fig. 1) was more significantly decreased by the addition of somatostatin to the incubation. The level of labeling from [γ-^{32}P]ATP was $-46.0 \pm 3.9\%$ for the 18 000 dalton band, $-35.7 \pm 2.9\%$ for the 17 000 dalton band and $-32.0 \pm 3.5\%$ for the 14 000 dalton band, compared to the labeling of the same bands in control incubations.

When somatostatin-28 and [D-Trp^8]somatostatin were added to the SPM preparations in vitro the inhibition of endogenous protein phosphorylation was enhanced further (fig. 2). These effects included not only inhibition of B-50 phosphorylation and the phosphorylation of the low molecular weight proteins, but especially, with [D-Trp^8]-somatostatin, a general decrease in the labeling of the high molecular weight bands (> 52 000 daltons).

Because B-50 is the predominant protein whose phosphorylation is sensitive to somatostatin and related peptides in hippocampal SPM preparations, and to facilitate comparisons between somatostatin and ACTH with regard to SPM protein phosphorylation, the following data are presented as effects of somatostatin and analogs in vitro on endogenous phosphorylation of B-50 in SPM preparations. A quantitative comparison of the phosphorylation of B-50 in response to ACTH-(1-24), somatostatin, somatostatin-28 and [D-Trp^8]somatostatin is shown in table 1A. The order of potency for inhibition of B-50 phosphorylation was found to be ACTH-(1-24) ($-68.6 \pm 10.7\%$) > [D-Trp^8]somatostatin ($-48.9 \pm 3.7\%$) > somatostatin-28 ($-37.2 \pm 3.4\%$) > somatostatin ($-11.5 \pm 3.8\%$).

TABLE 1

Effect of somatostatin, related peptides and ACTH-(1-24) on endogenous phosphorylation of B-50 in synaptic membrane fractions in vitro.

Fraction	Peptide [b]	Concentration (M)	% Decrease in B-50 phosphorylation [c]	n
(A) Hippocampal SPM	Somatostatin	10^{-4}	−11.5 ± 3.8	12
	Somatostatin-28	10^{-4}	−37.2 ± 3.4	9
	[D-Trp^8]somatostatin	10^{-4}	−48.9 ± 3.7	24
	ACTH-(1-24)	10^{-4}	−68.6 ± 10.7	9
(B) Cortex SPM	[D-Trp^8]somatostatin	10^{-4}	−43.0 ± 2.1	8
Cortex ASP	[D-Trp^8]somatostatin	10^{-5}	−10.0 ± 3.7	5
Cortex ASP	ACTH-(1-24)	10^{-5}	−65.0 ± 4.2	9

[a] Abbreviations used: SPM, synaptic plasma membranes; ASP 55-80, ammonium sulfate-precipitated extract from cortex. See Materials and methods for details of preparation. n: number of incubations.

[b] Added 15 s prior to initiation of the protein phosphorylation assay with [γ-^{32}P]ATP.

[c] Calculated as[(Experimental − Control)/Control]× 100.

3.2. Regional specificity of the effect of [D-Trp^8]-somatostatin on SPM protein phosphorylation

The effect of 10^{-4} M [D-Trp^8]somatostatin on phosphorylation of B-50 from various brain regions was compared (fig. 3). Membrane preparations from the hippocampus were most sensitive to addition of the peptide to assays of endogenous protein kinase activity. 10^{-4} M [D-Trp^8]somatostatin inhibited hippocampal B-50 phosphorylation by −48.9 ± 3.7%. SPM preparations from the amygdala (−46.0 ± 8.1%) and cortex (-43.0 ± 2.1%) showed considerable decreases in B-50 phosphorylation in the presence of this somatostatin analog. B-50 phosphorylation in hypothalamic SPM was inhibited the least by the addition of [D-Trp^8]somatostatin (−32.1 ± 1.6%). The degree of inhibition in response to [D-Trp^8]somatostatin seen in the hypothalamus was statistically different from the other values ($P < 0.005$ level, Student's t-test).

3.3. Comparison of effects of [D-Trp^8]somatostatin with known effects of ACTH-(1-24)

Because the inhibition of SPM protein phosphorylation seen in response to [D-Trp^8]somatostatin closely resembles that seen in response to ACTH-(1-24) (Zwiers et al., 1976, 1980), the question arises as to whether the two peptides act by a common mechanism. We performed therefore several experiments to determine if the somatostatin analog was interacting with the ACTH-sensitive B-50 protein kinase of SPM preparations.

First, [D-Trp^8]somatostatin was incubated with an extract of a crude mitochondrial-synaptosomal pellet fraction from cortex which had been shown to contain B-50 protein kinase in a more purified form. This fraction, termed the ASP 55-80 frac-

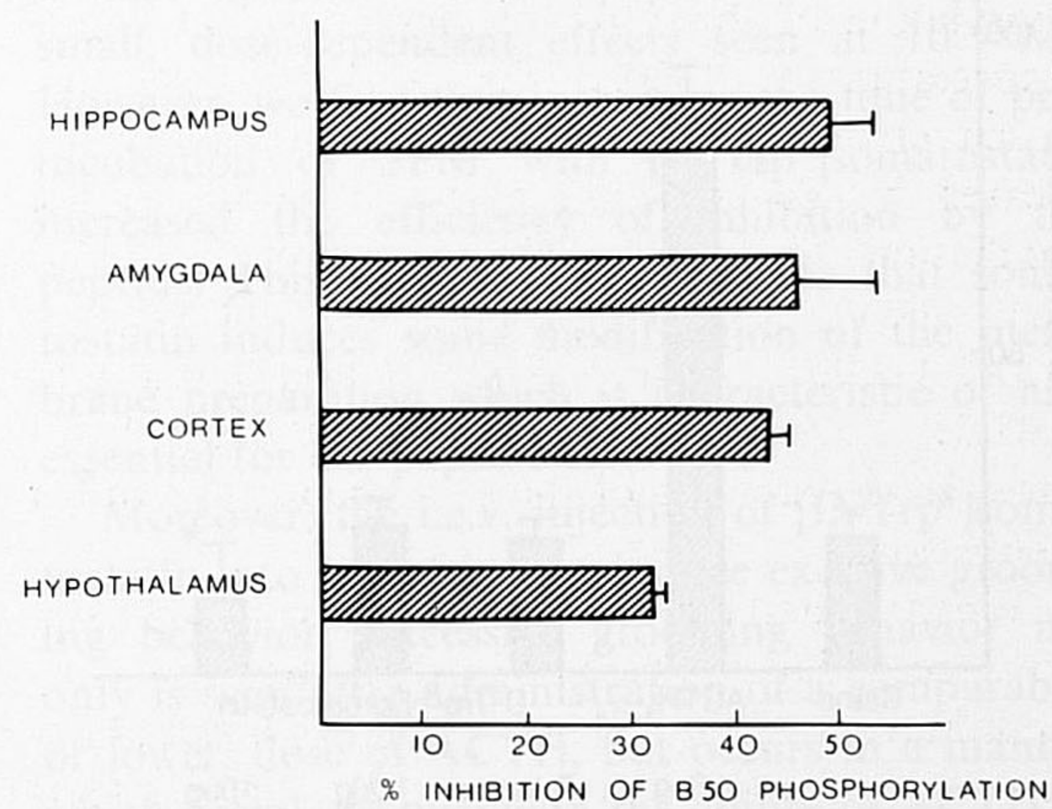

Fig. 3. Effect of [D-Trp^8]somatostatin on the phosphorylation of B-50 in the SPM fraction from various brain regions. Assay conditions were as described in fig. 2 and Materials and methods. The results are expressed as the mean ± S.E.M. of 3–8 individual experiments.

tion, contains B-50 and only one protein kinase that is B-50 protein kinase and is more sensitive to ACTH than the original SPM preparation (Zwiers et al., 1980). In duplicate assays, 10^{-4} M [D-Trp^8]somatostatin inhibited B-50 phosphorylation by -27%. The addition of 10^{-5} M [D-Trp^8]somatostatin to an ASP 55-80 fraction inhibited B-50 phosphorylation by only $-10.0 \pm 3.7\%$ (table 1B). This was in contrast to a decrease of $-65.0 \pm 4.2\%$ in B-50 phosphorylation in response to 10^{-5} M ACTH-(1-24). Thus, the more purified B-50 protein kinase system is only slightly affected by the somatostatin analog.

Secondly, ACTH-(1-24) is known to induce excessive grooming behavior in rats when injected i.c.v. The structure-activity characteristics of ACTH-related peptides with regard to induction of this grooming behavior correlates with the ability of these peptides to inhibit B-50 protein phosphorylation in vitro (Gispen and Isaacson, 1981). We therefore tested [D-Trp^8]somatostatin to see if it would cause excessive grooming behavior in rats. Rats with preimplanted cannulas received [D-Trp^8]somatostatin (0.1, 1.0 and 10.0 μg in 3 μl of saline), ACTH-(1-24) (1.0 μg in 3 μl saline) or saline (3 μl). The injection of ACTH-(1-24) induced nearly maximal excessive grooming activity, whereas no induction of excessive grooming behavior was seen in the rats treated with doses of the somatostatin analog (fig. 4). However, administration of 10 μg (6.1 nmol) of [D-Trp^8]somatostatin induced barrel rotation in the rats. This response was not observed with the lower doses of [D-Trp^8]somatostatin. The results demonstrate that [D-Trp^8]somatostatin, which has an activity similar to that of ACTH in producing inhibition of B-50 phosphorylation in intact hippocampal SPM preparations, does not resemble ACTH in terms of interaction with a more purified B-50 protein kinase fraction and in induction of excessive grooming behavior in vivo.

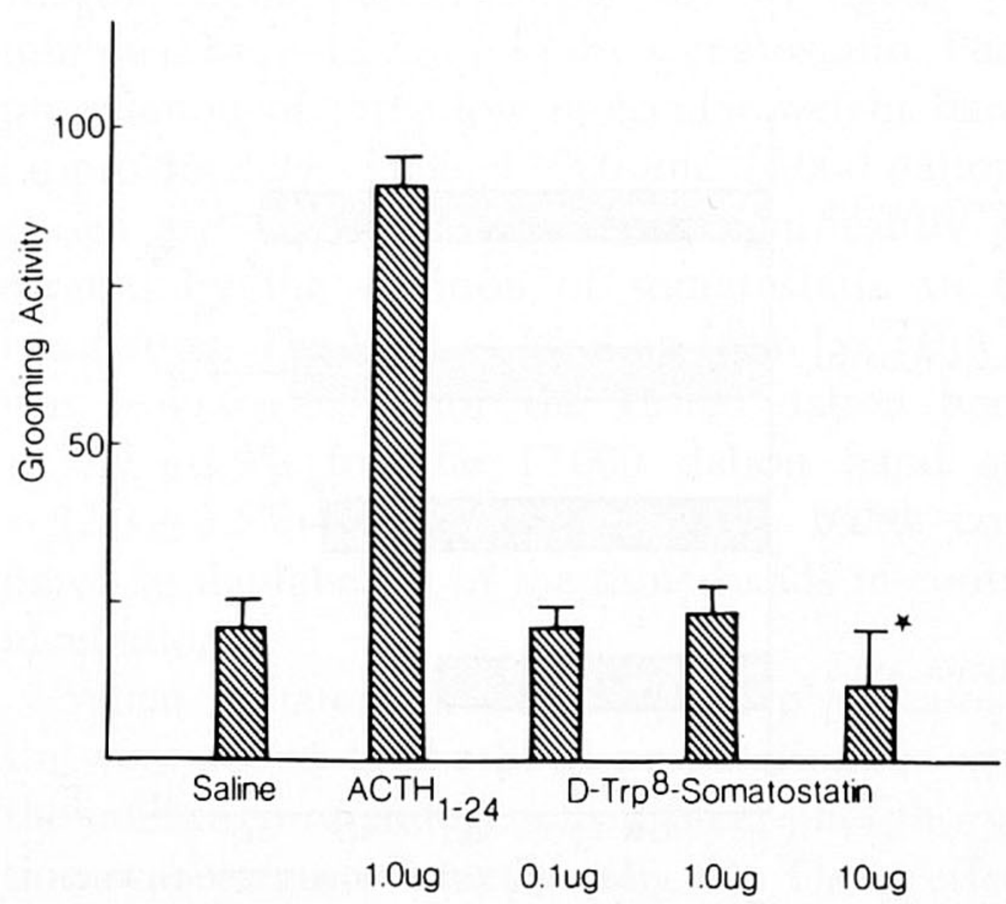

Fig. 4. Effects of i.c.v. injected peptides on excessive grooming behavior in rats. Grooming activity is expressed as the percentage of the maximal possible grooming score $\pm$S.E.M. (n = 4). The asterisk indicates that the animals displayed episodes of 'barrel rotation'.

4. Discussion

Given the evidence that the hippocampus is a functional site of action for somatostatin, we have examined whether somatostatin may affect one neurochemical process, SPM protein phosphorylation, believed to modulate synaptic efficacy (Williams and Rodnight, 1977). We found that somatostatin and two related peptides, somatostatin-28 and [D-Trp^8]somatostatin inhibited the phosphorylation of specific SPM proteins. The characteristics of the inhibition differ from those seen in response to ACTH and suggest the effects are mediated by interaction of the peptides with previously described somatostatin membrane binding sites (Reubi et al., 1981).

The difference in degree of inhibition of hippocampal B-50 protein phosphorylation in response to somatostatin, somatostatin-28 and [D-Trp^8]somatostatin in vitro seems to correspond to the relative affinities of these peptides for rat brain membrane binding sites. Reubi et al. (1981) determined that somatostatin-28 had 1.87 $\times$ and [D-Trp^8]somatostatin had 4.22 $\times$ the potency of somatostatin in a radioligand membrane binding assay. Similarly, Srikant and Patel (1981b) report [D-Trp^8]somatostatin to have 4.7 $\times$ the relative affinity of somatostatin for binding to somatostatin receptors in synaptosomal membranes. Our data show the ratio of activity for somatostatin, somatostatin-28 and [D-Trp^8]somatostatin in pro-

ducing inhibition of B-50 phosphorylation to be 1 : 3.2 : 4.3, in reasonable agreement with the binding studies.

Quantitative data on the regional distribution of specific binding sites for a somatostatin-28 analog in rat brain show the relative binding capacity to be hippocampus ⩾ amygdala ⩾ cortex > hypothalamus (Reubi et al., 1981). Our results describing the inhibition of B-50 phosphorylation in response to [D-Trp^8]somatostatin correlate with this data in that we find effects of this peptide in membrane preparations from the hippocampus, amygdala and cortex, followed by less inhibition in membrane preparations from the hypothalamus. The data are also compatible with the idea that the amount of inhibition in B-50 phosphorylation which was observed is related to the number of binding sites available in each region for interaction with the peptide.

Based on electrophysiological and behavioral observations, Rezek et al. (1976) had postulated that the hippocampus was a primary site of action for somatostatin. Administration of somatostatin in low doses into the hippocampus produced stereotyped behaviors, alterations of sleep-waking cycles and of the EEG. At higher doses (25 μg), somatostatin induced barrel rotation, an effect also noted by Cohn and Cohn (1975), Garcia-Sevilla et al. (1978) and Wood et al. (1979). Our own results with [D-Trp^8]somatostatin are in agreement with these observations. The i.c.v. injection of 10 μg (6.1 nmol) of [D-Trp^8]somatostatin produced barrel rotation in rats. Our ability to induce this behavior with a somewhat lower dose of peptide than that used in previous studies probably reflects our use of a somatostatin analog with an enhanced affinity for brain binding sites.

The induction of barrel rotation by somatostatin in rats is accompanied by an increased turnover rate of acetylcholine in the hippocampus (Malthe-Sorenssen, 1978; Wood et al., 1979). The muscarinic receptor blockers, atropine (Cohn and Cohn, 1977) and trihexiphenidyl (Malthe-Sorenssen et al., 1978) block somatostatin-induced barrel rotation. The synaptic interaction responsible for these effects may be localized to the dense population of nerve terminals surrounding hippocampal CA1 and CA2 pyramidal cells, shown by Perutz et al. (1977) to contain high levels of somatostatin-like immunoreactivity. The iontophoretic application of somatostatin onto hippocampal CA1 and CA2 pyramidal cells causes excitation of a glutamate-like nature (Dodd and Kelly, 1978). A functional association between somatostatin and acetylcholine neurons is also suggested by the observations of Davies et al. (1980) and Davies and Terry (1981) that levels of both somatostatin and acetylcholine in the hippocampus fall markedly during Alzheimer's disease (presenile dementia). Taken together with all of the above studies, our data constitute preliminary evidence for a somatostatin-induced neurochemical correlate that is related to both the function of a specific neurotransmitter system within the hippocampus and a resultant behavioral effect.

The mechanism by which somatostatin and analogs inhibit SPM protein phosphorylation remains unclear, but our results suggest that the effects are not due to a direct inhibition of B-50 protein kinase, as is seen in response to ACTH (Zwiers et al., 1979, 1980). This assumption is based on the fact that [D-Trp^8]somatostatin does not inhibit the purified B-50 protein kinase/B-50 fraction which has a more enhanced sensitivity to ACTH in vitro than intact SPM preparations (Zwiers et al., 1980). Inhibition of SPM phosphorylation by somatostatin and analogs requires a high concentration of peptide (10^{-4} M, with small, dose-dependent effects seen at 10^{-5} M). However, we find that increasing the time of preincubation of SPM with [D-Trp^8]somatostatin increased the efficiency of inhibition by the peptide. This fact may also indicate that somatostatin induces some modification of the membrane preparation which is characteristic of and essential for the peptide effect.

Moreover, the i.c.v. injection of [D-Trp^8]somatostatin into rats does not induce exessive grooming behavior. Excessive grooming behavior not only is seen after administration of a comparable, or lower, dose of ACTH, but occurs in a manner which correlates well with the ability of ACTH to inhibit B-50 phosphorylation (Gispen and Isaacson, 1981).

In the present report we have used inhibition of B-50 phosphorylation by somatostatin and analogs

as a criterion of effect in order to draw comparisons between the effects of these peptides and of ACTH. However, although B-50, under the incubation conditions used here, is the predominant phosphorylated protein in hippocampal SPM membranes, it should be noted that B-50 may not be the primary functional phosphoprotein in these membranes with regard to the action of somatostatin peptides. The aforementioned dissociation of somatostatin-induced effects from those of ACTH, which is known to alter the function of the SPM B-50 protein kinase, is evidence of this fact. The greater somatostatin-induced decrease in phosphorylation, relative to B-50, of three low molecular weight proteins in hippocampal SPM implies that the latter may be involved in the action of the peptide. Although the membrane preparation used is enriched in SPM (Burbach et al., 1981; Kristjansson et al., 1982), it is possible that the low molecular weight phosphoprotein bands are derived from some contamination of these light membranes with myelin as myelin is known to contain phosphoproteins in the low molecular weight range (Sulakhe et al., 1980). Further characterization is needed to accurately define the endogenous SPM substrates and enzyme activities which are modified in response to somatostatin and its analogs.

Acknowledgements

The authors wish to acknowledge the excellent technical assistance of Jan Brakkee and Mariola Klis. The Sprague-Dawley rats used in some of these experiments were donated by Dr. Melvyn Soloff and Mats Fernstrom. Beverly Lockwood, Carlene Thomas and Lia Claessens are thanked for their help in typing this manuscrupt. This research was supported by a grant from the Netherlands Organization for the Advancement of Pure Research (ZWO, The Hague) and by Grants NS 11718 and BRS RR 05700 to L.A.D. and by Grant AM 18370 to D.H.C. from the National Institutes of Health.

References

Bär, P.R., P. Schotman and W.H. Gispen, 1980, Enkephalins affect hippocampal membrane phosphorylation, European J. Pharmacol. 65, 165.

Bär, P.R., A.M. Thielen, F.H. Lopes da Silva, H. Zwiers and W.H. Gispen, 1982, Membrane phosphoproteins of rat hippocampus: sensitivity to tetanic stimulation and enkephalin, Brain Res. 245, 69.

Brakkee, J.H., V.M. Wiegant and W.H. Gispen, 1979, A simple technique for rapid implantation of a permanent cannula into the rat brain ventricular system, Laboratory Animal Sci. 29, 78.

Burbach, J.P.H., E.R. De Kloet, P. Schotman and D. De Wied, 1981, Proteolytic conversion of β-endorphin by brain synaptic membranes, J. Biol. Chem. 256, 12463.

Cohn, M.C. and M. Cohn, 1975, 'Barrel rotation' induced by somatostatin in the nonlesioned rat, Brain Res. 96, 138.

Davies, P., R. Katzman and R.D. Terry, 1980, Reduced somatostatin-like immunoreactivity in cerebral cortex from cases of Alzheimer disease and Alzheimer senile dementia, Nature 288, 279.

Davies, P. and R.D. Terry, 1981, Cortical somatostatin-like immunoreactivity in cases of Alzheimer's disease and senile dementia of the Alzheimer's type, Neurobiol. Aging 2, 9.

DeRobertis, E. and G. Rodriguez de Lores Arnaiz, 1969, Structural components of the synaptic region, in: Handbook of Neurochemistry, Vol. 11, ed. A. Lajtha (Plenum Press, New York) p. 365.

Dodd, J. and J.S. Kelly, 1978, Is somatostatin an excitatory transmitter in the hippocampus?, Nature 273, 674.

Elde, R. and T. Hökfelt, 1979, Localization of hypophysiotrophic peptides and other biologically active peptides within the brain, A. Rev. Physiol. 41, 587.

Ehrlich, Y.H., L.G. Davis, P. Keen and E.G. Brunngraber, 1980, Endorphin-regulated protein phosphorylation in brain membranes, Life Sci. 26, 1765.

Garcia-Sevilla, J.A., T. Magnusson and A. Carlsson, 1978, Effect of intracerebroventricularly administered somatostatin on brain monoamine turnover, Brain Res. 155, 159.

Gispen, W.H. and R.L. Isaacson, 1981, ACTH-induced excessive grooming in the rat, Pharmacol. Ther. 12, 209.

Gispen, W.H., V.M. Wiegant, H.M. Greven and D. De Wied, 1975, The induction of excessive grooming in the rat by intraventricular application of peptides derived from ACTH: structure activity studies, Life Sci. 17, 645.

Gispen, W.H., H. Zwiers, V.M. Wiegant, P. Schotman and J.E. Wilson, 1979, The behaviorally active neuropeptide ACTH as neurohormone and neuromodulator: The role of cyclic nucleotides and membrane phosphoproteins, Adv. Exp. Med. Biol. 116, 199.

Kristjansson, G.I., H. Zwiers, A.B. Oestreicher and W.H. Gispen, 1982, Evidence that the synaptic phosphoprotein B-50 is localized exclusively in nerve tissue, J. Neurochem. 39, 371.

Lowry, O.J., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951, Protein measurements with the folin phenol reagent, J. Biol. Chem. 193, 265.

Luine, V.N., R.I. Khylchevskaya and B.S. McEwen, 1974, Oestrogen effects on brain and pituitary enzyme activities, J. Neurochem. 23, 925.

Malthe-Sorensson, D., P.L. Wood, D.L. Cheney and E. Costa, 1978, Modulation of the turnover rate of acetylcholine in

rat brain by intraventricular injections of thyroptropin-releasing hormone, somatostatin, neurotensin and angiotensin II, J. Neurochem. 31, 685.

Meyers, C.A., D.H. Coy, M.W. Huang, A.V. Schally and T.W. Redding, 1978, Highly active position eight analogs of somatostatin and separation of peptide diastereomers by partition chromatography, Biochemistry 17, 2326.

Meyers, C.A., W.A. Murphy, T.W. Redding, D.H. Coy and A.V. Schally, 1980, Synthesis and biological actions of prosomatostatin, Proc. Natl. Acad. Sci. U.S.A. 77, 6171.

Perutz, P., M. Sar, G.H. Grossmann and J.S. Kizer, 1977, Synaptic terminals with somatostatin-like immunoreactivity in the rat brain, Brain Res. 137, 181.

Reubi, J.-C., M.H. Perrin, J.E. Rivier and W. Vale, 1981, High affinity binding sites for a somatostatin-28 analog in rat brain, Life Sci. 28, 2191.

Rezek, M., V. Havlicek, K.R. Hughes and H. Friesen, 1976, Central site of action of somatostatin (SRIF): role of hippocampus, Neuropharmacol. 15, 499.

Sorensen, R.G., L.P. Kleine and H.R. Mahler, 1981, Presynaptic localization of phosphoprotein B-50, Brain Res. Bull. 7, 57.

Srikant, C.B. and Y.C. Patel, 1981a, Somatostatin analogs. Dissociation of brain receptor binding affinities and pituitary actions in the rat, Endocrinology 108, 341.

Srikant, C.B. and Y.C. Patel, 1981b, Somatostatin receptors. Identification and characterization in rat brain membranes, Proc. Natl. Acad. Sci. U.S.A. 78, 3930.

Sulakhe, P.V., E.H. Petrali, E.R. Davis and B.J. Thiessen, 1980, Calcium ion stimulated endogenous protein kinase catalyzed phosphorylation of basic proteins in myelin subfractions and myelin-like membrane fraction from rat brain, Biochemistry 19, 5363.

Whittaker, V.P., I.A. Michaelson and R.J.A. Kirkland, 1964, The separation of synaptic vesicles from nerve-ending vesicles, Biochem. J. 90, 293.

Wiegant, V.M., H. Zwiers, P. Schotman and W.H. Gispen, 1978, Endogenous phosphorylation of rat brain synaptosomal plasma membranes in vitro: some methodological aspects, Neurochem. Res. 3, 443.

Williams, M. and R. Rodnight, 1977, Protein phosphorylation in nervous tissue: Possible involvement in nervous tissue function and relationship to cyclic nucleotide metabolism, Progr. Neurobiol. 8, 183.

Wood, P.L., D.L. Cheney and E. Costa, 1979, Modulation of the turnover rate of hippocampal acetylcholine by neuropeptides: possible site of action of α-melanocyte-stimulating hormone, adrenocorticotrophic hormone and somatostatin, J. Pharmacol. Exp. Ther. 209, 97.

Zwiers, H., V.J. Aloyo and W.H. Gispen, 1981, Behavioral and neurochemical effects of the new opioid peptide dynorphin-(1-13): Comparison with other neuropeptides, Life Sci. 28, 2545.

Zwiers, H., P. Schotman and W.H. Gispen, 1980, Purification and some characteristics of an ACTH-sensitive protein kinase and its substrate protein in rat brain membranes, J. Neurochem. 34, 1689.

Zwiers, H., J. Tonnaer, V.M. Wiegant, P. Schotman and W.H. Gispen, 1979, ACTH-sensitive protein kinase from rat brain membranes, J. Neurochem. 33, 247.

Zwiers, H., D. Veldhuis, P. Schotman and W.H. Gispen, 1976, ACTH, cyclic nucleotides and brain protein phosphorylation in vitro, Neurochem. Res. 1, 669.