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Research report

Hippocampal somatostatin receptors and modulation of adenylyl cyclase activity in histamine-treated rats

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Abstract

In the present study, the effects of an intracerebroventricular (i.c.v.) dose of histamine (0.1, 1.0 or 10.0 μg) on the hippocampal somatostatin (SS) receptor/effector system in Wistar rats were investigated. In view of the rapid onset of histamine action, the effects of histamine on the somatostatinergic system were studied 2 h after its administration. Hippocampal SS-like immunoreactivity (SSLI) levels were not modified by any of the histamine doses studied. SS-mediated inhibition of basal and forskolin (FK)-stimulated adenylyl cyclase (AC) activity was markedly increased in hippocampal membranes from rats treated with 10 μg of histamine (23% $\pm$ 1% vs. 17% $\pm$ 1% and 37% $\pm$ 2% vs. 23% $\pm$ 1%, respectively). In contrast, neither the basal nor the FK-stimulated enzyme activities were affected by histamine administration. The functional activity of the hippocampal guanine-nucleotide binding inhibitory protein (G_i protein), as assessed by the capacity of the stable GTP analogue 5'-guanylylimidodiphosphate (Gpp[NH]p) to inhibit FK-stimulated AC activity, was not modified by histamine administration. These data suggest that the increased response of the enzyme to SS was not related to an increased functional activity of G_i proteins. In fact, the increased AC response to SS in hippocampal membranes from histamine (10 μg)-treated rats was associated with quantitative changes in the SS receptors. Equilibrium binding data obtained with [^{125}I]Tyr^{11}-SS indicate an increase in the number of specific SS receptors (541 $\pm$ 24 vs. 365 $\pm$ 16 fmol/mg protein, $P < 0.001$) together with a decrease in their apparent affinity (0.57 $\pm$ 0.04 vs. 0.41 $\pm$ 0.03 nM, $P < 0.05$) in rat hippocampal membranes from histamine (10 μg)-treated rats as compared to control animals. With the aim of determining if these changes were related to histamine binding to its specific receptor sites, the histaminergic H_1 and H_2 receptor antagonists mepyramine and cimetidine, respectively, were administered 1 h before histamine injection. The pretreatment with mepyramine or cimetidine induced an increase in the number and affinity constant of the SS receptors whereas the simultaneous pretreatment with both histamine antagonists prevented the histamine-induced changes in SS binding to its receptors. Since the hippocampal SS receptor/effector system is modulated by histamine, it is tempting to speculate that in the hippocampus, SS could be involved as a mediator of the histamine effects on behaviors such as learning and memory.

Keywords: Histamine; Somatostatin receptor; Adenylyl cyclase; G-protein; Hippocampus

1. Introduction

Somatostatin (SS) is a neuropeptide widely distributed in the brain [11]. In the hippocampus, immunohistochemical studies have revealed many SS-containing interneurons and a profuse network of intrinsic and extrinsic SS-containing fibers that appear to project to pyramidal and granule neurons [3,18]. Specific SS receptors are present in the hippocampus [39], suggesting that SS may be a neurotransmitter or neuromodulator in this brain area. Biochemical analysis demonstrated that SS receptors are coupled to GTP-binding proteins (G-proteins) [22]. The G-proteins then couple the SS receptors with multiple effector proteins to either inhibit them, as occurs with adenylyl cyclase (AC) [44] and voltage-dependent Ca^{2+} channels [52], or to activate them, as occurs with different K^+ channels [51]. Several findings suggest an important role for SS in cognitive processes such as learning and memory by modulating neuronal activity in the hippocampus [2,16]. Recently, it has been shown that SMS 201-995, a specific peptidase-resistant SS analog, has anticonvulsant activity on EEG seizures induced by glutamate analogs in the hippocampus [50], thus suggesting an inhibitory role of hippocampal SS on seizures.

There are data supporting the hypothesis that the so-

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matostatinergic and histaminergic systems are interrelated and that reciprocal regulation occurs. Centrally administered SS produces a dose-related decrease in histamine levels in the rat hippocampus [7]. Ample evidence exists for histaminergic projections to the hippocampus [33,53], a brain area rich in SS content and receptors [21,40]. These and other subcortical afferents could play a decisive role in the expression of long-term potentiation. Furthermore, several studies suggest that the histamine H_1 and H_2 receptors may mediate together the actions of histamine in the hippocampus [46]. SS and histamine participate in a similar manner in the regulation of a number of behaviors, including those subserving memory and learning [2,16,19,46,49] and motor activity [11,46]. It has been suggested that SS [24] and histamine [43] may act as endogenous anticonvulsants in the hippocampus. In view of all the above, the aim of this study was to elucidate the role of histamine in modulating the SS receptor/effector system in the rat hippocampus. Since it appears that SS modulation of neuronal function involves the inhibition of AC activity by the occupation of receptor sites negatively coupled, via G-proteins, to this enzyme [32], we have assessed the integrity of SS receptor function by assaying both the levels of receptor recognition sites and the ability of SS to inhibit AC activity. In addition, we assessed the functional activity of the guanine nucleotide-binding inhibitory protein (G_i) and determined the effect of histamine on SS-like immunoreactivity (SSLI) content in the hippocampus. The effects of pretreatment with the histamine H_1 and H_2 receptor antagonists mepyramine and cimetidine, respectively, on the above-cited parameters were also evaluated.

2. Materials and methods

2.1. Chemicals

Synthetic Tyr^{11}-SS and SS-14 were purchased from Universal Biologicals Ltd (Cambridge, U.K.); carrier-free $Na^{125}I$ (IMS 30, 100 mCi/ml) and rabbit antibody were purchased from the Radiochemical Centre (Amersham, UK); histamine hydrochloride, mepyramine maleate, cimetidine, bacitracin, bovine serum albumin (BSA), forskolin (FK), phenylmethylsulfonyl fluoride (PMSF), 3-isobutyl-1-methylxanthine (IBMX), GTP and Gpp[NH]p were supplied by Sigma Química (Madrid, Spain). The rabbit antibody used in the radioimmunoassay technique was purchased from the Radiochemical Centre (Amersham, UK). This antiserum was raised in rabbits against SS-14 conjugated to BSA and is specific for SS, but since SS-14 constitutes the C-terminal portions of both SS-25 and SS-28, the antiserum does not distinguish between these three forms. Cross-reactivity with other peptides was less than 0.5%. Cross-reaction with several SS analogues demonstrated that neither the N-terminal glycine nor the C-terminal cysteine residue is required for antibody binding, suggesting that the antigen site is directed towards the central part of the molecule containing the tryptophan residue. The binding of SS-14 to this antibody does not depend on an intact disulfide bond in the molecule as breaking of the disulfide bond by reaction with 0.1% mercaptoethanol (boiling water bath, 5 min) did not change the immunoreactivity of the peptide.

2.2. Experimental animals

The animals used in this study were Wistar rats ($n = 60$) weighing between 200 and 250 g. Rats were maintained on a 12 h light/dark cycle (07.00–19.00) and allowed free access to food. Histamine was dissolved in 0.9% NaCl and administered intracerebroventricularly (i.c.v.) in a volume of 10 μl according to the method described by Noble et al. [27] at a dose of 0.1, 1.0 or 10.0 μg of free base, as previously described [41]. Mepyramine (30 mg/kg) [28] was dissolved in saline and cimetidine (20 μg/rat) [26] was first dissolved in dimethyl sulfoxide and then diluted with saline to the concentration required. Mepyramine and/or cimetidine were administered via an intraperitoneal (i.p.) and i.c.v. injection, respectively, 1 h before histamine administration. In all experiments, control animals received equivalent volumes of the corresponding vehicle. Rats were killed by decapitation 2 h after the last drug injection. The brain was rapidly removed and the hippocampus was dissected over ice according to the method of Głowinski and Iversen [14].

2.3. Tissue extraction and SS radioimmunoassay

For SSLI measurements, the hippocampus was rapidly homogenized in 1 ml 2 M acetic acid using a Brinkman polytron (setting 5, 30 s). The extracts were boiled for 5 min in a water bath, chilled in ice, and aliquots (100 μl) were removed for protein determination [34]. The homogenates were subsequently centrifuged at $15000 \times g$ for 15 min at 4°C and the supernatant was neutralized with 2 M NaOH. The extracts were then stored at −70°C until assay. The SSLI content was determined in tissue extracts by a modified radioimmunoassay method [34], with a sensitivity limit of 10 pg/ml. Incubation tubes prepared in duplicate contained 100 μl samples of unknown or standard solutions of 0–500 pg cyclic SS tetradecapeptide diluted in phosphate buffer (0.05 M, pH 7.2 containing 0.3% BSA, 0.01 M EDTA), 200 μl of appropriately diluted anti-SS serum, 100 μl of freshly prepared [^{125}I]Tyr^{11}-SS diluted in buffer to give 6000 cpm/assay tube (equivalent to 5–10 pg), and enough buffer to give a final volume of 0.8 ml. All reagents, as well as the assay tubes, were kept chilled in ice before their incubation for 48 h at 4°C. Separation of bound and free hormone was accomplished by the addition of 1 ml dextran-coated charcoal (dextran T-70: 0.2% w/v, Pharmacia, Uppsala, Swe-

den; charcoal Norit A: 2% w/v, Serva Feinbiochemica, Heidelberg, Germany). Serial dilution curves for the hippocampus were parallel to the standard curve. The intra- and inter-assay variation coefficients were 6.2 and 8.6%, respectively.

2.4. Binding assay

Tyr^{11}-SS was radioiodinated by chloramine-T iodination according to the method of Greenwood [15]. The tracer was purified in a Sephadex G-25 fine column (1 × 100 cm) equilibrated with 0.1 M acetic acid containing BSA 0.1% (w/v). The specific activity of the purified labelled peptide was about 600 Ci/mmol.

Hippocampal membranes were prepared as previously described by Reubi et al. [37]. Protein concentration was assayed by the method of Lowry et al. [23], with BSA as a standard. Specific SS binding was measured according to the modified method of Czernik and Petrack [8]. Briefly, the membranes (0.15 mg protein/ml) were incubated in 250 μl of a medium containing 50 mM Tris-HCl buffer (pH 7.5), 5 mM $MgCl_2$, 0.2% (w/v) BSA and 0.1 mg/ml bacitracin with 250 pM [^{125}I]Tyr^{11}-SS either in the absence or presence of 0.01–10 nM unlabelled SS. After a 60 min incubation at 30°C, bound and free ligand were separated by centrifugation at 11 000 × *g* for 2 min and the radioactivity in the resultant pellet was measured. Nonspecific binding was obtained from the amount of radioactivity bound in the presence of 10^{-7} M SS and represented about 20% of the binding observed in the absence of unlabelled peptide. This nonspecific component was subtracted from the total bound radioactivity in order to obtain the corresponding specific binding.

2.5. Evaluation of radiolabelled peptide degradation

The inactivation of [^{125}I]Tyr^{11}-SS in the incubation medium after exposure to membranes was studied by measuring the ability of preincubated peptide to rebind to fresh membranes [1]. Briefly, [^{125}I]Tyr^{11}-SS (250 pM) was incubated with membranes from rat hippocampus (0.15 mg protein/ml) for 60 min at 30°C. After this preincubation, aliquots of the medium were added to fresh membranes and incubated for an additional 60 min at 30°C. The fraction of the added radiolabelled peptide which was specifically bound during the second incubation was measured and expressed as a percentage of the binding that had been obtained in control experiments performed in the absence of membranes during the preincubation period.

2.6. Adenylyl cyclase assay

AC activity was measured as previously reported [17] with minor modifications. Briefly, hippocampal membranes (0.06 mg/ml) were incubated with 1.5 mM ATP, 5 mM $MgSO_4$, 10 μM GTP, an ATP-regenerating system (7.5 mg/ml creatine phosphate and 1 mg/ml creatine kinase), 1 mM IBMX, 0.1 mM PMSF, 1 mg/ml bacitracin, 1 mM EDTA, and test substances (10^{-4} M SS or 10^{-5} M FK) in 0.1 ml of 0.025 M triethanolamine/HCl buffer (pH 7.4). After a 15 min incubation at 30°C, the reaction was stopped by heating the mixture for 3 min. After cooling, 0.2 ml of an alumina slurry (0.75 g/ml in triethanolamine/HCl buffer, pH 7.4) was added and the suspension was centrifuged. The supernatant was taken for assay of cyclic AMP (cAMP) by the method of Gilman [13]. The SS concentration used was that necessary to achieve inhibition of rat [44] and human [5,12] brain AC activity. FK was used at a concentration that could effectively stimulate the catalytic subunit of rat AC [44].

2.7. Data analysis

The computer program LIGAND [25] was used to analyze the binding data. The use of this program enabled models of receptors that best fit the given sets of data to be selected. The same program was also used to present the data in the form of Scatchard plots and to compute values for receptor affinity (K_d) and density (B_{max}) that best fit the sets of binding data for each rat. Statistical comparisons of all the data were carried out with one way analysis of variance (ANOVA) and the Student Newman-Keuls test. Means among groups were considered significantly different when the P value was less than 0.05. Each individual experiment was performed in duplicate.

3. Results

As shown in Table 1, SS-mediated inhibition of basal and FK-stimulated AC activity was markedly increased in

Table 1
Effect of somatostatin (SS) (10^{-4} M) and forskolin (FK) (10^{-5} M) on brain adenylyl cyclase activity (pmol cAMP/min/mg protein) in hippocampal membranes from control rats ($n = 15$) and rats treated with 0.1 μg ($n = 5$), 1.0 μg ($n = 5$) or 10.0 μg ($n = 5$) of histamine

	Control	Histamine		
		0.1 μg	1.0 μg	10 μg
Basal activity	119 ± 4	123 ± 1	118 ± 10	121 ± 2
+ 10^{-4} M SS	99 ± 4	100 ± 3	97 ± 4	93 ± 2
% SS inhibition of basal activity	17 ± 1	19 ± 2	18 ± 1	23 ± 1 **
+ 10^{-5} M FK	634 ± 16	682 ± 51	606 ± 11	631 ± 38
Fold FK stimulation over basal	5.2 ± 0.3	5.5 ± 0.3	5.1 ± 0.2	5.4 ± 0.3
+ 10^{-5} M FK + 10^{-4} M SS	488 ± 11	487 ± 26	478 ± 6	398 ± 13
% SS inhibition of FK stimulation	23 ± 1	28 ± 3	21 ± 3	37 ± 2 ***

Values represent the mean ± SEM of five separate experiments, each performed in duplicate. In the control group, the results express the mean value of a pool of the control animals. Statistical comparison versus control: ** $P < 0.01$, *** $P < 0.001$.

Table 2
Effect of somatostatin (10^{-4} M) and forskolin (10^{-5} M) on brain adenylate cyclase activity (pmol/cAMP/min/mg protein) in hippocampal membranes from control rats ($n = 10$) and rats pretreated with mepyramine (30 mg/kg, i.p.) ($n = 5$) or cimetidine (20 μg, i.c.v.) ($n = 5$) 1 h before histamine (10 μg, i.c.v.) injection

	Control	Mepyramine plus histamine	Cimetidine plus histamine
Basal activity	120 ± 3	125 ± 6	124 ± 7
$+10^{-4}$ M SS	101 ± 2	97 ± 4	95 ± 3
% SS inhibition of basal activity	16 ± 1	22 ± 2 **	23 ± 3 *
$+10^{-5}$ M FK	636 ± 32	640 ± 26	653 ± 23
$+10^{-5}$ M FK + 10^{-4} M SS	489 ± 28	422 ± 34	424 ± 31
% SS inhibition of FK stimulation	23 ± 3	34 ± 4 *	35 ± 5 *

Values represent the mean ± SEM of five separate experiments, each performed in duplicate. In the control group, the results express the mean value of a pool of the control animals. Statistical comparison versus control: * $P < 0.05$, ** $P < 0.01$.

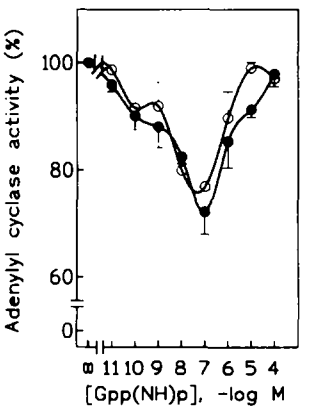

Fig. 1. Dose effect curves for 5′-guanylylimidodiphosphate (Gpp[NH]p)-mediated inhibition of adenylyl cyclase (AC) activity in rat hippocampal membranes from control (●) ($n = 5$) and histamine (10.0 μg)-treated (○) ($n = 5$) rats. Curves for the action of Gpp[NH]p on AC activity were carried out in the presence of 3×10^{-6} M forskolin (FK) and the indicated concentrations of Gpp[NH]p. Data are expressed as a percentage of FK-stimulated AC activity in the absence of Gpp[NH]p (100%). The results are given as the mean ± SEM of five separate determinations, each performed in duplicate. No statistically significant differences were obtained between the control and histamine-treated rats.

hippocampal membranes from rats treated with 10.0 μg of histamine as compared with control rats. The response of AC to the diterpene forskolin (FK), which is assumed to act directly upon the catalytic subunit of AC, was also examined. The results obtained indicate that the ability of the diterpene to stimulate the enzyme activity was not altered after histamine administration. In addition, no significant differences were observed for the basal and FK-stimulated AC activities between control and histamine-treated (0.1, 1.0 or 10.0 μg) rats (Table 1). Pretreatment with the histamine H_1 or H_2 receptor antagonists mepyramine and cimetidine, respectively, also induced an increase in SS-mediated inhibition of basal and FK-stimulated AC activity (Table 2).

Hippocampal G_i function, as assessed by inhibiting FK (3×10^{-6} M)-stimulated AC activity with the stable GTP analog Gpp[NH]p, was similar in control and histamine (10 μg)-treated rats (Fig. 1).

Preliminary experiments confirmed that the specific binding of [^{125}I]Tyr^{11}-SS to hippocampal membranes changed linearly with protein concentration and was time-dependent in all experimental groups. An apparent equilibrium was observed between 50 and 180 min at 30°C (data not shown). All subsequent binding experiments were therefore conducted at 30°C for 60 min. Hippocampal membranes from all experimental groups showed a similar peptide degradation capacity and the values varied by no more than 10% among the groups.

SS binding to hippocampal membranes was found to be markedly increased after histamine administration at a dose of 10.0 μg as compared with controls (Fig. 2). This increase was due to a rise in the maximal number of SS receptors (48.2%), as revealed by Scatchard plots of the binding data (Table 3; Fig. 2). In addition, a significant decrease in the affinity of these receptors (39%) was observed (Table 3; Fig. 2). No changes in the number or affinity of the SS receptors were detected at the lower doses of 0.1 or 1.0 μg of histamine (Table 3). The addition of 10^{-5} M histamine to the incubation medium changed neither the number nor the affinity of the SS receptors in hippocampal membranes from normal rats (data not shown). With the aim of determining if the changes in SS binding were related to histamine binding to its specific receptor sites, the histamine H_1 and H_2 recep-

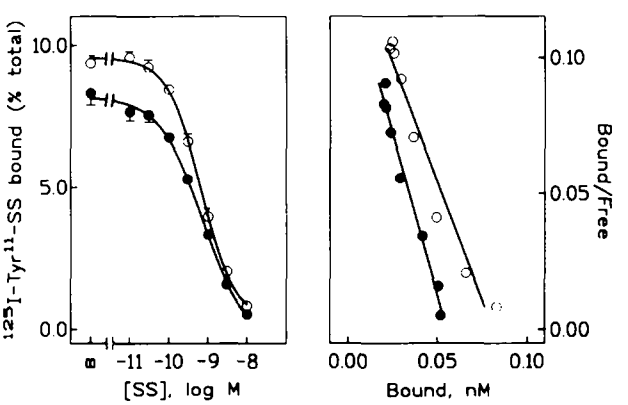

Fig. 2. Effect of histamine (10.0 μg, i.c.v.) on somatostatin (SS) binding to rat hippocampal membranes. Left panel: Competitive inhibition of specific [^{125}I]Tyr^{11}-SS binding by unlabelled SS to hippocampal membranes. Membranes (0.15 mg protein/ml) were incubated for 60 min at 30°C in the presence of 250 pM [^{125}I]Tyr^{11}-SS and increasing concentrations of native peptide. Points correspond to values for the control rats (●) ($n = 5$) and rats treated with 10.0 μg of histamine (○) ($n = 5$). Each point represents the mean ± SEM of five experiments, each performed in duplicate. Right panel: Scatchard analysis of the binding data.

Table 3
Effect of increasing concentrations of histamine (0.1 μg, 1.0 μg or 10.0 μg) and of pretreatment with cimetidine (20 μg/rat) plus mepyramine (30 mg/kg) administered 1 h before histamine (10.0 μg) on equilibrium parameters for somatostatin (SS) binding to rat hippocampal membranes and on SS-like immunoreactivity (SSLI) concentration in the rat hippocampus

Group	SS receptors		SSLI	n
	B_{max}	K_d		
Control	393 ± 15	0.32 ± 0.03	16.14 ± 1.81	5
Histamine 0.1 μg	428 ± 16	0.30 ± 0.03	15.46 ± 2.09	5
Control	408 ± 14	0.38 ± 0.03	13.08 ± 2.19	5
Histamine 1.0 μg	418 ± 26	0.34 ± 0.04	14.21 ± 0.60	5
Control	365 ± 16	0.41 ± 0.03	11.06 ± 1.27	5
Histamine 10.0 μg	541 ± 24 ***	0.57 ± 0.04 *	12.59 ± 0.86	5

Binding parameters were calculated from Scatchard plots by linear regression. Units for SSLI are ng of SS per mg of protein, units for K_d are nM and units for B_{max} are fmol of SS bound per mg of protein. The results are represented as the mean ± SEM of five separate experiments, each performed in duplicate. Statistical comparison versus control: * $P < 0.05$, *** $P < 0.001$.

tor antagonists mepyramine and cimetidine, respectively, were administered 1 h before histamine administration. A 29.5% and 27.2% increase in the number of SS receptors accompanied by a 69.6% and 72.7% decrease in their apparent affinity was observed in rats pretreated with mepyramine or cimetidine, respectively (Table 4). The pretreatment with mepyramine and cimetidine administered simultaneously prevented the histamine-induced changes in SS binding to its receptors (Table 4).

The i.c.v. administration of histamine at a dose of 0.1, 1.0 or 10.0 μg did not significantly affect SSLI content in the hippocampus as compared with the control groups (Table 3).

Table 4
Effect of pretreatment with mepyramine (30 mg/kg, i.p.), cimetidine (20 μg, i.c.v.) or mepyramine plus cimetidine administered 1 h before histamine (10 μg, i.c.v.) injection on equilibrium parameters for somatostatin (SS) binding to rat hippocampal membranes

Group	SS receptors		n
	B_{max}	K_d	
Control	389 ± 13	0.33 ± 0.04	15
Mepyramine plus histamine	504 ± 20 *	0.56 ± 0.04 *	5
Cimetidine plus histamine	495 ± 23 *	0.57 ± 0.04 *	5
Mepyramine plus cimetidine plus histamine	395 ± 21	0.33 ± 0.01	5

Binding parameters were calculated from Scatchard plots by linear regression. Units for K_d are nM and units for B_{max} are fmol of SS bound per mg of protein. The results are represented as the mean ± SEM of five separate experiments, each performed in duplicate. In the control group, the results express the mean value of a pool of the controls ($n = 15$), since the B_{max} and K_d values were not affected by the vehicle. Statistical comparison versus control: * $P < 0.05$.

4. Discussion

This study demonstrates for the first time that the number of SS receptors and the AC response to SS are increased in the hippocampus of rats treated with 10.0 μg of histamine, an effect that is mediated by both H_1 and H_2 histaminergic receptors. The hippocampus was chosen to examine the modulation of the SS receptor/effector system due to the high concentration of SS and its receptors [21,40] in spite of the moderate density of histaminergic fibers [33,53] present in this brain area. This moderate density of histaminergic fibers may explain the fact that changes in the somatostatinergic system were only observed at the highest dose tested.

The increased capacity of SS to inhibit basal and FK-stimulated AC activity in rats treated with 10.0 μg of histamine, as compared with the control group, is most likely related to the observed rise in the number of SS receptors. Indeed, the basal and FK-stimulated enzyme activities are not altered by histamine, suggesting that the catalytic subunit of AC is not involved in the increased response to SS. Furthermore, the functional activity of the G_i protein was similar in the control and histamine (10.0 μg)-treated group, which suggests that there is no abnormality in the coupling of the SS receptor to AC.

A relatively high concentration of SS (10^{-4} M) was required to produce inhibition of AC activity. This same SS concentration was used by Schettini et al. [44], Bergström et al. [5] and Garlind et al. [12] in their studies on SS-mediated inhibition of AC activity in the rat and human brain. This concentration is three log units higher than that necessary to displace [^{125}I]Tyr^{11}-SS binding. A possible explanation for this discrepancy may lie in the observation that G-proteins can modulate the affinity of SS receptors and/or the coupling to the effector system (AC among others). In this respect, Enjalbert et al. [9] and Koch and Schonbrunn [20] have demonstrated that the mobilization of the G-protein by GTP reduces the SS receptor affinity for the neuropeptide in cerebral cortical cells and GH_4C_1 pituitary cell clones. Indeed, in the presence of GTP necessary to couple the SS receptor to the AC catalytic subunit, the SS receptor may shift from an apparent high-affinity state (observed in binding studies) to an apparent low-affinity state (observed in AC studies).

The SSLI content and the equilibrium parameters of the SS receptors in the hippocampus of control rats were similar to those previously reported by others [36,47]. Scatchard analysis of the stoichiometric data suggests the existence of only one type of SS receptor. This finding agrees with some studies in rat brain membranes in which [^{125}I]Tyr^{11}-SS was also used as a tracer [10,47] but differs from other reported data where different SS analogs were used [38,48]. Recently, five different SS receptor subtypes have been cloned [4] and the tissue distribution of the messenger ribonucleic acid for each subtype has been studied in the rat [6,35]. The fact that this study with

[^{125}I]Tyr^{11}-SS shows only one type of SS receptor might be explained by the hypothesis that this radioligand binds to all types of SS receptors with dissociation constants that are virtually identical and cannot be discriminated by Scatchard analysis.

Overall, the present data are suggestive of a possible role of histamine as a modulator of the SS receptor/effector system in the hippocampus. The molecular mechanism underlying the increase in SS receptors and SS-mediated AC inhibition in hippocampal membranes of histamine (10.0 μg)-treated rats is unknown. This effect, however, seems to be due to a direct interaction of histamine with histaminergic receptors which are present in high density in this brain area [31,42] since the changes induced by this biogenic amine were prevented by pretreatment with the histamine H_1 and H_2 receptor antagonists administered simultaneously. In addition, blockade of the histamine H_1 or H_2 receptors by pretreatment with mepyramine or cimetidine, respectively, induced an increase in the number of SS receptors and in SS-mediated inhibition of AC activity, indicating that both postsynaptic histaminergic receptors, H_1 and H_2, are implicated in the histamine effects on the hippocampal somatostatinergic system observed in this study. The increase in tracer binding was not due to a direct effect of histamine on SS receptors since no change in [^{125}I]Tyr^{11}-SS binding was detected following incubation of fresh hippocampal membranes with histamine. The dose response concentration of histamine eliciting an increase in the specific binding of SS in the hippocampus is in good agreement with effective doses of histamine which have been shown to produce a physiological effect [41]. Examples of positive control mechanisms of receptor-receptor interactions include insulin activation of the IGF-II receptor [30], thyrotropin-releasing hormone activation of SS binding [45] and relaxin activation of [^{125}I]Tyr^{11}-insulin binding [29].

The role of SS receptors in the hippocampus, a structure involved in the regulation of behavior, especially cognitive functions [55], still remains to be elucidated. However, there are several lines of evidence suggesting that SS can play a physiological role in the modulation of this limbic structure [54]. Since the hippocampus receives histaminergic innervation [33,53] and SS receptors are modulated by histamine, it is tempting to speculate that in the hippocampus, SS could be involved as a mediator of the effects of histamine on behaviors such as learning and memory. The importance of this observation for basic science and clinical research requires further exploration.

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