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α-Fluoromethylhistidine influences somatostatin content, binding and inhibition of adenylyl cyclase activity in the rat frontoparietal cortex

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Abstract

Slow-wave sleep, wakefulness, locomotor activity and learning and memory are regulated in similar ways by somatostatin (SS) and histamine. To clarify the possible role of endogenous histamine on the somatostatinergic system of the rat frontoparietal cortex, we studied the effect of 50 μg of α-fluoromethylhistidine (α-FMH), a specific inhibitor of histidine decarboxylase, administered intracerebroventricularly (i.c.v.) at 1, 4 and 6 h, on somatostatin-like immunoreactivity (SSLI) content and the SS receptor/effector system. The histamine content in the frontoparietal cortex decreased to about 67, 60 and 72% of control values at 1, 4 and 6 h after α-FMH administration, respectively. At 6 h after α-FMH injection, there was an increase in SSLI content and a decrease in the number of SS receptors, with no change in the apparent affinity. No significant differences were seen for the basal and forskolin (FK)-stimulated adenylyl cyclase (AC) activities in the frontoparietal cortex of α-FMH-treated rats when compared to the control group at all times studied. At 6 h after α-FMH administration, however, the capacity of SS to inhibit basal and FK-stimulated AC activity in the frontoparietal cortex was significantly lower than in the control group. The ability of the stable GTP analogue 5'-guanylylimidodiphosphate (Gpp(NH)p) to inhibit FK-stimulated AC activity in frontoparietal cortex membranes was the same in the α-FMH-treated (6 h) and control animals. Therefore, the decreased SS-mediated inhibition of AC activity observed in the α-FMH-treated rats is not due to an alteration at the guanine nucleotide-binding inhibitory protein (G_i) level but rather may be due to the decrease in the number of SS receptors. Taken together, these data suggest that α-FMH influences the sensitivity to SS in the rat frontoparietal cortex.

Keywords: α-Fluoromethylhistidine; Somatostatin receptor; Adenylyl cyclase; Rat; Frontoparietal cortex

1. Introduction

It is generally accepted that the somatostatinergic and histaminergic systems exert similar effects on sleep–wakefulness [1–4], memory and learning [5–7] and motor activity [1,8]. An anatomical basis for the interaction between the somatostatinergic and histaminergic systems has also been provided by the observations of histaminergic nerve terminals in cerebral regions rich in SS content and SS receptors [9–11]. Although the effects of somatostatin (SS) on brain histamine content have been studied [12], the

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effects of endogenous histamine on the rat somatostatinergic system are mainly unknown. In a recent study carried out by this laboratory, we observed that exogenous histamine increases the SS receptor/effector system in the rat frontoparietal cortex, inducing both an increase in the number of SS receptors and in SS-mediated inhibition of AC activity [13]. These effects were shown to be specifically mediated by histamine receptors since they were prevented by pretreatment with the histamine H_1 and H_2 receptor antagonists mepyramine and cimetidine, respectively. The aim of the present study, thus, was to clarify the possible role of endogenous histamine on the rat cerebral somatostatinergic system. Therefore, we examined the effects of inhibition of histamine synthesis by the histidine decarboxylase inhibitor α-fluoromethylhistidine (α-FMH) [14,15] on SS receptors in rat frontoparietal cortex membranes. Since the post-receptor mechanism of action of SS includes, at least in part, the inhibition of adenylyl cyclase (AC) activity via GTP binding 'G proteins' [16,17], we have studied SS-inhibited AC activity in frontoparietal cortex membranes from control and α-FMH-treated rats. Low concentrations of the stable GTP analogue 5'-guanylylimidodiphosphate (Gpp(NH)p) were employed to detect functional guanine nucleotide-binding inhibitory protein (G_i) activity by inhibiting basal AC activity previously amplified by forskolin (FK). In addition, SSLI and histamine content were determined.

2. Materials and methods

2.1. Chemicals

Synthetic Tyr^{11}-somatostatin (Tyr^{11}-SS) and SS-14 were purchased from Universal Biologicals Ltd. (Cambridge, UK); carrier-free Na ^{125}I (IMS 30, 100 mCi/ml) was purchased from the Radiochemical Centre (Amersham, UK); bacitracin, phenylmethylsulfonyl fluoride (PMSF), 3-isobutyl-1-methylxanthine (IBMX), bovine serum albumin (BSA), GTP, FK and Gpp(NH)p were supplied by Sigma Química (Madrid, Spain). α-FMH was kindly supplied by Merck, Sharp and Dome Research Laboratories (Rathway, NJ, USA). The rabbit antibody used in the radioimmunoassay technique was purchased from the Radiochemical Center (Amersham, UK). This antiserum was raised in rabbits against SS-14 conjugated to BSA and is specific for SS, but since SS-14 also constitutes the C-terminal portions of both SS-25 and SS-28, the antiserum does not distinguish between these 3 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 its antibody does not depend on an intact disulfide in the molecule since breaking of the disulfide bond by reaction with 0.1% mercaptoethanol (boiling water bath, 5 min) did not change peptide immunoreactivity.

2.2. Experimental animals

Thirty female Wistar rats weighing 200–250 g were used in this study since previous studies with male Wistar rats carried out by our laboratory showed similar values of SS receptors and SS content in the control group [18]. All rats used were at the same stage of the estrous cycle (beginning of the cycle). Rats were maintained on a 12-h light/dark cycle (07.00–19.00 h) and allowed free access to food. α-FMH was dissolved in saline and administered intracerebroventricularly (i.c.v.) in a volume of 10 μl according to the method described by Noble et al. [19] at a dose of 50 μg as previously described [20]. In all experiments, control animals received 10 μl of saline. The rats were decapitated 1, 4, and 6 h after α-FMH administration since it has been previously reported that a significant decrease in histamine content in the rat frontoparietal cortex is achieved at these time periods [20]. The brain was rapidly removed and the frontoparietal cortex was dissected over ice according to the method of Glowinski and Iversen [21].

2.3. Determination of brain histamine

After homogenization of frontoparietal cortex in 3% perchloric acid containing 5 mM Na_2-EDTA with a Polytron homogenizer at the maximum setting for 10 s in an ice bath, the homogenate was cen-

trifuged at 10,000 *g* for 30 min at 4°C and stored at −80°C until use. Fifty μl of the clear supernatant were injected into an HPLC-fluorometric system and histamine was measured by the α-phthalaldehyde method as described by Yamatodani et al. [22]. The minimum detection limit of this method was 0.05 pmol, and the intra- and inter-assay coefficients of variation were 3.3 and 6.8%, respectively.

2.4. Tissue extraction and SS radioimmunoassay

For SSLI measurements, the frontoparietal cortex was rapidly homogenized in 1 ml 2 M acetic acid using a Brinkman polytron (setting 5, 30 s). Extracts were boiled for 5 min in a water bath, chilled in ice, and aliquots (100 μl) were removed for protein determination [23]. The homogenates were subsequently centrifuged at 15,000 *g* for 15 min at 4°C and the supernatant was neutralized with 2 M NaOH. Extracts were immediately stored at −70°C until assay. The SSLI content was measured in tissue extracts by a modified radioimmunoassay method [23], 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 incubation at 4°C for 48 h. Separation of bound and free hormone was accomplished by the addition of 1 ml dextran-coated charcoal (dextran T70: 0.2% w/v, Pharmacia, Uppsala, Sweden; charcoal: Norit A 2% w/v, Serva, Feinbiochemica, Heidelberg, Germany). Dilution curves for each brain area were parallel to the standard curve. The intra- and inter-assay variation coefficients were 6.8 and 8%, respectively.

2.5. Binding assay

Tyr^{11}-SS was radioiodinated by chloramine-T iodination [24]. Separation of iodinated SS from unincorporated iodine was carried out on a Sephadex G-25 (fine) column equilibrated and eluted with 0.1 M acetic acid containing BSA (0.1%, w/v). The specific activity of the radioligand was 600 Ci/mmol.

Membranes from rat frontoparietal cortex were prepared as previously described by Reubi et al. [25]. Membrane protein was determined by the method of Lowry et al. [26] using BSA as a standard. Specific SS binding was measured according to the modified method of Czernik and Petrack [27]. 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 unlabeled SS. After incubation for 60 min 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 in a Kontron gamma-counter. Non-specific binding, that is, binding occurring in the presence of a high concentration (10^{-7} M) of unlabeled SS, represented about 20% of the binding observed in the absence of unlabeled peptide. This non-specific component was subtracted from the total bound radioactivity in order to obtain the corresponding specific binding.

2.6. Evaluation of radiolabeled peptide degradation

To determine the extent of tracer degradation during incubation, we measured the ability of preincubated peptide to bind to fresh membranes as previously described [28]. Briefly, ^{125}I-Tyr^{11}-SS (250 pM) was incubated with membranes from rat frontoparietal cortex (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 radiolabeled 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.

2.7. Adenylyl cyclase assay

AC activity was measured as previously reported [29] with minor modifications. Briefly, rat frontoparietal cortex membranes (0.06 mg/ml) were in-

cubated 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 [30]. The SS concentration used was that necessary to achieve inhibition of rat [17] and human [31,32] brain AC. FK was used at a concentration that could effectively stimulate the catalytic subunit of rat AC [17].

2.8. Data analysis

The computer program LIGAND [33] was used to analyze the binding data. The use of this program enabled models of receptors which best fit a given set of binding data to be selected. The same program was also used to present data in the form of Scatchard plots [34] and to compute values for receptor affinity (k_d) and density (B_{max}). Statistical comparisons of all the data were carried out by a two-way analysis of variance (ANOVA). Bonferroni-corrected post hoc *t*-tests were used after a significant overall treatment effect was found. Means among groups were considered significantly different when the *P*-value was less than 0.05. Each individual experiment was performed in duplicate.

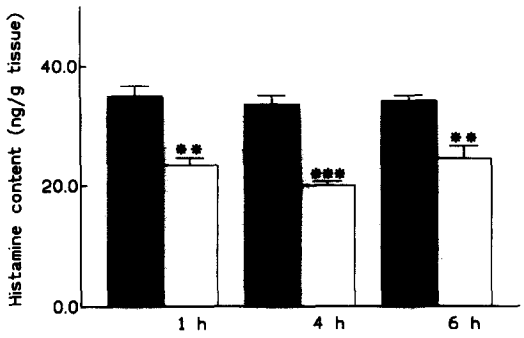

Fig. 1. Effect of a single intracerebroventricular injection of α-fluoromethylhistidine (α-FMH) (50 μg) on histamine content in the rat frontoparietal cortex at 1, 4 and 6 h after drug administration. Solid bars correspond to control animals ($n = 5$ rats in each group) and open bars correspond to rats treated with α-FMH at 1 ($n = 5$), 4 ($n = 5$) or 6 h ($n = 5$) after injection. Values are expressed as the mean ± S.E.M. of 5 separate determinations performed in duplicate. Statistical comparison versus control: ** $P < 0.01$; *** $P < 0.001$.

3. Results

The i.c.v. administration of α-FMH at a dose of 50 μg significantly decreased histamine content in the rat frontoparietal cortex to about 67, 60 and 72% of control values at 1, 4 and 6 h after its administration, respectively (Fig. 1). This decrease in histamine content was associated with an increase in SSLI

Table 1
Effect of α-Fluoromethylhistidine (α-FMH) (50 μg) administered intracerebroventricularly (i.c.v.) at 1, 4 or 6 h on somatostatin-like immunoreactivity (SSLI) content and equilibrium parameters for somatostatin (SS) binding to rat frontoparietal cortex membranes

Groups	SS receptors		SSLI	*n*
	B_{max}	K_d		
Control 1 h	363 ± 26	0.40 ± 0.03	9.70 ± 0.91	5
α-FMH 1 h	365 ± 24	0.38 ± 0.04	11.25 ± 1.74	5
Control 4 h	359 ± 17	0.34 ± 0.03	11.92 ± 0.99	5
α-FMH 4 h	369 ± 20	0.29 ± 0.02	10.79 ± 0.84	5
Control 6 h	363 ± 17	0.38 ± 0.04	10.4 ± 1.0	5
α-FMH 6 h	249 ± 13 [b]	0.29 ± 0.03	20.2 ± 1.1 [a]	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 femtomoles of SS bound per mg of protein. The results are represented as the means ± S.E.M. of five separate experiments performed in duplicate. *n* = number of animals in each experimental group.
Statistical comparison versus control: [a] $P < 0.05$, [b] $P < 0.01$.

content at 6 h after the drug administration, with no changes being observed at either 1 or 4 h (Table 1).

The specific binding of ^{125}I-Tyr^{11}-SS to frontoparietal cortex membranes was time-dependent; 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. Peptide degradation was determined to rule out the possibility of different SS degrading activities which might have affected the interpretation of the results. Membranes from each experimental group, however, showed a similar peptide degradation capacity and the values varied by no more than 10% among the experimental groups, thus making it possible to compare their type values.

Increasing concentrations of unlabeled SS inhibited the specific binding of ^{125}I-Tyr^{11}-SS to rat frontoparietal cortex membranes from both the control and the α-FMH-treated groups (Fig. 2, left panel). The specific binding of the tracer to frontoparietal cortex membranes, however, was significantly lower in rats treated with α-FMH at 6 h as compared with controls (Fig. 2, left panel) whereas no changes were observed at 1 or 4 h. Scatchard analysis of the binding data indicate that the decrease in tracer binding observed at 6 h was due to a decrease in the maximal number of SS receptors, with no change in the apparent affinity (Table 1; Fig. 2, right panel).

SS has been shown to inhibit AC activity through interaction with specific SS receptors coupled to the enzyme via a G_i protein [16,17]. Therefore, we studied SS-modulated AC activity by incubating frontoparietal cortex membranes with SS (10^{-4} M)

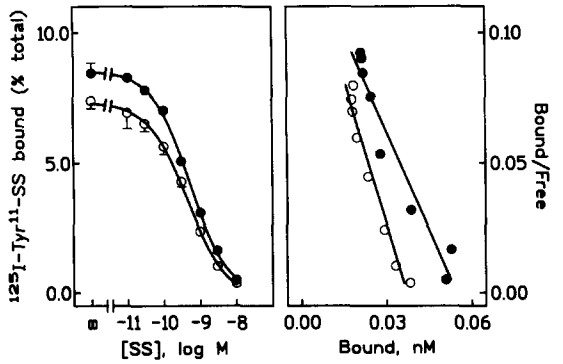

Fig. 2. Left panel: competitive inhibition of specific ^{125}I-Tyr^{11}-somatostatin (^{125}I-Tyr^{11}-SS) binding by unlabeled SS to membranes from rat frontoparietal cortex. Membranes (0.15 mg protein/ml) were incubated for 60 min at 30°C in the presence of 250 pM ^{125}I-Tyr^{11}-SS and increasing concentrations of native peptide. Points correspond to control rats (●) ($n = 5$) and α-fluoromethylhistidine (α-FMH)-treated rats at 6 h after α-FMH injection (○) ($n = 5$). Each point is the mean of 5 individual experiments performed in duplicate. Right panel: Scatchard analysis of the binding data.

either in the presence or absence of FK (10^{-5} M), a direct AC activator. No significant differences in basal or FK-stimulated AC activity were observed between the control and the α-FMH-treated rats at all times studied. At 6 h after α-FMH administration, however, the capacity of SS to inhibit both basal and FK-stimulated AC activity in frontoparietal cortex membranes was significantly lower than in the control group (Table 2; Fig. 3).

Functional G_i activity was examined in frontoparietal cortex membranes of control and α-FMH

Table 2
Effect of somatostatin (SS) (10^{-4} M) and forskolin (FK) (10^{-5} M) on adenylyl cyclase (AC) activity (pmol cAMP/min/mg protein) in frontoparietal cortex membranes from control rats ($n = 5$) and α-fluoromethylhistidine-treated rats ($n = 5$)

	α-Fluoromethylhistidine (6 h)	
	Control	Treated
Basal activity	120 ± 2	132 ± 3
Basal activity + 10^{-4} M SS	94 ± 2	119 ± 3 [c]
% SS inhibition of basal activity	22 ± 1	10 ± 1 [b]
Basal activity + 10^{-5} M FK	664 ± 15	694 ± 21
Fold FK estimulation of basal activity	5.4 ± 0.3	5.3 ± 0.3
Basal activity + 10^{-5} M FK + 10^{-4} M SS	516 ± 20	614 ± 21 [a]
% SS inhibition of FK-stimulated activity	22 ± 2	12 ± 2 [b]

Values represent the mean ± S.E.M. of 5 separate experiments performed in duplicate. n = number of animals in each experimental group. Statistical comparison versus control: [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

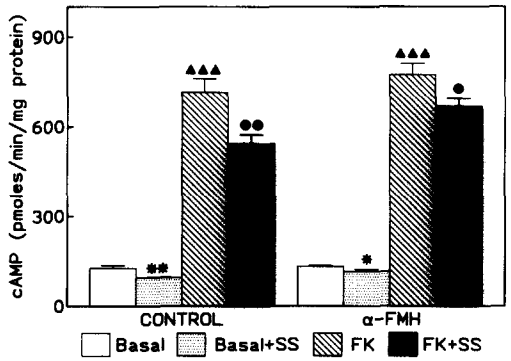

Fig. 3. Comparison of the ability of somatostatin (SS) to inhibit adenylyl cyclase (AC) activity in frontoparietal cortex membranes of control rats ($n = 5$) and α-fluoromethylhistidine (α-FMH)-treated rats at 6 h after α-FMH administration ($n = 5$). Membrane preparations were incubated with or without SS (10^{-4} M) in the absence (basal) or presence of 10^{-5} M forskolin (FK). Data are expressed as the mean ± S.E.M. of 5 separate experiments performed in duplicate. Statistical analysis was performed by ANOVA. ▲▲▲ $P < 0.001$: comparison between basal and FK-stimulated groups. * $P < 0.05$; ** $P < 0.01$: comparison between basal and SS-treated groups. ● $P < 0.05$; ●● $P < 0.01$: comparison between FK and FK + SS groups.

(6 h)-treated rats in order to determine whether the decrease in SS receptors and/or SS-modulated AC activity observed at 6 h after drug administration was due to an alteration at the G_i level. In the presence of FK (3×10^{-6} M), which amplifies basal AC activity, increasing concentrations of Gpp(NH)p produce an inhibitory effect on AC activity that has been used by several investigators as a measure of G_i function [35]. The ability of Gpp(NH)p to inhibit FK-stimulated AC activity was similar in frontoparietal cortex membranes from control and α-FMH (6 h)-treated rats (Fig. 4).

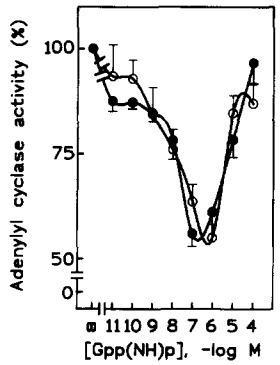

Fig. 4. Dose–effect curves for 5′-guanylylimidodiphosphate [Gpp(NH)p]-mediated inhibition of adenylyl cyclase (AC) activity in rat frontoparietal cortex membranes from control rats (●) ($n = 5$) and α-fluoromethylhistidine (α-FMH)-treated rats (○) ($n = 5$) at 6 h after α-FMH administration. Curves for 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 ± S.E.M. of 5 separate experiments performed in duplicate. No statistically significant differences were obtained between the control and α-FMH-treated rats.

4. Discussion

α-FMH is an irreversible and specific inhibitor of histidine decarboxylase [14,15] and even a single administration can deplete neuronal histamine in the animal brain [36,37]. Thus, α-FMH has been considered a useful tool to examine the functions of neuronal histamine in the brain. As shown in Fig. 1, a significant reduction in histamine content after α-FMH injection was observed between 1 and 6 h in the cerebral cortex, in agreement with other authors [19]. In the mammalian brain, histamine is thought to be stored in two anatomically distinct pools, that is, the neuronal pool, with a rapid turnover, and the mast cell pool, with a very slow turnover [38–41]. The half-life of neuronal histamine in the rat brain is reported to be approximately 60 min, which is much shorter than that in mast cells (almost 4 days) [38–43]. In the present study, α-FMH caused a 33%, 40% and 28% reduction in histamine levels in the frontoparietal cortex at 1, 4 and 6 h after its administration, respectively. Garbarg et al. [42] have shown that blockade of histidine decarboxylase activity by α-FMH injection decreases brain histamine only in the neuronal pool and does not affect the non-neuronal sources (mainly mast cells). In addition, Maeyama et al. [44] found almost complete depletion of brain histamine in W/W^v mice, which are devoid of mast cells. Therefore, it is probable that the residual histamine in the rat frontoparietal cortex after α-FMH administration may be due to the histamine content present in mast cells.

An i.c.v. administration of α-FMH was chosen in this study instead of an intraperitoneal (i.p.) injection for several reasons. Firstly, Garbarg et al. [42] reported that i.p. injection of α-FMH decreased histidine decarboxylase activity not only in various brain

regions, but also in gastric tissues. In addition, Sakurai et al. [45] demonstrated that α-FMH concentrations in the peripheral tissues were much higher than that determined in the brain after intravenous injection. Thus, an i.c.v. route of administration would eliminate any possible systemic effects of the drug which might affect the interpretation of the results.

Because ^{125}I-Tyr^{11}-SS acts biologically like the native peptide [46], it was chosen as the tracer. The extent of SS degradation in this study was relatively important, but it reached similar values in all the experimental groups, so it was possible to compare their type values. The values calculated for k_d and binding can be modified by ^{125}I-Tyr^{11}-SS degradation and so they must be considered as apparent.

In the control rats, the SSLI content was similar to that previously reported by others [47,48]. The inhibition of histamine synthesis induced by α-FMH was accompanied by a rise in SSLI content at 6 h after α-FMH administration, although to date, the mechanism is unknown. The studies of the effect of histamine on SS release or SS intracellular content are not clear [49,50]. In some studies, histamine seems to act as a mild inhibitor of SS release in fetal cultured cerebral cortical cells from rat brain, although this effect is not significant at concentrations of up to 10^{-5} M [49,50]. In the present study, no change in SSLI content was detected at either 1 or 4 h, which suggests that a certain time is required for the effect on SSLI content to take place. It is possible that the change in SSLI content at 6 h following α-FMH administration may be secondary to the decrease in SS receptors. This is consistent with studies on adrenergic receptors in which changes in the turnover of dopamine following clonidine administration were shown to be secondary to its effects on adrenergic receptors [51,52].

The treatment with α-FMH decreased ^{125}I-Tyr^{11}-SS binding in membranes from the rat frontoparietal cortex at 6 h after its administration. Scatchard plot analysis of the SS-receptor interaction demonstrated a 32% reduction in the maximal binding capacity in the α-FMH-treated group and an association constant in the same magnitude range as that of the control rats. The fact that the Scatchard plots are linear in all the experimental groups is not proof of a single binding site. In effect, 5 different subtypes of SS receptors have recently been cloned [53], all of which appear to be expressed in the rat brain [54] and which have high affinity for SS-14. Thus, a linear Scatchard plot reveals only that all SS receptor subtypes have similar affinity for the radioligand used.

The mechanisms by which α-FMH administration reduces the number of SS receptors in the frontoparietal cortex is open to speculation, although both direct and indirect effects can be considered. At present, we can only speculate that the effects of α-FMH on the number of SS receptors in the frontoparietal cortex are caused by changes in histaminergic transmission which then transsynaptically regulate the number of SS receptors. The presence of SS receptors in parts of the brain that have histaminergic nerve fibers in close proximity lends support to this notion [9–11]. In addition, the results of a variety of recent experiments suggest that neural input may regulate the concentration of peptides, proteins and enzymes in a variety of systems [55,56].

Alternatively, the regulation of the somatostatinergic system may be indirect. The inhibition of histamine synthesis caused by α-FMH produces neuroendocrine alterations that may induce changes in the SS receptors. It has been demonstrated that α-FMH administration leads to an inhibition of corticosterone secretion [57] and prolactin secretion by restraint stress [58] as well as an increase in plasma vasopressin [59]. The reduction in the binding capacity after α-FMH (6 h) administration was not due to a deleterious effect of α-FMH on total protein synthesis since Slotkin et al. [60] have shown that α-FMH does not inhibit protein synthesis.

The rat AC activity was inhibited by SS, which is in agreement with the literature [17,30,31]. A relatively high concentration of SS (10^{-4} M) was required to produce this inhibition. This concentration is the same as that used by other authors in their experiments on rat [17] and human [30,31] AC activity. Despite this relatively high concentration, however, several lines of evidence suggest that the effect of SS is receptor-mediated and is not a non-specific inhibitory effect. In this regard, the GTP dependency of the inhibitory effect suggests the involvement of a G protein in the response. This finding is consistent with binding studies on postmortem human and on rat brain tissue which have shown that the binding of SS to its recognition site is affected by GTP in a

manner consistent with the involvement of a G protein [30,31]. In addition, Nagao et al. [61] and Schettini et al. [17] have shown that SS-reduced cAMP formation in the rat brain occurs via a G protein coupled to AC. These findings, plus the lack of an inhibitory effect of SS (1 μM) on basal AC activity in primary cultures of mouse embryonic glial cells reported by Chneiweiss et al. [62] would argue against a non-specific inhibitory effect of the neuropeptide.

The attenuation of the inhibitory activity of SS on AC activity in the α-FMH (6 h)-treated rats is most likely related to the observed loss of SS receptors since the basal and FK-stimulated AC activities were similar in the control and α-FMH-treated group, indicating that the catalytic subunit of AC is intact. In addition, functional G_i activity, as determined by the effect of Gpp(NH)p on FK-stimulated AC activity in frontoparietal cortex membranes, was similar in the control and α-FMH-treated group, suggesting that there is no impairment in the coupling of the SS receptor to AC.

In conclusion, some of the effects of α-FMH may well depend on a decrease in the SS receptor/effector system. Thus, SS administered i.c.v. decreases slow-wave sleep [3,4], induces motor excitation [8] and potentiates learning and memory [6,7], whereas α-FMH has opposite effects on these activities [19,36,63]. From the present results, it would appear that endogenous histamine located in neuronal cells plays a role in modulating the sensitivity to SS in the frontoparietal cortex.

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