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Effect of Dimaprit and Cimetidine on the Somatostatinergic System in the Rat Frontoparietal Cortex

Lilian Puebla, Ph.D., and Eduardo Arilla, M.D.

*A recent study carried out by this laboratory demonstrated that exogenous histamine increases the somatostatin (SS) receptor/effector system in the rat frontoparietal cortex (Puebla and Arilla, 1995). In the present study we examined the participation of the H_2-histaminergic system in this modulation by use of the H_2-receptor agonist and antagonist dimaprit and cimetidine, respectively. Dimaprit administration [20 μg/rat, intracerebroventricularly (ICV)] to rats 2 hours before decapitation increased the number of SS receptors in the frontoparietal cortex without changing the affinity constant. Pretreatment with cimetidine (20 μg/rat, ICV) prevented the dimaprit-induced changes in SS binding in the frontoparietal cortex, whereas cimetidine alone (20 μg/rat, ICV) had no observable effect on this parameter. The in vitro addition of dimaprit or cimetidine to frontoparietal cortex membranes from untreated rats did not markedly affect the SS binding characteristics. Somatostatin caused a significantly higher inhibition of basal and forskolin (FK)-stimulated adenylyl cyclase (AC) activity in frontoparietal cortex membranes from dimaprit-treated rats than in controls, an effect that was prevented by pretreatment with cimetidine. No significant differences, however, were detected for the basal or FK-stimulated AC enzyme activity in the control, dimaprit-, and/or cimetidine-treated groups, which suggests no impairment of the AC catalytic subunit. In addition, the functional activity of the guanine nucleotide-binding inhibitory protein G_i, as measured by the capacity of the stable GTP analogue 5'-guanylylimidodiphosphate [Gpp(NH)p] to inhibit FK-stimulated AC activity, was not altered by dimaprit. Thus, the increased SS-mediated inhibition of AC activity observed in the dimaprit-treated rats may be caused by the increase in the number of SS receptors. Neither dimaprit nor cimetidine affected somatostatinlike immunoreactivity (SSLI) content. The present results, together with the fact that SS and histamine have been shown to influence locomotor activity and nociception in a similar manner, suggest that some of the neurotransmitter effects of SS may be modulated by histamine via H_2-histaminergic receptors. [**Neuropsychopharmacology 14:367–374, 1996**]*

KEY WORDS: *Dimaprit; Cimetidine; Somatostatin receptor; Adenylyl cyclase; rat; Frontoparietal cortex*

There are several sets of data supporting the hypothesis that the somatostatinergic and histaminergic systems are interrelated and that reciprocal regulation occurs. The cerebral cortex is one of the brain areas with the highest concentration of somatostatin (SS) and SS receptors (Patel and Reichlin 1978; Srikant and Patel 1981). It has been demonstrated that histaminergic fibers ascending from histaminergic neurons present in the tuberomammillary nucleus of the posterior hypothalamus innervate all areas and layers of the cerebral cortex (Panula et al. 1989). In addition, centrally administered

From the Molecular Neuroendocrinology Unit, Department of Biochemistry and Molecular Biology, Alcalá University Medical School, Madrid.

Address correspondence to: Dr. E. Arilla, Molecular Neuroendocrinology Unit, Department of Biochemistry and Molecular Biology, Alcalá University Medical School, Alcalá de Henares, 28871 Madrid, Spain.

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SS has been reported to produce a dose-related decrease in histamine levels in the rat cerebral cortex (Cacabelos et al. 1988). A recent study by our laboratory demonstrated that exogenous histamine increases the SS receptor/effector system in the rat frontoparietal cortex (Puebla and Arilla 1995). Because a high density of histamine H_2-receptors has been detected in the cerebral cortex (Ruat et al. 1990, 1991), it was of substantial interest to examine the participation of the H_2-histaminergic system in the histamine-induced modulation of the somatostatinergic system in the rat frontoparietal cortex. Furthermore, histamine, acting via H_2-receptors, and SS seem to play synergistic roles in regulating locomotor activity, both inducing hypoactivity (Rezek et al. 1977; Cacabelos et al. 1988; White and Rumbold 1988) and nociception (Chrubasik et al. 1984; Bhattacharya and Parmar 1985; Malmberg-Aiello et al. 1994). In view of these findings, dimaprit, an H_2-receptor agonist, and cimetidine, an H_2-receptor antagonist, were used to analyze whether the H_2-histaminergic system exerts some effect on somatostatinlike immunoreactivity (SSLI) content and on the specific binding of SS to its receptors. Considering that the postreceptor mechanism of action of SS includes, at least in part, the inhibition of adenylyl cyclase (AC) activity following the activation of SS receptors coupled via guanine nucleotide-binding inhibitory protein G_i to the enzyme (Schettini et al. 1989), the ability of SS to inhibit basal and forskolin (FK)-stimulated AC activity in the rat frontoparietal cortex was also measured. Functional activity of the inhibitory GTP-binding regulatory protein G_i was also assessed by the inhibitory action of low concentrations of the stable GTP analog 5'-guanylylimidodiphosphate [Gpp(NH)p] on FK-stimulated AC activity in frontoparietal cortex membranes.

MATERIALS AND METHODS

Chemicals

Synthetic Tyr^{11}-SS and SS-14 were purchased from Universal Biologicals Ltd (Cambridge UK); carrier-free $Na^{125}I$ (IMS 300, 100 mCi/ml) was purchased from the Radiochemical Centre (Amersham, UK). Bacitracin, phenylmethylsulfonylfluoride (PMSF), 3-isobutyl-1-methylxanthine (IBMX), bovine serum albumin (BSA), GTP, FK, Gpp(NH)p, and cimetidine were supplied by Sigma Química (Madrid, Spain). Dimaprit dihydrochloride was supplied by ICN Biochemicals (Cleveland, USA). The rabbit antibody used in the radioimmunoassay technique was raised in rabbits against SS-14 conjugated to BSA and is specific for SS, but because SS-14 also constitutes the C terminal portions of SS-28, the antiserum does not distinguish between these two forms (Penman et al. 1979).

Experimental Animals

The animals used in this study were Wistar rats ($n = 30$) weighing between 200 and 250 g. Rats were maintained on a 12-hour light/dark cycle (0700–1900) and allowed free access to food. Dimaprit (20 μg/rat) was dissolved in 0.9% NaCl, and cimetidine (20 μg/rat) was dissolved first in dimethyl sulfoxide and then diluted with 0.9% NaCl to the concentration desired. Drug doses were selected according to the effective doses reported in previous studies (Netti et al. 1981; Kraly and Arias 1990). All drugs were administered intracerebroventricularly (ICV) according to the method described by Noble et al. (1967) in a volume of 10 μl. In one experimental group, cimetidine (20 μg/rat, ICV) was administered 30 minutes before dimaprit (20 μg/rat, ICV) injection. Control animals for each group were injected with equivalent volumes of the corresponding vehicle. Rats were killed by decapitation 2 hours after the last drug injection. The brain was rapidly removed, and the frontoparietal cortex was dissected over ice according to the method of Glowinski and Iversen (1966).

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 minutes in a water bath, chilled in ice, and aliquots (100 μl) were removed for protein determination (Patel and Reichlin 1978). The homogenates were subsequently centrifuged at 15,000/g for 15 minutes at 4°C, and the supernatant was neutralized with 2 M NaOH. Extracts were immediately stored at −70°C until assayed. The SSLI content was measured in tissue extracts by a modified radioimmunoassay method (Patel and Reichlin 1978) with a sensitivity limit of 10 pg/ml. The intra- and interassay variation coefficients were 6.8% and 8%, respectively.

Binding Assay

Tyr^{11}-SS was radioiodinated by chloramine-T iodination (Greenwood et al. 1963). 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 purified labeled peptide was determined by the method of Singh et al. (1985). The specific activity of radioiodinated SS was approximately 600 Ci/mmol.

Membranes from rat frontoparietal cortex were prepared as previously described by Reubi et al. (1981). Membrane protein was determined by the method of Lowry et al. (1951) using BSA as a standard. Specific SS

binding was measured according to the method of Czernik and Petrack (1983) modified from Srikant and Patel (1981). 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 to 10 nM unlabeled SS. After incubation for 60 minutes at 30°C, bound and free ligand were separated by centrifugation at 11,000/g for 2 minutes, and the radioactivity in the resultant pellet was measured in a Kontron gamma counter. Nonspecific 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 nonspecific component was subtracted from the total bound radioactivity in order to obtain the corresponding specific binding. Peptide degradation was determined by the method of Aguilera et al. (1982).

Adenylyl Cyclase Assay

Adenylyl cyclase activity was measured as previously reported (Houslay et al. 1976) with minor modifications. Briefly, rat frontoparietal cortex 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 creatin phosphate and 1 mg/ml creatine kinase), 1 mM IBMX, 0.1 mM PMSF, 1 mg/ml bacitracin, 1 mM EDTA, and test substances (SS or FK) in 0.1 ml of 0.025 M triethanolamine/HCl buffer (pH 7.4). After a 15-minute incubation at 30°C, the reaction was stopped by heating the mixture for 3 minutes. After cooling, 0.2 ml of an alumina slurry (0.75 g/ml in triethanolamine/HCl buffer, pH 7.4) were added and the suspension was centrifuged. The supernatant was taken for assay of cyclic AMP (cAMP) by the method of Gilman (1970).

Table 1. Effect of Dimaprit, Cimetidine, and Cimetidine plus Dimaprit on SS Receptors and SSLI Content[a]

	SS Receptors		SSLI
	B_{max}	K_d	($n = 5$)
Control	396 ± 12	0.27 ± 0.03	8.66 ± 1.40
Dimaprit	572 ± 34[b]	0.34 ± 0.04	8.14 ± 0.51
Control	396 ± 21	0.24 ± 0.02	10.72 ± 0.64
Cimetidine plus dimaprit	423 ± 21	0.29 ± 0.03	13.72 ± 0.46
Control	401 ± 10	0.34 ± 0.03	10.55 ± 0.53
Cimetidine	405 ± 13	0.39 ± 0.02	10.21 ± 0.94

[a]Binding parameters were calculated from Scatchard plots by linear regression. Units for K_d are in nM, units for B_{max} are femtomoles of SS bound per mg of protein, and units for SSLI are ng SS per mg protein. The results are represented as the means ± SEM of five separate experiments performed in duplicate.

[b]Statistical comparison versus control: $p < .001$.

Data Analysis

The computer program LIGAND (Munson and Rodbard 1980) was used to analyze the binding data. The use of this program enabled models of receptors that 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 (Scatchard 1949) and to compute values for receptor affinity (K_d) and density (B_{max}). Statistical comparison of all the data were carried out with one-way analysis of variance (ANOVA) and Student's Newman-Keuls test. Means among groups were considered significantly different when the p value was less than .05. Each individual experiment was performed in duplicate.

RESULTS

Previous experiments confirmed that the specific binding of ^{125}I-Tyr^{11}-SS to frontoparietal cortex membranes changed linearly with protein concentration and was time dependent in all the experimental groups. An apparent equilibrium was observed between 50 and 180 minutes at 30°C (data not shown). All subsequent binding experiments were therefore conducted at 30°C for 60 minutes.

Dimaprit administration was associated with an increase in SS binding (Table 1 and Figure 1). Scatchard plots of the binding of ^{125}I-Tyr^{11}-SS to membranes indicate that dimaprit administration increased the number of SS receptors without changing their apparent affinity. Pretreatment with cimetidine reversed the effect of dimaprit on frontoparietal cortex SS receptors, whereas cimetidine alone had no observable effect on SS binding. To assess whether dimaprit exerted a direct action on SS receptors, 10^{-5} M dimaprit was included in the incubation medium at the time of the binding assay with membranes from the frontoparietal cortex of normal rats. The addition of dimaprit to the incubation medium changed neither the number nor the affinity of the SS receptors in the frontoparietal cortex membranes (data not shown).

The stability of the radioligand in membranes from different groups was measured to examine the possibility that decreased ^{125}I-Tyr^{11}-SS degradation might account for increased SS binding by these membranes. Frontoparietal cortex membranes from control and dimaprit- or cimetidine-treated rats showed a similar peptide degradation capacity and the values varied by no more than 10% in all experimental groups.

As shown in the concentration-response curve for the effect of SS on AC activity (Figure 2), inhibition of basal and FK-stimulated AC activity was only significant at a concentration of 10^{-4} M SS in frontoparietal cortex membranes from control rats. Therefore, this

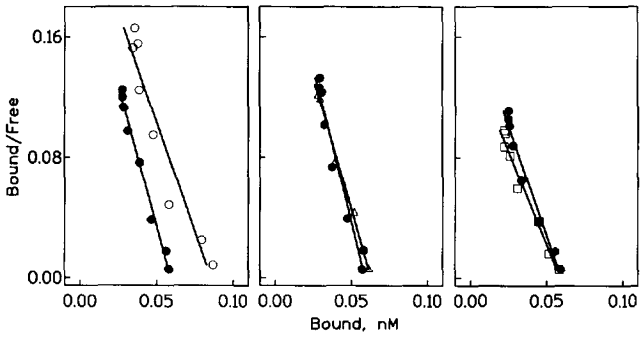

Figure 1. Scatchard plots of the specific binding of ^{125}I-Tyr^{11}-somatostatin (^{125}I-Tyr^{11}SS) to frontoparietal cortex membranes. Membranes (0.15 mg protein/ml) were incubated for 60 minutes at 30°C in the presence of 250 pM ^{125}I-Tyr^{11}-SS and increasing concentrations of native peptide. *Solid circles,* control rats (n = 5); *open circles,* dimaprit-treated rats (n = 5) (left panel); *open squares,* cimetidine-treated rats (n = 5) (right panel); *open triangles,* cimetidine plus dimaprit-treated rats (n = 5) (center panel). Values are expressed as the mean ± SEM of five separate experiments performed in duplicate. The corresponding equilibrium binding parameters are included in Table 1.

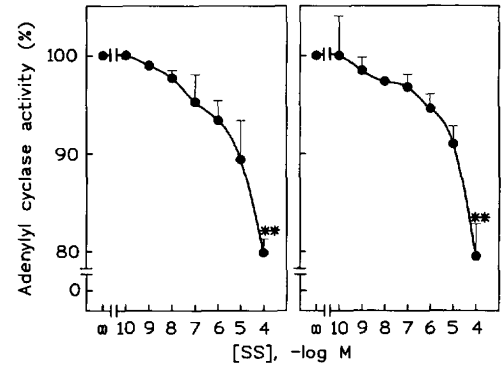

Figure 2. Effect of increasing concentrations of SS on basal (*left panel*) and FK-stimulated (*right panel*) adenylyl cyclase activity in frontoparietal cortex membranes from control rats. The effect of SS was studied in the absence or presence of 10^{-5} M FK and the indicated concentrations of SS. Data are expressed as a percentage of the basal or FK-stimulated adenylyl cyclase activity in the absence of SS (100%). Basal and FK-stimulated AC activity was 163 ± 3 and 887 ± 48 pmol cAMP/min/mg protein, respectively. The results are given as the mean ± SEM of five separate determinations, each performed in duplicate. ** Statistical comparison versus AC activity in the absence of SS: $p < .01$.

concentration was used in subsequent experiments on SS-mediated inhibition of AC activity. No significant differences were seen for either the basal or the FK-stimulated AC enzyme activities in membranes from the frontoparietal cortex of the control, dimaprit, and/or cimetidine groups (Table 2). In all experimental groups a significant inhibition of basal and FK-stimulated AC activity by SS (10^{-4} M) was observed. In the dimaprit group, however, the capacity of SS (10^{-4} M) to inhibit the basal and FK-stimulated AC activity in the frontoparietal cortex was significantly higher than in the control group. Pretreatment with cimetidine prevented the increase in SS-mediated inhibition of AC activity induced by dimaprit, whereas cimetidine alone had no effect on this parameter.

The stable GTP analog Gpp(NH)p was employed to evaluate whether there were changes in the functional activity of G_i proteins which might explain the greater SS-mediated inhibition of AC activity observed in the present study. Low concentrations of Gpp(NH)p produced an inhibitory effect on FK-stimulated (3×10^{-6} M) AC activity in control and dimaprit-treated rats, achieving a maximum inhibition at a concentration of 10^{-7} M (Figure 3). This effect has been used by several investigators as a measure of G_i functional activity (Strassheim et al. 1991; Young et al. 1991). The Gpp(NH)p-mediated inhibition of FK-stimulated AC activity was similar in frontoparietal cortex membranes from control and dimaprit-treated rats (Figure 3).

Dimaprit or cimetidine produced no change in fron-

Table 2. Effect of SS (10^{-4} M) and FK (10^{-5} M) on Brain AC Activity (pmol cAMP/min/mg protein) in Frontoparietal Cortex Membranes from Control, dimaprit-, cimetidine-, and cimetidine plus dimaprit-treated rats[a]

	Dimaprit		Cimetidine		Cimetidine + Dimaprit	
	Control (n-5)	Treated (n-5)	Control (n-5)	Treated (n-5)	Control (n-5)	Treated (n-5)
Basal activity	157 ± 9	178 ± 7	141 ± 18	141 ± 7	146 ± 6	139 ± 10
Basal activity + 10^{-4} M SS	124 ± 7	120 ± 7	110 ± 5	112 ± 1	112 ± 5	105 ± 8
SS inhibition of basal activity (%)	21 ± 2	28 ± 1[b]	22 ± 3	21 ± 4	20 ± 3	24 ± 4
+ 10^{-5} M	926 ± 43	1068 ± 61	815 ± 30	880 ± 21	863 ± 46	784 ± 50
Fold FK stimulation over basal	5.9 ± 0.3	6.0 ± 0.2	5.8 ± 0.4	6.2 ± 0.3	5.9 ± 0.2	5.6 ± 0.2
10^{-5} M FK + 10^{-4} M SS	722 ± 42	748 ± 34	641 ± 26	667 ± 31	688 ± 40	608 ± 47
SS inhibition of FK stimulation (%)	22 ± 1	30 ± 1[c]	21 ± 1	24 ± 3	23 ± 1	22 ± 2

[a] Values represent the mean ± SEM of five separate experiments performed in duplicate.
[b] Statistical comparison versus control: $p < .05$.
[c] Statistical comparison versus control: $p < .001$.

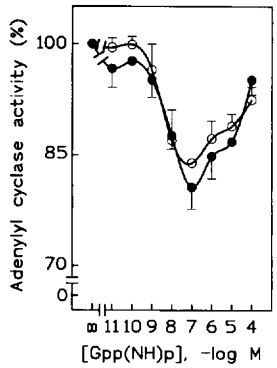

Figure 3. Dose-effect curves for 5'-guanylylimidodiphosphate [Gpp(NH)p]-mediated inhibition of AC activity in rat frontoparietal cortex membranes from control (*solid circles, n* = 5) and dimaprit-treated (*open circles, n* = 5) rats. Curves for the action of Gpp(NH)p on AC activity were carried out in the presence of 3×10^{-6} M 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 performed in duplicate. No statistically significant differences were obtained between the control and dimaprit-treated rats.

toparietal cortex SSLI levels in comparison with the control group (Table 1).

DISCUSSION

This study provides evidence that the number of SS receptors and the inhibitory effect of this neuropeptide on AC activity increase in frontoparietal cortex membranes from dimaprit-treated rats. The data extend previous observations carried out by our laboratory that demonstrate that exogenous histamine positively modulates the SS receptor/effector system in the rat frontoparietal cortex (Puebla and Arilla 1995) and indicate that the histamine H_2-receptor is involved in this modulation.

The SSLI content and the binding parameters of SS receptors in the frontoparietal cortex of control rats were similar to those previously reported by others (Patel and Reichlin 1978; Srikant and Patel 1981). Scatchard analysis of the stoichiometric data reveal linear plots. This, however, is not proof of the existence of one type of SS receptor. Indeed, five different SS receptor subtypes have recently been cloned (Hoyer et al. 1994), and the tissue distribution of the messenger ribonucleic acid for these SS receptor subtypes has been studied in the rat (Bruno et al. 1993; Pérez et al. 1994). The five SS receptor subtypes appear to be expressed in the rat brain and have similar high affinity for SS. Thus, a linear Scatchard plot reveals only that all SS receptor subtypes have similar affinity for the radioligand used in the study.

To date, the mechanism by which histamine H_2 receptors mediate the action of dimaprit on SS binding to its specific receptors in the rat frontoparietal cortex is unknown. A direct effect of dimaprit on the SS receptors can be excluded, however, because no change in tracer binding was detected following incubation of fresh frontoparietal cortex membranes with 10^{-5} M dimaprit. In addition, histamine H_2-receptors seem to mediate this effect because pretreatment with the H_2 antagonist cimetidine selectively antagonizes the increase in ^{125}I-Tyr^{11}-SS binding and SS-mediated inhibition of AC activity.

We cannot eliminate the possibility, however, that the effect of dimaprit on the SS receptor/effector system may be mediated by an indirect action on other neurotransmitter systems. It has recently been demonstrated that histamine stimulates acetylcholine release in the rat hippocampus through an action at H_2 receptors (Mochizuki et al. 1994). Our research group previously demonstrated that the activation of the cholinergic muscarinic and cholinergic nicotinic systems increases the number of SS receptors in the frontoparietal cortex and hippocampus, respectively (Barrios et al. 1990; Alvaro-Alonso et al. 1993).

A recent study carried out by our laboratory has demonstrated that the blockade of histamine H_3 autoreceptors by thioperamide, a selective H_3 antagonist that enhances histamine release, increases the number of somatostatin receptors and SS-mediated inhibition of AC activity. This effect was shown to be due to the activation of postsynaptic histamine H_1 and H_2 receptors by endogenous histamine released from the histaminergic nerve terminals by thioperamide. In addition, the activation of H_1 receptors by a selective agonist, 2-pyridylethylamine also increased the activity of the SS receptor/effector system (unpublished data).

Although some studies carried out in primary cell cultures have demonstrated that SS inhibits stimulated AC activity to a significant extent in a dose-dependent manner (Van Calker et al. 1980; Chneiweiss et al. 1984), in parallel with its ability to inhibit radioligand binding, other investigators required a relatively high concentration of SS (10^{-4} M) to produce inhibition of basal and FK-stimulated AC activity in rat or human cerebral membranes (Nagao et al. 1989; Schettini et al. 1989; Garlind et al. 1992), the same concentration as that used in the present study. Despite this high concentration, several lines of evidence suggest that the effect of SS is receptor-mediated and is not a nonspecific inhibitory effect. In this regard, the GTP dependence 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 rat brain tissue that 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 (Garlind et al. 1992). In addition, Nagao et al. (1989) and Schettini et al. (1989) 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. (1984), would argue against a nonspecific inhibitory effect of the neuropeptide.

In frontoparietal cortex membranes from control and dimaprit-treated rats similar levels of AC activity were noted both in basal conditions and when the enzyme was stimulated directly by the diterpene FK. Thus, the increased inhibitory effect of SS on AC activity in frontoparietal cortex membranes of dimaprit-treated rats is not due to an impairment of the catalytic subunit of AC but is most likely related to the observed increase in the number of SS receptors.

The functional activity of G_i proteins was measured in control and dimaprit-treated rats in order to determine whether the greater inhibition by SS observed in the dimaprit group was due to a change at this level. In both control and dimaprit-treated rats, the nonhydrolyzable GTP analog Gpp(NH)p elicited a characteristic biphasic effect on FK-stimulated AC activity, with low concentrations inhibiting FK-stimulated AC activity and higher concentrations stimulating it. This biphasic effect is due to the activation of G_1 and G_s proteins, respectively, due to the higher affinity of G_i proteins for Gpp(NH)p (Strassheim et al. 1991). The lack of changes in the inhibitory effect of Gpp(NH)p on FK-stimulated AC activity in frontoparietal cortex membranes from dimaprit-treated rats suggests that the increased SS inhibition of AC activity is not a result of an increase in $G_{i\alpha}$ protein AC coupling but is also probably related to the increase in the number of SS receptors observed in this study.

Dimaprit or cimetidine produced no change in frontoparietal cortical SSLI levels in comparison with the control group. This result is not unexpected given a recent finding that exogenous histamine affects neither SS content nor SS release in cultured rat fetal cerebrocortical cells (De los Frailes et al. 1993). In addition, since SS inhibits growth hormone (GH) secretion, the fact that the H_2-histaminergic antagonist cimetidine has little effect on GH secretion (Zanaboni et al. 1984) suggests that there is no tonic activation of H_2 receptors modulating the release of SS by histamine. Although administration of cimetidine plus dimaprit appears to increase SSLI levels in the frontoparietal cortex, this difference is not statistically significant.

The functional significance of the present results has yet to be established. Since SS and H_2-histaminergic agonists have been shown to induce hypoactivity (Rezek et al. 1977; White and Rumbold 1988) and to control nociception (Chrubasik et al. 1984; Bhattacharya and Parmar 1985; Malmberg-Aiello et al. 1994) when administered ICV, our results suggest that histamine potentiates somatostatinergic neurotransmission and thus may control the sensitivity of target neurons to SS in the cerebral cortex.

Although the implications of the present findings for psychopharmacology are not well-known, several investigators have observed a decrease in histamine levels (Mazurkiewicz-Kwilecki and Nsonwah 1989) and SS content and receptors (Beal 1990) in the cerebral cortex of patients with Alzheimer's disease. As both neurotransmitters modulate locomotor activity (Rezek et al. 1977; White and Rumbold 1988) and learning and memory (De Noble et al. 1980; Vécsei et al. 1983; Kamei et al. 1993) and this neurodegenerative disease is characterized by cognitive and motor deficits, the regulation of the somatostatinergic system by the H_2-histaminergic system may be important in understanding the pathophysiology of this disease.

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REFERENCES

Aguilera G, Parker DS, Catt KJ (1982): Characterization of somatostatin receptors in the rat adrenal glomerulosa zone. Endocrinology 111:1376–1384

Alvaro-Alonso I, Prieto JC, Arilla E (1993): Effect of acute and chronic diisopropylfluorophosphate and atropine administration on somatostatin binding in the rat frontoparietal cortex and hippocampus. Psychopharmacology 110:103–109

Barrios V, Rodríguez-Sánchez MN, Colás B, Arilla E (1990): Effects of acute nicotine and mecamylamine administration on somatostatin concentration and binding in the rat brain. Eur J Pharmacol 179:263–170

Beal MF (1990): Somatostatin in neurodegenerative illnesses. Metabolism 39(suppl 2):116–119

Bhattacharya SK, Parmar SS (1985): Antinociceptive effect of intracerebroventricularly administered histamine in rats. Res Commun Chem Pathol Pharmacol 49:125–136

Bruno JF, Xu Y, Song J, Berelowitz M (1993): Tissue distribution of somatostatin receptor subtype ribonucleic acid in the rat. Endocrinology 133:2561–2567

Cacabelos R, Niigawa H, Yamatodani A, Gomez-Pan A, Nishimura T, Wada H (1988): Antagonist effects of growth hormone-releasing factor and somatostatin on brain histamine. Endocrinology 122:1269–1276

Chneiweiss J, Glowinski J, Prémont J (1984); Vasoactive intestinal polypeptide receptors linked to an adenylate cyclase and their relationship with biogenic amine and somatostatin-sensitive adenylate cyclases on central neu-

ronal and glial cells in primary cultures. J Neurochem 44:779–786

Chrubasik J, Meynandier J, Blond S, Scherpereel P, Ackerman E, Weinstock M, Bonath K, Cramer H, Wünsch E (1984): Somatostatin, a potent analgesic. Lancet 2: 1208–1209

Czernik AJ, Petrack B (1983): Somatostatin receptor binding in rat cerebral cortex. Characterization using a nonreducible somatostatin analog. J Biol Chem 285:5525–5530

De los Frailes MT, Cacicedo L, Lorenzo MJ, Tolón RM, Fernández G, Sánchez Franco F (1993): Neurotransmitter regulation of somatostatin secretion by fetal rat cerebral cortical cells in culture. J Endocrinol Invest 15:661–668

De Noble VJ, Hepler DJ, Barto RA (1980): Cysteamine-induced depletion of somatostatin produces differential cognitive deficits in rats. Brain Res 482:42–48

Garlind A, Fowler CJ, Alafuzoff I, Winblad R, Cowburn RF (1992): Neurotransmitter-mediated inhibition of postmortem human brain adenylyl cyclase. J Neural Transm 87:113–124

Gilman AG (1970): A protein binding assay for adenosine 3′,5′-cyclic monophosphate. Proc Natl Acad Sci U S A 67:305–312

Glowinski J, Iversen LL (1966): Regional studies of catecholamines in the rat brain I. The disposition of [^{3}H]norepinephrine. [^{3}H]dopamine and [^{3}H]DOPA in various regions of the brain. J Neurochem 13:655–669

Greenwood FC, Hunter WM, Glover JS (1963): The preparation of ^{131}I-labeled human growth hormone of high specific radioactivity. Biochem J 89:114–123

Houslay MD, Metcalfe JC, Warren GB, Hesketh TR, Smith GA (1976): The glucagon receptor of rat liver plasma membranes can couple to adenylate cyclase without activating it. Biochim Biophys Acta 436:489–494

Hoyer D, Lübbert H, Bruns C (1994): Molecular pharmacology of somatostatin receptors. Naunyn Schmiedebergs Arch Pharmacol 350:441–453

Kamei C, Okumura Y, Tasaka Y (1993): Influence of histamine depletion on learning and memory recollection in rats. Psychopharmacology 111:376–382

Kraly FS, Arias RL (1990): Histamine in brain may have no role for histaminergic control of food-related drinking in the rat. Physiol Behav 47:5–9

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951): Protein measurement with Folin phenol reagent. J Biol Chem 193:265–275

Malmberg-Aiello P, Lamberti C, Ghelardini C, Giotti A, Bartolini A (1994): Role of histamine in rodent antinociception. Br J Pharmacol 11:1269–1279

Mazurkiewicz-Kwilecki IM, Nsonwah S (1989): Changes in the regional brain histamine and histidine levels in postmortem brains of Alzheimer patients. Can J Physiol Pharmacol 67:75–78

Mochizuki T, Okakura-Mochizuki K, Horii A, Yamamoto Y, Yamatodani A (1994): Histaminergic modulation of hippocampal acetylcholine release in vivo. J Neurochem 62:2275–2282

Munson PJ, Rodbard D (1980): LIGAND: a versatile computerized approach for characterization of ligand binding systems. Anal Biochem 107:220–239

Nagao M, Sakamoto C, Matozaki T, Nishizaki H, Konda Y, Nakano O, Baba S (1989): Coupling of inhibitory GTP binding proteins to somatostatin receptors on rat cerebrocortical membranes. Folia Endocrinol Jpn 65:1357–1366

Netti C, Guidobono F, Olgiati VR, Sibilia V, Pecile A (1981): Histamine agonist and antagonist drugs: Interference with CNS control of GH release in rats. Hormone Res 14:180–191

Noble EP, Wurtman RJ, Axelrod J (1967): A simple and rapid method for injecting H^3-norepinephrine into the lateral ventricle of the rat brain. Life Sci 6:281–291

Panula P, Pirvola U, Auvinen S, Airaksinen MS (1989): Histamine immunoreactive nerve fibers in the rat brain. Neuroscience 28:585–610

Patel JC, Reichlin S (1978): Somatostatin in hypothalamus, extrahypothalamic brain and peripheral tissues of the rat. Endocrinology 102:523–531

Penman E, Wass AH, Lund A, Lowry PJ, Stewart J, Dawson AM, Besser GM, Rees LH (1979): Development and validation of a specific radioimmunoassay for somatostatin in human plasma. Ann Clin Biochem 16:15–25

Pérez J, Rigo M, Kaupmann K, Brunns C, Yasuda K, Bell GI, Lübbert H, Hoyer D (1994): Localization of somatostatin mRNA in rat brain by in situ hybridization Naunyn Schmiedebergs Arch Pharmacol 349:145–160

Puebla L, Arilla E (1995): Exogenous histamine increases the somatostatin receptor/effector system in the rat frontoparietal cortex. Eur J Pharmacol 289:361–368

Reubi JC, Perrin MH, Rivier JE, Vale V (1981): High affinity binding sites for the somatostatin-28 analogue in rat brain. Life Sci 28:2191–2198

Rezek M, Havlicek V, Hughes KR, Friesen H (1977): Behavioural and motor excitation and inhibition induced by the administration of small and large doses of somatostatin into the amygdala. Neuropharmacology 16:157–162

Ruat M, Traiffort E, Bouthenet ML, Schwartz JC, Hirschfeld J, Buschauer A, Schunack W (1990): Reversible and irreversible labeling and autoradiographic localization of the cerebral histamine H_2-receptor using ^{125}I-iodinated probes. Proc Natl Acad Sci U S A 87:1658–1662

Ruat M, Traiffort E, Arrang JM, Leurs R, Schwartz JC (1991): Cloning and tissue expression of a rat histamine H_2-receptor gene. Biochem Biophys Res Commun 179:1470–1478

Scatchard (1949): The attractions of proteins for small molecules and ions. Ann NY Acad Sci 51:660–671

Schettini G, Florio T, Meucci O, Landolfi E, Grimaldi M, Ventra C, Marino A (1989): Somatostatin inhibition of adenylate cyclase activity in different brain areas. Brain Res 492:65–71

Singh P, Rae-Venter B, Townsend CM Jr, Khalil T, Thompson JC (1985): Gastrin receptors in normal and malignant gastrointestinal mucosa: Age-associated changes. Am J Physiol 249:G761–G769

Srikant CB, Patel YC (1981): Somatostatin receptors: Identification and characterization in rat brain membranes. Proc Natl Acad Sci U S A 78:3920–3934

Strassheim D, Palmer T. Milligan G, Houslay MD (1991): Alterations in G-Protein expression and the hormonal

regulation of adenylate cyclase in the adipocytes of obese (fa/fa) Zucker rats. Biochem J 276:197–202

Van Calker D, Müller M, Hamprecht B (1980): Regulation by secretin, vasoactive intestinal peptide, and somatostatin of cyclic AMP accumulation in cultured brain cells. Proc Natl Acad Sci U S A 77:6907–6911

Vécsei L, Bollok I, Telegdy G (1983): Comparative studies with cyclic and linear somatostatin on active avoidance behaviour and open-field activity in rats. Acta Physiol Hung 61:43–49

White JM, Rumbold GR (1988): Behavioral effects of histamine and its antagonists: A review. Psychopharmacology 95:1–14

Young P, Kirkham DM, Murphy GJ, Cawthorne MA (1991): Evaluation of inhibitory nucleotide regulatory protein G_i function in hepatocytye and liver membranes from obese Zucker (fa/fa) and their lean (fa/?) littermates. Diabetologia 34:565–569

Zanaboni A, Galmozzi G, Mariononi S, Muciaccia WZ (1984): Inhibitory effect of cimetidine on L-dopa-stimulated growth hormone release in normal man. Clin Endocrinol 21:535–540