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Research report

Influence of fluoxetine and *p*-chloroamphetamine on the somatostatin receptor–adenylyl cyclase system in the rat frontoparietal cortex

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Abstract

There is evidence that suggests a reciprocal functional link between the serotonergic and the somatostatinergic system in the rat frontoparietal cortex. However, to date, the role of endogenous 5-hydroxytryptamine (serotonin) on the regulation of the somatostatin (SS) receptor–adenylyl cyclase (AC) system remains unclear. In the present study, the administration of fluoxetine (10 mg/kg i.p.), a 5-hydroxytryptamine uptake inhibitor in a single dose or administered daily for 14 days increased the number of specific $[^{125}I]Tyr^{11}$-SS receptors, with no change in the receptor affinity, in rat frontoparietal cortical membranes. However, the capacity of SS to inhibit forskolin (FK)-stimulated AC activity in these membranes was lower than in the control groups. The ability of the stable GTP analogue 5′-guanylylimidodiphosphate (Gpp(NH)p) to inhibit FK-stimulated AC activity in frontoparietal cortical membranes was also decreased in rats acutely and chronically treated with fluoxetine. *p*-Chloroamphetamine (5 mg/kg i.p.), which leads to a lasting reduction of 5-hydroxytryptamine innervation, administered on days 1, 3 and 5 and the rats sacrificed 1 or 3 weeks after the first injection, decreased the number of SS receptors without changing the receptor affinity. In this experimental group, SS also caused a significantly lower inhibition of FK-stimulated AC activity. *p*-Chloroamphetamine had no effect on the ability of Gpp(NH)p to inhibit FK-stimulated AC activity in frontoparietal cortical membranes at all the time periods studied. The present results suggest that under normal circumstances some SS receptors are under a tonic stimulatory control through the serotonergic system. © 1997 Elsevier Science B.V.

Keywords: Fluoxetine; *p*-Chloroamphetamine; Somatostatin receptor; Adenylyl cyclase; Rat; Frontoparietal cortex

1. Introduction

There is evidence that suggests a reciprocal functional link between the serotonergic and the somatostatinergic system in the rat frontoparietal cortex. This cerebral area is rich in somatostatin (SS)-containing neurons [36] and SS receptors [8] and receives a dense serotonergic innervation [22]. In the brain, both SS and 5-hydroxytryptamine (serotonin) have been implicated in motor and sensory functions [10,18,22,40]. SS mediates its various functions through receptors that are coupled to GTP-binding proteins (G-proteins) [34,46], inducing inhibition of adenylyl cyclase (AC) [6,7,46], reduction in calcium currents [54] and increases in potassium channel conductance [53]. In the rat cerebral cortex, both 5-hydroxytryptamine 5-HT_1 and 5-HT_2 receptors have been described [11], with an apparent prevalence of the latter class. The binding capacity of 5-hydroxytryptamine 5-HT_1 receptors was significantly increased in the cerebral cortex of rats treated for 10 days with SS, whereas the affinity of 5-hydroxytryptamine 5-HT_1 receptors was significantly decreased [37]. Recently, serotonergic axon terminals have been demonstrated to be present on SS-immunoreactive neurons of the rat paraventricular nucleus [26]. Recent studies have also shown that selective serotonergic lesions in rats increase preprosomatostatin mRNA expression in the striatum [2]. In addition, long-term treatment with 5-hydroxytryptamine uptake inhibitors, such as clomipramine and zimelidine, or with a 5-hydroxytryptamine precursor reduces cerebral SS-like immunoreactivity content [24]. On the other hand, SS facilitates 5-hydroxytryptamine release [52] and stimulates both its synthesis and utilization [13]. Alterations of the somatostatinergic and serotonergic systems have been associated with depression, Alzheimer's disease, schizophre-

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nia and Huntington's chorea [23,42]. To clarify the possible role of endogenous 5-hydroxytryptamine on the regulation of the SS receptor–AC system in the rat brain, we examined the effect of the enhancement of serotonergic neurotransmission by increasing 5-hydroxytryptamine availability in the vicinity of the receptor site with fluoxetine (3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine hydrochloride), a highly selective 5-hydroxytryptamine re-uptake inhibitor [55]. The selectivity and potency of this drug as an inhibitor of the 5-hydroxytryptamine re-uptake pump has aroused considerable interest and is being widely used as a tool for studying the functional role of the serotonergic system and for the treatment of mental depression [50]. In addition, we studied the effects of a decrease in serotonergic neurotransmission by reduction of 5-hydroxytryptamine innervation in the neocortex with the amphetamine derivate *p*-chloroamphetamine [12]. Therefore, the present study assessed the acute and chronic effect of fluoxetine and *p*-chloroamphetamine on the specific binding of SS to its receptors, the ability of SS to inhibit forskolin (FK)-stimulated AC activity and the functional activity of guanine nucleotide-binding inhibitory proteins (G_i-proteins) in rat frontoparietal cortical membranes.

2. Materials and methods

2.1. Materials

Synthetic Tyr^{11}-SS was purchased from Universal Biologicals (Cambridge, UK); carrier-free Na^{125}I (IMS 300, 100 mCi/ml) was purchased from the Radiochemical Center (Amersham, UK); fluoxetine, *p*-chloroamphetamine, bacitracin, phenylmethylsulphonyl fluoride, guanosine triphosphate (GTP), 5′-guanylylimidodiphosphate (Gpp(NH)p); 3-isobutyl-1-methylxanthine and bovine serum albumin were purchased from Sigma (St. Louis, MO, USA). All other reagents were of the highest purity commercially available.

2.2. Experimental animals

The animal experiments performed in the present study were conducted under the guidelines of the Animal Care Committee of Alcalá University and the experimental protocols have been approved. Wistar rats weighing 200–250 g at the beginning of the treatment were used in all experiments. The rats were housed in groups of five under controlled room temperature (22°C), in a constant 12-h light–dark cycle, with food and water given ad libitum. Fluoxetine and *p*-chloroamphetamine dissolved in saline were injected i.p. in a volume of 2 ml/kg. In the acute treatment, a single fluoxetine dose of 10 mg/kg i.p. was injected as previously described [55]. In the chronic treatment, fluoxetine was injected at a dose of 10 mg/kg i.p. once daily for 14 days. Animals were sacrificed by decapitation 24 h after the last injection as previously described [47]. The fluoxetine dose of 10 mg/kg i.p. was observed by Wong et al. [55] to alter the response of rat forebrain neurons to 5-hydroxytryptamine. *p*-Chloroamphetamine treatment was carried out as previously described [51] according to the following schemes: a dose of 5 mg/kg of *p*-chloroamphetamine was administered on days 1, 3 and 5 and the rats were decapitated at 1 or 3 weeks after the first injection. Control animals for each group were injected with saline at identical intervals. The brain was rapidly removed and the frontoparietal cortex was dissected over ice according to the method of Glowinski and Iversen [16].

2.3. Measurement of 5-hydroxytryptamine

To assess the destruction of serotonergic neurons in *p*-chloroamphetamine-treated rats, frontoparietal cortical 5-hydroxytryptamine levels in control and treated rats were measured by high-performance liquid chromatography (HPLC) with electrochemical detection [48].

2.4. Binding assay

Tyr^{11}-SS was radioiodinated by chloramine-T iodination according to the method of Greenwood et al. [17]. The tracer was purified in a Sephadex G-25 (fine) column which had been equilibrated and eluted with 0.1 M acetic acid containing bovine serum albumin 0.1% (w/v). The specific activity of the purified labelled peptide was ≈ 600 Ci/mmol.

Frontoparietal cortical membranes were prepared as previously described by Reubi et al. [38]. The frontoparietal cortex was homogenized in 10 mM HEPES-KOH buffer (pH 7.5) with a Brinkmann polytron homogenizer (setting 5, 15 s). The homogenate was spun at $600 \times g$ for 5 min at 4°C and the supernatant centrifuged at $48\,000 \times g$ for 30 min at 4°C. The resulting pellet was suspended in 10 mM HEPES-KOH (pH 7.5) and centrifuged as before. The new pellet was then re-suspended in 50 mM Tris–HCl buffer (pH 7.5). Samples were stored at −70°C until assay. Proteins were assayed by the method of Lowry et al. [29], with bovine serum albumin as a standard. Specific SS binding was measured according to the modified method of Czernik and Petrack [8]. 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) bovine serum albumin 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, the free radioligand was separated from the bound radioligand by centrifugation at $12\,000 \times g$ (Beckman microcentrifuge) for 1.5 min and the resultant pellet was counted in a Beckman γ counter. Non-specific binding, i.e. binding occurring in the presence of a high concentration (10^{-7} M) of unlabelled SS, represented

Table 1
Effects of fluoxetine or *p*-chloroamphetamine on the equilibrium parameters of SS binding to rat frontoparietal cortical membranes

Groups	B_{max}	K_d
Control	531 ± 28	0.43 ± 0.07
Fluoxetine (4 h)	672 ± 39 [b]	0.42 ± 0.06
Control	534 ± 30	0.43 ± 0.06
Fluoxetine (14 days)	896 ± 50 [a]	0.39 ± 0.05
Control	507 ± 38	0.58 ± 0.06
p-Chloroamphetamine (1 week)	373 ± 19 [b]	0.43 ± 0.05
Control	516 ± 36	0.57 ± 0.02
p-Chloroamphetamine (3 weeks)	422 ± 17 [b]	0.52 ± 0.02

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/mg protein. The results are presented as the mean ± S.E.M. of five separate experiments, each performed in duplicate. Statistical comparison vs. control: [a] $P < 0.001$, [b] $P < 0.05$.

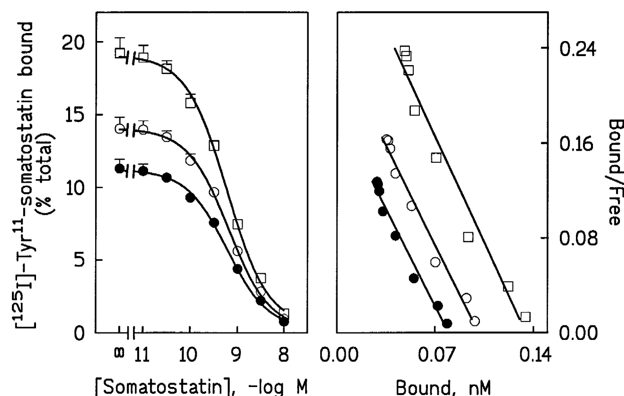

Fig. 1. Effect of acute and chronic fluoxetine administration (10 mg/kg i.p.) on SS binding to rat frontoparietal cortical membranes. Left panel: competitive inhibition of specific [^{125}I]Tyr^{11}-SS binding by unlabelled SS to frontoparietal cortical membranes. Membranes (0.15 mg protein/ml) were 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 animals in the control group pool (●) and acute (○) and chronic (□) fluoxetine-treated rats. In the control group, the results express the mean value of a pool of the control groups since B_{max} and K_d values of the controls were not affected by the vehicle. Each point is the mean ± S.E.M. of five separate experiments, each performed in duplicate. Right panel: Scatchard analysis of the binding data.

≈ 20% of the binding observed in the absence of native peptide and was subtracted from the total bound radioactivity in order to obtain the corresponding specific binding. The inactivation of [^{125}I]Tyr^{11}-SS in the incubation medium after exposure to membranes was studied by observing the ability of the peptide to re-bind to fresh membranes [1].

2.5. AC assay

AC activity was measured as previously reported [19] with minor modifications. Briefly, rat frontoparietal cortical membranes (0.06 mg/ml) were incubated with 1.5 mM ATP, 5 mM $MgSO_4$, 10 μM GTP and an ATP-regenerating system (7.5 mg/ml creatine phosphate and 1 mg/ml creatine kinase), 1 mM 3-isobutyl-1-methylxanthine, 0.1 mM phenylmethylsulphonyl fluoride, 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 refrigeration, 0.2 ml of an alumina slurry (0.75 g/ml in triethanolamine/HCl buffer, pH 7.4) was added and the suspension centrifuged. The supernatant was taken to assay cyclic adenosine 3′,5′-monophosphate (cAMP) by the method of Gilman [15].

2.6. Data analysis

The computer program LIGAND [32] 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 [45] 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 values were

Table 2
Effect of SS (10^{-4} M) and FK (10^{-5} M) on AC activity (pmol cAMP/min/mg protein) in frontoparietal cortical membranes from control ($n = 10$), fluoxetine (4 h) ($n = 5$)- and fluoxetine (14 days) ($n = 5$)-treated rats

	4 h		14 days	
	Control	Fluoxetine	Control	Fluoxetine
Basal activity	168 ± 15	170 ± 17	172 ± 12	166 ± 15
+ 10^{-4} M SS	109 ± 12	110 ± 10	108 ± 15	106 ± 15
+ 10^{-5} M FK	480 ± 20	486 ± 26	489 ± 23	474 ± 22
10^{-4} M SS + 10^{-5} M FK	355 ± 30	403 ± 20	347 ± 24	398 ± 23
% SS inhibition of FK stimulation	26 ± 2	17 ± 3 [a]	29 ± 5	16 ± 2 [a]

Experiments were performed as described in Materials and methods. Values represent the mean ± S.E.M. of the determinations performed. Statistical comparison vs. control: [a] $P < 0.05$.

Table 3
Effect of *p*-chloroamphetamine on the 5-hydroxytryptamine (serotonin) levels in the rat frontoparietal cortex at 1 and 3 weeks after its administration

	5-Hydroxytryptamine	
	1 week	3 weeks
Frontoparietal cortex		
Control	412 ± 13	421 ± 13
p-Chloroamphetamine	251 ± 7 [a]	184 ± 7 [a]

Data are mean ± S.E.M. of 5 rats in each group. Values are ng of 5-hydroxytryptamine/g wet tissue weight. [a] $P < 0.001$ compared to control.

< 0.05. Each individual experiment was performed in duplicate.

3. Results

The acute and chronic administration of fluoxetine increased $[^{125}I]Tyr^{11}$-SS binding to rat frontoparietal cortical membranes as compared with controls. This increase was due to a rise in the maximal number of SS receptors as revealed by Scatchard plots of the binding data, without any change in the binding affinity (Table 1, Fig. 1).

To assess the functional consequences of the changes in SS binding, experiments were designed to examine the effect of SS on FK-stimulated AC activity in control rats and rats acutely treated with fluoxetine. No significant differences were seen for either the basal or FK-stimulated AC activities between the control and fluoxetine-treated rats (Table 2). As previously observed, SS inhibited FK-stimulated AC activity. When rats were treated with fluoxetine acutely or chronically, the effect of SS on FK-stimulated AC activity was markedly decreased in frontoparietal cortical membranes (Table 2).

Further experiments explored the effect of fluoxetine on G_i-proteins in rat frontoparietal cortical membranes by determining the ability of low Gpp(NH)p concentrations to inhibit FK-stimulated AC activity. The inhibitory effect of Gpp(NH)p on FK-stimulated AC activity was markedly decreased in frontoparietal cortical membranes from rats acutely or chronically treated with fluoxetine (Fig. 2).

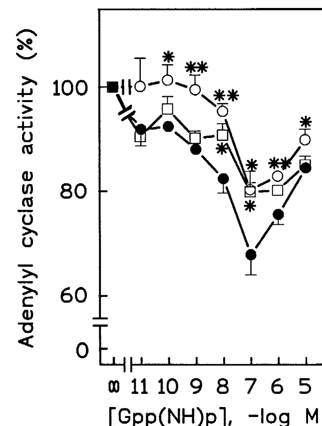

Fig. 2. Dose–effect curves for 5′guanylylimidodiphosphate (Gpp(NH)p) on FK-stimulated AC activity in rat frontoparietal cortical membranes from controls (●) and acute (○) and chronic (□) fluoxetine-treated rats. The results express the values of a pool of the control groups since no differences among them were found. The effect of Gpp(NH)p on AC activity was studied 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 the mean ± S.E.M. of three separate determinations, each performed in duplicate. Statistical comparison vs. control: * $P < 0.05$, ** $P < 0.01$.

With the aim of determining the effect of decreased serotonergic neurotransmission on the SS receptor–AC system, a group of rats were treated with *p*-chloroamphetamine, which depletes 5-hydroxytryptamine levels in the rat brain (Table 3), leading to a lasting reduction of 5-hydroxytryptamine innervation in the neocortex and other forebrain structures. *p*-Chloroamphetamine administration leads to a decrease in the number of SS receptors in the frontoparietal cortical membranes, with no change in the binding affinity (Table 1).

No significant differences were seen for either the basal or FK-stimulated AC activities between the control rats and rats treated with *p*-chloroamphetamine. The capacity of SS to inhibit basal and FK-stimulated AC activity in these experimental groups is shown in Table 4. The effect

Table 4
Effect of SS (10^{-4} M) and FK (10^{-5} M) on AC activity (pmol cAMP/min/mg protein) in frontoparietal cortical membranes from control ($n = 10$) and *p*-chloroamphetamine ($n = 5$ in each group)-treated rats

	1 week		3 weeks	
	Control	*p*-Chloroamphetamine	Control	*p*-Chloroamphetamine
Basal activity	160 ± 10	168 ± 16	163 ± 20	160 ± 23
+ 10^{-4} M SS	103 ± 21	105 ± 14	93 ± 15	100 ± 11
+ 10^{-5} M FK	474 ± 7	453 ± 20	455 ± 22	464 ± 20
10^{-5} M FK + 10^{-4} M SS	354 ± 29	381 ± 20	343 ± 25	398 ± 8
% SS inhibition of FK stimulation	25 ± 3	15 ± 2 [a]	24 ± 3	14 ± 2 [a]

Experiments were performed as described in Materials and methods. Values represent the mean ± S.E.M. of the determinations performed. Statistical comparison vs. control: [a] $P < 0.05$.

of SS on FK-stimulated AC activity was decreased in frontoparietal cortical membranes from *p*-chloroamphetamine-treated rats as compared to control animals (Table 4).

Frontoparietal cortical membranes from control and *p*-chloroamphetamine-treated rats showed similar G_i-protein activity (data not shown) at 1 and 3 weeks after its administration.

4. Discussion

In the present study, we have shown that the increase of serotonergic transmission induced by acute or chronic administration of fluoxetine, a selective 5-hydroxytryptamine re-uptake inhibitor, leads to a rise in the number of SS receptors, without altering their affinity, in the rat frontoparietal cortex. However, this treatment results in a reduction of the ability of SS to inhibit FK-stimulated AC activity. In addition, the decrease of serotonergic transmission induced by reduction of 5-hydroxytryptamine innervation in the neocortex due to systemic *p*-chloroamphetamine administration [12] leads to a decrease of the number of SS receptors and of the ability of SS to inhibit FK-stimulated AC activity in frontoparietal cortical membranes.

The binding parameters of SS receptors in the control rats were similar to those previously reported by others [49]. Recently, five different SS receptor subtypes have been cloned [20] and the tissue distribution of the messenger ribonucleic acid for each subtype has been studied in the rat [4]. 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.

The increase in the number of SS receptors was observed at a concentration of fluoxetine that produces a significant increase in the extraperikaryal 5-hydroxytryptamine concentration in the rat brain [55], leading to an enhanced activation of synaptic 5-hydroxytryptamine receptors as observed in functional studies [47].

5-hydroxytryptamine is known to bind and activate receptors coupled to different signaling pathways. In the rat cerebral cortex, both 5-hydroxytryptamine 5-HT_1 and 5-HT_2 receptors have been described [11,21]. Several 5-hydroxytryptamine receptor subtypes, such as 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1E}, are negatively coupled to AC, whereas 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} are positively coupled to phospholipase C [21]. On the other hand, it has been shown that cAMP analogs or agents whose action increases cell cAMP levels modulate SS binding by decreasing the maximal binding capacity of the receptors [31]. Since activation of the 5-hydroxytryptamine receptors 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1E} exerts a negative control on AC, it seems likely that the increase in the number of SS receptors following acute and chronic fluoxetine administration is the result, at least in part, of a decreased activation of a phosphorylation pathway via the cAMP-dependent protein kinase A (PKA). Recently, we found that the administration of a single dose of 5-hydroxytryptamine was accompanied by an increase in the number of SS receptors in the rat frontoparietal cortex [33].

It has been shown that chronic treatment with similar doses of fluoxetine induces an increased serotonergic function [43]. In vitro binding and quantitative autoradiographic studies have shown that neither 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} nor 5-HT_3 receptor-binding sites in various brain areas are affected by chronic fluoxetine treatment [27]. However, other authors have shown that chronic fluoxetine treatment up-regulates 5-HT uptake sites and 5-HT_2 receptors in rat brain [5].

The changes in SS binding were not due to a direct effect of 5-hydroxytryptamine on SS receptors since no change in tracer binding was detected following incubation of fresh frontoparietal cortical membranes with fluoxetine or 5-hydroxytryptamine [33]. This is consistent with the absence of any apparent structural analogy between these molecules.

Daily administration of fluoxetine to rats did not change radioligand binding to α_1-, or α_2-adrenergic, muscarinic cholinergic, H_1-histaminergic or opiate receptors [56], suggesting that these neurotransmitter systems are not implicated in the increase of the number of SS receptors found following long-term fluoxetine administration.

Several studies suggest that a central serotonergic mechanism plays a role in the regulation of SS-containing neurons. However, to date, these studies have frequently seemed contradictory. There are studies suggesting that 5-hydroxytryptamine may inhibit SS release [9]. This mechanism could be involved in the increase of the number of SS receptors observed following acute and chronic fluoxetine administration. In contrast, other authors [2,24] have postulated that augmentation of serotonergic activity probably stimulates SS release in the cerebral cortex and hippocampus. If this is so, the increase of serotonergic neurotransmission induced by fluoxetine administration could lead to a rise of SS levels in the synaptic cleft that, together with the elevation of the number of SS receptors, would increase somatostatinergic neurotransmission. In addition, 5-hydroxytryptamine was found to have no influence on SS release either from cultured cerebral cortical cells [39] or from hypothalamic tissues [30].

The effects of *p*-chloroamphetamine are characterized by an acute release of 5-hydroxytryptamine followed by a marked decrease in forebrain levels of this neurotransmitter; many 5-hydroxytryptamine axon terminals undergo subsequent degeneration, leading to a lasting reduction of 5-hydroxytryptamine innervation in the neocortex and other forebrain structures [12]. A possible mechanism for the decrease of the number of SS receptors after *p*-chloro-

amphetamine administration is that it is secondary to a decreased serotonergic activity; the serotonergic lesion could be removing serotonergic input to somatostatinergic neurons, altering SS release and thereby changing the number of post-synaptic SS receptors. Alternatively, it is possible that some SS receptors are localized pre-synaptically on serotonergic axons and, therefore, the degradation of these axons due to *p*-chloroamphetamine administration would be responsible for the observed decrease of SS binding.

Basal cAMP levels were unaffected by *p*-chloroamphetamine and fluoxetine which is in agreement with previous studies [51]. Basal and FK-stimulated AC activity was inhibited by SS in all the experimental groups studied which is in agreement with the literature [3,35,46]. A very high concentration of SS (10^{-4} M) was required to produce this inhibition although the same concentration was used by Schettini et al. [46], Bergström et al. [3], Garlind et al. [14] and Nagao et al. [35] in their studies on SS-mediated AC activity in rat and human brain. However and despite this high concentration, several lines of evidence suggest that the effect of SS is receptor-mediated and is not due to a non-specific inhibitory effect. In this regard, the GTP dependency of the inhibitory effect suggests the involvement of a G-protein in this response. This finding is consistent with binding studies on post-mortem human and rat brain tissues 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. In addition, Nagao et al. [35] and Shettini et al. [46] have shown that SS-reduced cAMP formation in the rat brain occurs via a G-protein coupled to AC. Overall results, plus the lack of a SS (1 μM) inhibitory effect on basal adenylate cyclase activity in primary cultures of mouse embryonic glial cells reported by Chneiweiss et al. [6] would argue against a non-specific inhibitory effect of the neuropeptide.

The ability of SS to inhibit FK-stimulated AC activity is significantly attenuated in frontoparietal cortical membranes from fluoxetine or *p*-chloroamphetamine-treated rats as compared with their controls. We have assessed possible involvement of AC catalytic subunit and the functional activity of G-protein coupling in this enzymatic response to SS in both treatments. FK-stimulated AC activity was not affected by fluoxetine or *p*-chloroamphetamine administration which allows us to exclude the possibility that both substances affect the functioning of the AC catalytic subunit. We have also examined the response of AC to the GTP analogue Gpp(NH)p as an index of the functional activity of the coupling G-protein. The inhibitory effect of Gpp(NH)p on FK-stimulated AC activity was markedly decreased in frontoparietal cortical membranes from rats acutely and chronically treated with fluoxetine which suggests that there is a less functional G_i-protein in these membranes that would explain the decreased inhibition of AC by SS after acute or chronic fluoxetine administration.

The results obtained following acute and chronic fluoxetine administration are consistent with data of Rovescalli et al. [41] who found that in vitro exposure of cortical slices to 5-hydroxytryptamine results in reduced SS inhibition of FK-stimulated AC activity. In addition, Rovescalli et al. [41] confirmed that 5-hydroxytryptamine may trigger protein kinase C (PKC) activation which seems to be a prerequisite for sensitizing AC towards Gpp(NH)p. It has been shown that PKC, by phosphorylating the G_i-proteins [44], can suppress hormonal inhibition of AC [25]. Therefore, the fact that the ability of SS to inhibit FK-stimulated AC activity is reduced following acute fluoxetine administration supports the hypothesis that 5-hydroxytryptamine might functionally inactivate G_i-proteins through PKC-mediated phosphorylation. Recently, the findings of Lesch et al. [28] indicate a direct effect of chronic antidepressant administration on steady-state concentrations of G-protein subunits, decreasing the $G_i\,\alpha$-protein in several brain regions. This $G_i\,\alpha$-protein mediates inhibition of AC by SS receptors.

The present results suggest that under normal circumstances some SS receptors are under a tonic stimulatory control through the serotonergic system. Both the serotonergic and somatostatinergic systems have been implicated in depression as well as in other central nervous system disorders [23,42]. These results indicate the existence of serotonergic and somatostatinergic interactions in the rat frontoparietal cortex and suggest that the SS receptor–AC system is implicated in the mechanism of action of the antidepressant drug fluoxetine. Therefore, it is tempting to speculate that the somatostatinergic system may play a role in the pathophysiology of depression.

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References

[1] Aguilera, G., Parker, D.S. and Catt, K.J., Characterization of somatostatin receptor in the rat adrenal glomerulose zone, *Endocrinology*, 111 (1982) 1376–1384.

[2] Bendotti, C., Tarizzo, G., Fumagalli, F., Baldessari, S. and Samanin, R., Increased expressions of preproneuropeptide Y and preprosomatostatin mRNA in striatum after selective serotonergic lesion in rats, *Neurosci. Lett.*, 160 (1993) 197–200.

[3] Bergström, L., Garlind, A., Nilsson, L., Alafuzoff, I., Fowler, C.J., Winblad, B. and Cowburn, R.F., Regional distribution of somatostatin receptor binding and modulation of adenylyl cyclase activity in Alzheimer's disease brain, *J. Neurol. Sci.*, 105 (1991) 225–233.

[4] Bruno, J.F., Xu, Y., Song, J. and Berelowitz, M., Tissue distribution of somatostatin receptor subtype messenger ribonucleic acid in the rat, *Endocrinology*, 133 (1993) 2561–2567.

[5] Cadogan, A.K., Marsden, C.A., Tulloch, I. and Kendall, D.A.,

Evidence that chronic administration of paroxetine and fluoxetine anhances 5-HT_2 receptor function in the brain of the guinea pig, *Neuropharmacology*, 22 (1993) 249–256.

[6] Chneiweiss, H., Glowinski, J. and Premont, J., Modulation by monoamines of somatostatin-sensitive adenylate cyclase on neuronal and glial cells from the mouse brain in primary cultures, *J. Neurochem.*, 44 (1985) 1825–1831.

[7] Chneiweiss, H., Glowinski, J. and Premont, J., Vasoactive intestinal polypeptide receptors linked to an adenylate cyclase and their relationship with biogenic amine and somatostatin-sensitive adenylate cyclase on central neuronal and glial cells in primary cultures, *J. Neurochem.*, 44 (1985) 779–786.

[8] Czernik, A.J. and Petrack, V., Somatostatin receptor binding in rat cerebral cortex. Characterization using a nonreducible somatostatin analog, *J. Biol. Chem.*, 285 (1983) 5525–5530.

[9] De los Frailes, M.T., Cacicedo, L., Lorenzo, M.J., Tolón, R.M., Fernández, G. and Sánchez-Franco, F., Neurotransmitter regulation of somatostatin secretion by fetal rat cerebral cortical cells in culture, *J. Endocrinol. Invest.*, 16 (1993) 661–668.

[10] Epelbaum, J., Somatostatin in the central nervous system: physiology and pathological modifications, *Prog. Neurobiol.*, 27 (1986) 63–100.

[11] Frazer, A. and Mayaani, S., Subtypes of receptors for serotonin, *Annu. Rev. Pharmacol. Toxicol.*, 30 (1990) 307–348.

[12] Fuller, R.W., Effects of *p*-chloroamphetamine on brain serotonin neurons, *Neurochem. Res.*, 17 (1992) 449–456.

[13] García-Sevilla, J.A., Magnusson, T. and Carlsson, A., Effect of intracerebroventricularly administered somatostatin on brain monoamine turnover, *Brain Res.*, 155 (1978) 159–164.

[14] Garlind, A., Fowler, C.J., Alafuzoff, I., Winlanb, B. and Cowburn, R.F., Neurotransmitter-mediated inhibition of post-morten human brain adenylyl cyclase, *J. Neural. Transm.*, 87 (1992) 113–124.

[15] Gilman, A.G., A protein binding assay for adenosine 3′5′-cyclic monophosphate, *Proc. Natl. Acad. Sci. USA*, 67 (1970) 305–312.

[16] Glowinski, J. and Iversen, L.L., 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 (1966) 655–669.

[17] Greenwood, F.C., Hunter, W.M. and Glover, J.S., The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity, *Biochem. J.*, 89 (1963) 114–123.

[18] Haroutunian, V., Martin, R., Campbell, G., Tsuboyama, G. and Davis, K., Cysteamine-induced depletion of central somatostatin-like immunoreactivity: effects on behavior, learning, memory and brain neurochemistry, *Brain Res.*, 403 (1976) 234–242.

[19] Houslay, M.D., Metcalfe, J.C., Warren, G.B., Hesketh, T.R. and Smith, G.A., The glucagon receptor of rat liver plasma membrane can couple to adenylate cyclase without activating it, *Biochim. Biophys. Acta*, 436 (1976) 489–494.

[20] Hoyer, D., Bell, G.I., Berelowitz, M., Epelbaum, J., Feniuk, W., Humphrey, P.P.A., O'Carroll, A.M., Patel, Y.C., Schonbrunn, A., Taylor, J.E. and Reisine, T., Classification and nomenclature of somatostatin receptors, *Trends Pharmacol. Sci.*, 16 (1995) 86–88.

[21] Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R. and Humphrey, P.P.A., VII International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin), *Pharmacol. Rev.*, 46 (1994) 157–203.

[22] Jacobs, B. and Azmitia, E.C., Structure and function of the brain serotonin system, *Physiol. Rev.*, 72 (1992) 165–229.

[23] Jacobs, B., Wilkinson, L. and Fornal, C., The role of brain serotonin – a neurophysiologic perspective, *Neuropsychopharmacology*, 3 (1990) 373–377.

[24] Kakigi, T. and Maeda, K., Effect of serotoninergic agents on regional concentration of somatostatin- and neuropeptide Y-like immunoreactivities in the rat brain, *Brain Res.*, 599 (1992) 45–50.

[25] Katada, T., Gilman, A.G., Watanabe, Y., Bauer, S. and Jacobs, K.H., Protein kinase C phosphorylates the inhibitory guanine nucleotide-binding regulatory component and apparently suppresses its function in hormonal inhibition of adenylate cyclase, *Eur. J. Pharmacol.*, 151 (1985) 431–437.

[26] Kiss, J., Csaky, A. and Halasz, B., Demonstration of serotonergic axon terminals on somatostatin-inmunoreactive neurons of the rat hypothalamus, *Brain Res.*, 442 (1988) 23–32.

[27] Le Poul, E., Lima, L., Laporte, A.M., Even, C., Doucet, E., Fattaccini, C.M., Laaris, N., Hamon, M. and Lanfumey, L., Recepteurs centraux de la serotonine et traitements chroniques avec un inhibiteur selectif de la recapture de la serotonine chez le rat: effects compares de la fluoxetine et de la paroxetine, *Encephale*, 21 (1995) 123–132.

[28] Lesch, K.P., Aulakh, C.S., Tolliver, T.J., Hill, J.L. and Murphy, D.L., Regulation of G proteins by chronic antidepressant drug treatment in rat brain: tricyclics but not clorgyline increase $G_o\alpha$ subunits, *Eur. J. Pharmacol.*, 207 (1991) 361–364.

[29] Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the Folin phenol reagent, *J. Biol. Chem.*, 193 (1951) 265–275.

[30] Maeda, K. and Frohman, L.A., Release of somatostatin and thyrotropin-releasing hormone from rat hypothalamic fragments in vitro, *Endocrinology*, 106 (1980) 1837–1842.

[31] Matozaki, T., Sakamoto, C., Nagao, M. and Baba, S., Pancreatic secretagogues regulate somatostatin binding to acinar cell membranes via two functionally distinct pathways, *Horm. Metab. Res.*, 20 (1988) 141–144.

[32] Munson, P.J. and Rodbard, D., A versatile computerized approach for characterization of ligand binding systems, *Anal. Biochem.*, 107 (1980) 220–239.

[33] Muñoz-Acedo, G. and Arilla, E., Modulation by 5-hydroxytryptamine of the somatostatin receptor–effector system and somatostatin levels in rat brain. *Mol. Brain Res.*, 37 (1996) 259–266.

[34] Murray-Whelan, R. and Schlegel, W., Brain somatostatin receptor-G protein interaction, *J. Biol. Chem.*, 267 (1992) 2960–2965.

[35] Nagao, M., Sakamoto, C., Matozaki, T., Nishizaki, H., Kondo, Y., Nakano, O. and Baba, S., Coupling of inhibitory GTP binding protein to somatostatin receptors on rat cerebrocortical membranes, *Folia Endocrinol. Jpn.*, 65 (1989) 1357–1366.

[36] Patel, Y.C. and Reichlin, S., Somatostatin in hypothalamus, extrahypotalamic brain, and peripheral tissues of the rat, *Endocrinology*, 102 (1978) 523–531.

[37] Popova, J., Ivanova, E., Tsheva, T. and Iavorska, N., Growth hormone and somatostatin treatment change 5-HT_1 receptor activity, *Gen. Pharmacol.*, 22 (1991) 1143–1146.

[38] Reubi, J.C., Perrin, M.H., Rivier, J.E. and Vale, W., High affinity binding sites for a somatostatin-28 analog in rat brain, *Life Sci.*, 28 (1981) 2191–2198.

[39] Robbins, R. Somatostatin and the cerebral cortex. In Y.C. Patel and G. Tannenbaum (Eds.), *Advances in Experimental Medicine and Biology, Vol. 188,* Plenum, New York, NY, 1985, pp. 201–215.

[40] Robertson, B., Baker, G.B. and Vanderwolf, C.H., The effects of serotonergic stimulation on hippocampal and neocortical slowwaves and behavior, *Brain Res.*, 555 (1991) 265–275.

[41] Rovescalli, A.C., Brunello, N., Pérez, J., Vitali, S., Steardo, L. and Racagni, G., Heterologous sensitization of adenylate cyclase activity by serotonin in the rat cerebral cortex, *Eur. Neuropsypharmacol.*, 3 (1993) 463–475.

[42] Rubinow, D.R., Davis, C.L. and Post, R.M., Somatostatin in neuropsychiatric disorders, *Prog. Neuropsychopharmacol. Biol. Psychol.*, 12 (1989) S137–S155.

[43] Rutter, J.J., Gundlah, C. and Auerbach, S.B., Increase in extracellular serotonin produced by uptake inhibitors is enhances after chronic treatment with fluoxetine, *Neurosci. Lett.*, 171 (1994) 183–186.

[44] Sagi-Eisenberg, R., GTP-binding proteins as possible targets for protein kinase C action, *Trends Biochem.*, 14 (1989) 355–357.

[45] Scatchard, G., The attractions of proteins for small molecules and ions, *Ann. NY Acad. Sci*, 51 (1949) 660–671.

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

[47] Schmidt, M.J., Fuller, R.W. and Wong, D.T., Fluoxetine, a highly selective serotonin reuptake inhibitor: a review of preclinical studies, *Br. J. Psychiatry*, 153 (1988) 40–46.

[48] Sperk, G., Simultaneous determination of serotonin, 5-hidroxyindoleacetic acid, 3,4-dihydroxyphenylacetic acid and homovanillic acid by high-performance liquid chromatography with electrochemical detection, *J. Neurochem.*, 38 (1982) 840–843.

[49] Srikant, C.B. and Patel, Y.C., Somatostatin receptors: identification and characterization in rat brain membranes, *Proc. Natl. Acad. Sci. USA*, 78 (1981) 3930–3934.

[50] Stark, P., Fuller, R.W. and Wong, D.T., The pharmacologic profile of fluoxetine, *J. Clin. Psychiatry*, 46 (1985) 7–13.

[51] Stockmeier, C.A., Martino, A.M. and Kellar, K.J., A strong influence of serotonin axons on β-adrenergic receptors in rat brain, *Science*, 230 (1985) 323–325.

[52] Tanaka, S. and Tsujimoto, A., Somatostatin facilitates the serotonin release from rat cerebral cortex, hippocampus and hypothalamus slices, *Brain Res.*, 208 (1981) 219–222.

[53] Wang, H., Reisine, T. and Dichter, M., Somatostatin-14 and somatostatin-28 induce opposite effects on potassium currents in rat neocortical neurons, *Proc. Natl. Acad. Sci. USA*, 86 (1989) 9616–9620.

[54] Wang, H., Reisine, T. and Dichter, M., Somatostatin-14 and somatostatin-28 inhibit calcium currents in rat neocortical neurons, *Neuroscience*, 38 (1990) 335–342.

[55] Wong, D.T., Bymaster, F.P., Horng, J.S. and Molloy, B.B., A new selective inhibitor for uptake of serotonin into synaptosomes of rat brain: 3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine, *J. Pharmacol. Exp. Ther.*, 193 (1975) 804–811.

[56] Wong, D.T., Reid, L.R., Bymaster, F.P. and Threlkeld, P.G., Chronic effects of fluoxetine, a selective inhibitor of serotonin uptake, on neurotransmitter receptors, *J. Neural. Transm.*, 64 (1985) 251–269.