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Desmethylimipramine pretreatment prevents 6-hydroxydopamine induced somatostatin receptor reduction in the rat hippocampus

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Summary

Several studies have shown anatomical and functional interconnections between catecholaminergic and somatostatinergic systems. To assess whether somatostatin (SS) may act presynaptically on catecholamine neurons, SS receptors were measured using radioligand test-tube binding assays on synaptosomes from hippocampus and frontoparietal cortex – areas that are innervated by catecholaminergic neurons with different densities and that have a high number of SS receptors – from control and 6-hydroxydopamine (6-OHDA)-treated rats. Intracerebroventricular (i.c.v.) injection of the catecholamine neurotoxin 6-OHDA (0.78 mg free base/kg of body weight in saline with 0.1% ascorbic acid) lowered hippocampal and frontoparietal cortical noradrenaline (NA) and dopamine (DA) levels at 1 week following the injection. Pretreatment of rats with desmethylimipramine (DMI) (40 mg/kg, intraperitoneal) prevented the drop in NA levels, but was not effective in attenuating DA depletion in the two brain areas studied. Treatment with 6-OHDA lowered the number of ^{125}I-Tyr^{11}-SS receptors in the hippocampus (130 ± 19 vs. 266 ± 16 fmol/mg protein, $P < 0.001$), whereas in the frontoparietal cortex a non significant 20% reduction in receptor number was found. The dissociation constants of ^{125}I-Tyr^{11}-SS binding to synaptosomes from frontoparietal cortex (0.65 ± 0.06 vs. 0.60 ± 0.04, P not significant) and hippocampus (0.44 ± 0.04 vs. 0.63 ± 0.14, P not significant) were similar in control and treated groups. Pretreatment with DMI reversed up to 80% of the effect of 6-OHDA on hippocampus

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SS receptors. DMI alone had no observable effect on the number and affinity of SS receptors. The 6-OHDA and the DMI treatment did not affect SLI levels in the brain areas studied. These results suggest that a portion of the hippocampal SS receptors may be localized presynaptically on the noradrenergic and dopaminergic nerve terminals.

Introduction

Somatostatin (SS), originally described as a hypothalamic neurohormone [1], has been thought in the past few years to function as a neurotransmitter in the central nervous system (CNS) [2]. SS containing neuronal pathways and autoradiographic receptor locations are largely known [3,4]. Several immunocytochemical studies have shown that SS-positive nerve terminals can be found in the various brain parts known to contain the nerve endings or the cell bodies of monoamine-containing neurons [5,6]. Furthermore, there is good evidence for the SS receptor on the locus coeruleus [7], a small homogeneous cluster of noradrenaline (NA)-containing neurons located in the dorsolateral pontine tegmentum. Most of the hippocampic NA innervation arises from this particular cell group [8–10]. Recently, concomitant storage of SS and noradrenaline (NA) has been demonstrated in some brain areas [11]. In addition, SS has been shown to increase brain monoamine turnover [12] suggesting that such action is probably mediated through specific SS receptors distributed in selected regions of the brain [11–15]. The hippocampus and cerebral cortex are the regions known to possess the most numerous SS receptors in the brain [16]. However, very little is known about the localization of SS receptors at the cellular level. To our knowledge it is unknown whether these SS receptors are located on catecholaminergic-containing nerve terminals at this level.

In order to investigate the possibility that, in the rat, some brain SS receptors may be localized presynaptically on the terminals of catecholaminergic neurons, we quantified SS binding in the hippocampus and frontoparietal cortex in control rats and in those that had been injected intracerebroventricularly (i.c.v.) with 6-hydroxydopamine (6-OHDA). Quantification was done at 1 week after the injection, since the injection of 6-OHDA into the lateral ventricle causes a specific degeneration of central catecholaminergic nerve terminals and a depletion of catecholamines [17]. Pretreatment with desmethylimipramine (DMI), an unspecific monoamine uptake blocker, was again used in order to evaluate whether the effects of 6-OHDA on SS receptors could be impeded by protecting these neurons. Concentrations of NA and dopamine (DA) were determined in the hippocampus and frontoparietal cortex to assess the effectiveness of the 6-OHDA treatment. Somatostatin-like immunoreactivity (SLI) in both brain areas was also examined.

Materials and Methods

Chemicals

Synthetic Tyr^{11}-SS and SS tetradecapeptide were purchased from Universal Biologicals Ltd. (Cambridge, UK); 6-OHDA hydrobromide, DMI, bacitracin and bovine serum albumin (fraction V) were from Sigma (St. Louis, MO, USA); and carrier-free $Na^{125}I$ (IMS 30; 100 mCi/ml) was from the Radiochemical Centre (Amersham, UK). Tyr^{11}-SS was radioiodinated by the chloramine-T method [18]. The tracer was purified in a Sephadex G-25 coarse column (1 × 100) which had been equilibrated with 0.1 M acetic acid containing bovine serum albumin 0.1% (w/v). The specific tracer radioactivity was about 400 Ci/g. The rabbit antibody used in the radioimmunoassay technique was purchased from the Radiochemical Centre (Amersham, UK). This antiserum was raised in rabbits against SS-14 conjugated to bovine serum albumin and is specific for SS but, since SS-14 constitutes the C-terminal portions of both SS-25 and SS-28, the antiserum does not distinguish between these three forms. All other chemicals were reagent grade.

Experimental animals

Wistar rats (200 to 250 g) were injected i.c.v. [19], under ether anesthesic with 0.78 mg/kg (free base) of 6-OHDA dissolved in 10 µl of 0.9 percent saline containing 0.1 percent ascorbic acid to retard oxidation of the neurotoxin [20]. The solution was made up just before use and was kept on ice. Similar doses of 6-OHDA have been used by a number of investigators in order to produce marked and long-lasting depletion of DA and NA in rodent brains [21]. One group of rats received DMI (40 mg/kg, intraperitoneal) 60 min before 6-OHDA injection [22], to protect noradrenergic terminals. Control animals were injected i.c.v. with 10 µl of vehicle, and were pretreated with an intraperitoneal saline injection when appropriate. Rats were killed by decapitation at 1 week after i.c.v. injection. The brains were rapidly removed and the frontoparietal cortex and whole hippocampus were rapidly dissected per Glowinski and Iversen [23].

Tissue extraction and somatostatin radioimmunoassay

SS was extracted from the hippocampus and frontoparietal cortex following the method of Patel and Reichlin [24], and since the yield of the overall extraction procedure was about 85% in the two brain areas studied, the recovery of SS was similar in the two brain areas. Protein was determined by the method of Lowry et al. [25]. SLI levels are measured by a modified specific radioimmunoassay method [24], with a sensitivity limit of 10 pg/ml. Incubation tubes prepared in triplicate 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% bovine serum albumin and 0.01 M EDTA, 200 µl appropriately diluted anti-SS serum and 100 µl freshly prepared ^{125}I-Tyr^{11}-SS diluted in buffer to give a final volume of 0.8 ml. All reagents and assay tubes were kept chilled in ice before the 48 h incubation at 4 °C. Separation of bound and free hormone was accomplished by addition of 1 ml dextran-coated

charcoal (dextran 0.2% w/v; charcoal 2% w/v). Dilution curves for each brain area were parallel to the standard curve. The intra and inter-assay variation coefficients were 5.2% and 7.1%, respectively.

Binding assay

Synaptosomes from the hippocampus and frontoparietal cortex were prepared as previously described [26]. Experimental conditions for SS binding were essentially as previously described for this laboratory [27]. Briefly, synaptosomes of either brain areas were separately incubated in 0.5 ml of 50 mM Tris-HCl buffer (pH 7.5) containing 5 mM $MgCl_2$, 30 mM NaCl, 1% bovine serum albumin, 0.1% bacitracin and 100 pM of ^{125}I-Tyr^{11}-SS in the absence or presence of 0.01–10 nM unlabelled SS. After 60 min incubation at 25 °C, synaptosome-bound peptide was isolated by centrifugation at 13000 *g* for 1.5 min, and the radioactivity determined in a Kontron gamma counter. Non-specific binding was obtained from the amount of radioactivity bound in the presence of 10^{-6} M SS and represented about 20% of the binding observed in the absence of unlabelled peptide. This nonspecific component was substracted from the total bound radioactivity in order to obtain the corresponding specific binding.

Evaluation of radiolabelled peptide degradation

To determine the extent of tracer degradation during incubation, we measured the ability of preincubated peptide to bind to fresh synaptosomes as previously described [28]. Briefly, ^{125}I-Tyr^{11}-SS (100 pM) was incubated with synaptosomes from rat frontoparietal cortex and hippocampus (1 mg protein/ml) for 60 min at 25 °C. After this preincubation, aliquots of the medium were added to fresh synaptosomes and incubated for an additional 60 min at 25 °C. The fraction of the added radiolabelled peptide which was specifically bound during the second incubation was measured and expressed as a percentage of the binding that had been obtained in control experiments performed in the absence of synaptosomes during the preincubation period.

Monoamine determinations

Concentrations of DA and NA were determined in the hippocampus and the frontoparietal cortex to assess the effectiveness of the 6-OHDA treatments. Both brain areas were rapidly removed, frozen on dry ice and stored at −80 °C. Tissue was weighed and homogenized in 0.2 N perchloric acid. After addition of an internal standard (dihydroxybenzylamine) and centrifugation of the homogenate, catecholamines in the supernatant were extracted and assayed by micropore high-performance liquid chromatography (HPLC) with electrochemical detection, as previously described [29].

Statistical analysis

The maximum binding capacity (B_{max}) and the dissociation constant (K_d) for the SS receptors were calculated after Scatchard analysis by linear regression [30]. Results were given in all cases as mean ± S.E.M. Student's *t*-test for unpaired variables was employed to assess differences between control and experimental groups, as indicated in the figures.

Results

I.c.v. 6-OHDA injection significantly reduced cortical and hippocampal levels of NA and DA (Table I). Pretreatment of rats with DMI prevented the drop in NA levels, but was not effective in attenuating the DA depletion (Table I). 6-OHDA and DMI treatments produced no change in SLI levels in the brain areas studied in comparison with the control group (Table I).

Binding experiments were performed at apparent equilibrium in all groups studied, as demonstrated by the corresponding kinetics of association (data not shown). Thus, a 1 h incubation period (at apparent equilibrium) was chosen for determining the stoichiometric parameters. The percentage of labelled SS degraded by synaptosomes during the binding experiments was similar in both treated and untreated animals, being 10.8, 9.2 and 11.3%, respectively, in 6-OHDA, DMI + 6-OHDA and DMI-treated rats and 12.0, 10.5 and 9.6% in the respective controls.

Addition of unlabelled SS reduced tracer binding to synaptosomes from hippocampus and frontoparietal cortex in all experimental groups (Fig. 1).

At 1 week after the 6-OHDA injection specific tracer-to-synaptosome binding was decreased in synaptosomes from the hippocampus but not in those from the frontoparietal cortex. Scatchard analysis of the binding of ^{125}I-Tyr^{11}-SS to synaptosomes from hippocampus and frontoparietal cortex indicated that i.c.v. 6-OHDA was associated with a decrease in the number of SS receptors in the hippocampus and with no change in the affinity constants (Figs. 1 and 2, Table II). Pretreatment with DMI re-

TABLE I

Effect of 6-hydroxydopamine (6-OHDA) and desmethylimipramine (DMI) on the levels of dopamine (DA) and noradrenaline (NA) and somatostatin-like immunoreactivity (SLI) in the hippocampus and frontoparietal cortex of the rat at 1 week after administration

For details of treatment see Materials and Methods.

	DA +	NA +	SLI + +
Hippocampus			
control	35 ± 9	252 ± 21	15.90 ± 3.13
6-OHDA	14 ± 4*	30 ± 9*	18.65 ± 3.20
DMI + 6-OHDA	17 ± 7*	256 ± 28	16.07 ± 3.79
DMI	37 ± 8	248 ± 25	14.64 ± 2.41
Frontoparietal cortex			
control	20 ± 5	125 ± 10	17.61 ± 2.80
6-OHDA	8 ± 2*	24 ± 7*	14.31 ± 0.71
DMI + 6-OHDA	10 ± 3*	127 ± 13	17.39 ± 4.14
DMI	23 ± 7	120 ± 14	15.14 ± 1.13

Data are mean ± S.E.M. of 6 rats.

+ Values are ng of amine/g tissue wet weight.

+ + Values are ng of SS/mg protein.

* $P < 0.01$ compared to control.

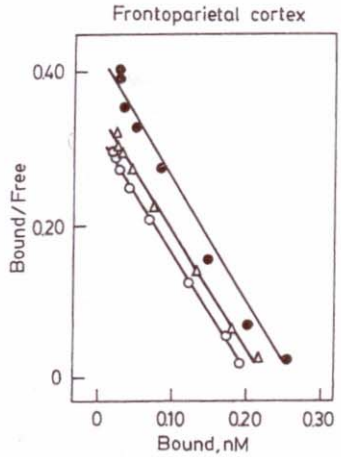

Fig. 1. Scatchard plots of specific ^{125}I-Tyr^{11}-somatostatin (^{125}I-Tyr^{11}-SS, 100 pM) binding to synaptosomes (1 mg protein/ml) of the frontoparietal cortex of controls (●), 6-OHDA-treated group (○) and desmethylimipramine (DMI)-plus 6-OHDA-treated group (△). The results express the values of a pool of the control medium groups since B_{max} and K_d values of the control groups were not affected by the saline or ascorbic acid media. Each point is the mean of five rats in each group. The corresponding equilibrium binding parameters are included in Table II.

versed up to 80% of the effect of 6-OHDA on hippocampus SS receptors (Table II). DMI alone had no observable effect on the binding of ^{125}I-Tyr^{11}-SS to synaptosomes from either brain area (Table II).

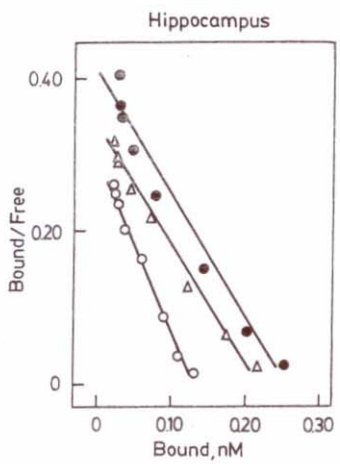

Fig. 2. Scatchard plots of specific ^{125}I-Tyr^{11}-somatostatin (^{125}I-Tyr^{11}-SS, 100 pM) binding to synaptosomes (1 mg protein/ml) of the hippocampus of controls (●), 6-OHDA-treated group (○) and desmethylimipramine (DMI)-plus 6-OHDA-treated group (△). The results express the values of a pool of the control medium groups since Bmax and Kd values of the control groups were not affected by the saline or ascorbic acid media. Each point is the mean of five rats in each group. The corresponding equilibrium binding parameters are included in Table II.

TABLE II

Effect of 6-hydroxydopamine (6-OHDA) and desmethylimipramine (DMI) on equilibrium parameters for somatostatin (SS) binding to synaptosomes from rat hippocampus and frontoparietal cortex 1 week after injection

	Hippocampus	Frontoparietal cortex
Control		
K_d	0.63 ± 0.14	0.60 ± 0.04
B_{max}	266 ± 16	254 ± 21
6-OHDA		
K_d	0.44 ± 0.04	0.65 ± 0.06
B_{max}	130 ± 8**	208 ± 19
Control		
K_d	0.62 ± 0.16	0.59 ± 0.02
B_{max}	247 ± 25	246 ± 18
DMI + 6-OHDA		
K_d	0.67 ± 0.17	0.66 ± 0.04
B_{max}	220 ± 42	224 ± 73
Control		
K_d	0.52 ± 0.06	0.54 ± 0.07
B_{max}	212 ± 10	268 ± 35
DMI		
K_d	0.44 ± 0.07	0.55 ± 0.03
B_{max}	216 ± 6	276 ± 9

Binding parameters were calculated after Scatchard analysis by linear regression [24]. Units for K_d are nM B_{max} units are fmol of SS bound per mg protein. Values represented the mean ± S.E.M. of five rats in each group. ** $P < 0.001$ versus control.

Discussion

The present results show that, without influencing apparent affinity, 6-OHDA administration in the rat results in a decrease in the number of SS receptors in hippocampus and a slight, although not significant, decrease in the frontoparietal cortex. Pretreatment with DMI prevented the 6-OHDA-induced changes in SS binding while DMI alone had no observable effect on SS binding.

The SLI content in the two brain areas studied as well as the binding parameters of brain SS receptors in the control rats were similar to those previously reported by others [14,31]. The Scatchard analysis of the stoichiometric data suggested the existence of only one type of SS receptor. This finding agrees with some studies in rat brain [13,14,16,32] but differs from other previously reported data [33,34]. It is conceivable that the use of small SS analogues [33] or their labelling with different isotopes [34] might explain the differences.

The present study shows that NA and DA pepletion in the hippocampus and the frontoparietal cortex provoked by 6-OHDA does not modify SS concentration in either area. This unchanging SLI level after 6-OHDA treatment is consistent with the

studies of Salin et al. [35] in which no change in striatal SLI levels was found after 6-OHDA administration. Recently, it has been demonstrated that striatal levels of mRNAs encoding SS are unaffected after similar 6-OHDA induced lesions [36]. However, all these results are in apparent conflict with other findings that NA and DA stimulate rat amygdala [37] and cortical [38] SS release, respectively. The lack of effects by the catecholamine neurotoxin 6-OHDA on hippocampal and cortical SS levels might be attributable to the putative long-term development of postlesional adaptive mechanisms that would counteract the effects of the suppression catecholamine action. In addition, it might be that even though the overall content of SLI in both brain areas did not vary, the rate of SS synthesis and release may have changed.

6-OHDA treatment significantly reduced the levels of DA and NA in the brain areas studied, in agreement with previously published values [7,22]. Pretreatment of rats with DMI prevented the drop in NA levels, but was not effective in attenuating the DA depletion, as already reported [22]. These results together with the fact that DMI reversed up to 80% of the effect of 6-OHDA on hippocampus SS receptors clearly indicate that a substantial proportion of SS receptors in the hippocampus could be located on the noradrenergic afferents to the hippocampus. While the possibility of transsynaptic degeneration cannot be entirely ruled out, similar chemical lesioning techniques have been used to suggest the presynaptic localization of opiate and other receptors on neuronal terminals [39,40]. The total decrease (about 47%) in the number of SS receptors in the hippocampus produced by 6-OHDA treatment is less than the drop in NA levels (88%) at this level, suggesting that only a portion of the SS receptors in the hippocampus are localized on noradrenergic terminals. The remaining SS receptors in the 6-OHDA treated rats must lie on intrinsic neurons since Palacios et al. [41] have shown that most SS receptors in the hippocampus are located on intrinisic neurons. Another possible explanation for the difference in magnitude between catecholamine depletion and the decrease in SS receptors is that these sites could be present on neuronal terminal membrane 'ghosts' that remain after 6-OHDA treatment. The presence of SS receptors on presynaptic noradrenergic terminals in the hippocampus correlates well with the known effects of SS on the monoamine metabolism in the hippocampus [12].

The changes in SS binding in the hippocampus and the frontoparietal cortex could accord with the significant regional variation in the density of catecholaminergic innervation of these two brain areas [8–10].

A better understanding of the physiological relevance of the localization of SS receptors for catecholaminergic terminals will probably increase our knowledge of the basic mechanism involved in the control of the activity of these pathways.

Quantification of SS binding in different hippocampal subfields in the 6-OHDA-treated rats merits further investigation.

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