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ACUTE NICOTINE ADMINISTRATION INCREASES SOMATOSTATIN CONTENT AND BINDING IN THE RAT HYPOTHALAMUS

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Summary

Within 4 minutes a single, intravenous injection of nicotine (0.3 mg/Kg) induced increases in somatostatin-like immunoreactivity concentrations in the rat hypothalamus but not in the striatum. These changes were associated with a significant increase in the specific binding of somatostatin to putative receptor sites in hypothalamic membranes, while no significant changes were found in striatum. The enhancement of somatostatin binding resulted from a rapid increase in the number of available receptors rather than a change in receptor affinity. This effect appears to be mediated by nicotinic cholinergic receptors, because pretreatment with a centrally active nicotinic receptor antagonist, mecamylamine (5.0 mg/Kg i.v.), prevented the nicotine-induced changes in somatostatin content and binding in the hypothalamus. Mecamylamine alone had no observable effect on the hypothalamic somatostatinergic system. These results suggest that the rat hypothalamic somatostatinergic system can be regulated by nicotine-like acetylcholine receptors.

Electrophysiological, autoradiographic and biochemical studies have demonstrated the presence of nicotinic cholinergic receptors in rat brain (1-4). The highest concentration of nicotinic cholinergic receptors is found in the hypothalamus (2-4). This brain region has been shown to contain a dense innervation of somatostatin (SS)-positive nerve terminals (5,6). In addition to their neurosecretory components, which project to the external layer of the median eminence, these neurons interconnect with various intrahypothalamic nuclei (7) and send some ascending and descending projections to other parts of the rat brain (6). SS receptors have been characterized (8,9) and visualized by autoradiography (10) in the hypothalamus. Recently, acetylcholine has been reported to potentiate the excitatory effect of SS on brain neurons (11). In addition, it is known that nicotine, a cholinergic drug, influences catecholamine turnover in the hypothalamus (12,13), in the same way that the neurotransmitter SS does (14). SS co-localizes with tyrosine hydroxylase in the nerve cells of discrete hypothalamic regions in rats (15). Taking these previous findings together, it becomes necessary to determine whether nicotinic cholinergic receptors modulate the SS hypothalamic receptors. And, in view of the rapid onset of nicotine action in the hypothalamus (13), it is of substantial interest to analyze whether nicotine can induce very rapid changes in SS receptors in the rat hypothalamus after intravenous injection. On the other hand, since SS neurons in the hypothalamus may receive a nicotinic cholinergic input (16), it would be interesting to know the effect of nicotine

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on the somatostatin-like immunoreactivity (SLI) concentration in this brain region. The experiments described in this report were designed to explore the action of nicotine on specific SS receptor binding and SLI in the hypothalamus. Pretreatment with mecamylamine, a centrally acting antagonist of nicotinic cholinergic receptors (17), was again used in order to evaluate whether the effects of nicotine on the somatostatinergic system involved the activation of nicotinic cholinergic receptors.

Materials and Methods

Chemicals

Synthetic Tyr11-SS and SS-14 were purchased from Universal Biologicals Ltd (Cambridge, U.K.), nicotine hydrogen tartrate, mecamylamine hydrochloride, bacitracin and bovine serum albumin (BSA) (fraction V) from Sigma (St. Louis, MO, U.S.A.) and carrier-free Na125I (IMS 30; 100 mCi/ml) was from the Radiochemical Centre (Amersham, U.K.). The SS antiserum used in the radioimmunoassay technique was purchased from the Radiochemical Centre (Amersham, U.K.). All other chemicals were reagent grade.

Experimental animals

Male Sprague Dawley rats weighing 230-250 g were divided into four groups. In the first group nicotine, as a tartrate salt, was administered intravenously (i.v.) into the right jugular vein at a dose of 0.3 mg/Kg (base) 4 min before decapitation, as previously described (13). In the second group mecamylamine HCl was administered at a dose of 5 mg/Kg i.v. 30 min before nicotine administration (18). In a third group the rats received mecamylamine HCl (5 mg/Kg/i.v.) 30 min before the saline injection. Nicotine and mecamylamine were administered dissolved in saline. In all experiments, the control animals received a saline injection via the same route as that used for drugs injected into the experimental animals. The rats were decapitated, the brains removed and the hypothalamus and striatum were dissected according to the method of Glowinski and Iversen (19).

Binding studies

Synthetic Tyr11-SS was radioiodinated by the method described by Greenwood et al (20), and purified by chromatography on a Sephadex G-25 column (100 x 1 cm) preequilibrated with 0.1 N acetic acid containing 0.1 % BSA. The specific activity of the purified labelled peptide was about 400 Ci/g. Membranes from the hypothalamus and striatum were prepared as described by Pitkänen et al (21). The proteins were determined by the method of Lowry et al (22). The binding of 125I-Tyr11-SS to membranes from the hypothalamus and striatum was performed according to the modified method of Srikant and Patel (9). Briefly, hypothalamic and striatal membranes (about 0.15 mg protein/ml) were incubated in 250 l of a medium containing 50 mM HEPES-KOH buffer (pH 7.5), 5 mM MgCl2, 0.1 % (wt/vol) BSA and 20 g/ml bacitracin with 250 pM 125I-Tyr11-SS in the absence or presence of 0.01-10 nM unlabelled SS. After 60 min incubation at 30°C, the free radioligand was separated from the bound radioligand by centrifugation at 12000 x g (Beckman microcentrifuge, Palo Alto, CA) for 1.5 min, and the resulting pellet was counted in a Beckman-counter. Nonspecific binding, i.e. binding in the presence of a high concentration (10-7 M) of unlabelled SS, represented about 25% of the binding observed in the absence of native peptide and was subtracted from the total bound

radioactivity to obtain the specific binding. The inactivation of 125I-Tyr11-SS in the incubation medium after exposure to membranes was studied by the ability of the peptide to rebind to fresh membranes (23).

Tissue extraction and somatostatin radioimmunoassay

SLI was extracted from hypothalamic and striatal tissue in 2 M acetic acid by sonication and boiling and measured by a radioimmunoassay (24) with a sensitivity limit of 10 pg/ml. The antiserum was raised in rabbits against SS-14 conjugated to BSA and is specific for SS, but since SS-14 also constitutes the C-terminal portion of SS-28, the antiserum does not distinguish between these two forms. Dilution curves for each brain region were parallel to the standard curve. The intra- and inter-assay variation coefficients were 5.7% and 7.5% respectively.

Data analysis

Estimation of the equilibrium dissociation constant (Kd) and the maximum number of receptors (Bmax), for binding of 125I-Tyr11-SS in Scatchard plots (25) were calculated on a Hewlett-Packard device with linear regression analysis. Statistical analysis of the significance of the difference between mean values were performed by Student's t-test. All data are presented as the mean ± S.E.M.

Results

As illustrated in Table I, nicotine at a dose of 0.3 mg/Kg given i.v. produced a significant increase in the SSLI levels in the hypothalamus but not in striatum at 4 min. When the rats were pretreated with mecamylamine (5 mg/Kg i.v.), the effects of nicotine on the SSLI levels in hypothalamus were completely inhibited (Table I). Mecamylamine treatment alone did not influence the hypothalamic SSLI levels (Table I).

TABLE I

Effect of acute treatment with nicotine and mecamylamine plus nicotine on somatostatin-like immunoreactivity concentration in the hypothalamus and striatum of the rat. For details on treatment see Materials and Methods.

Groups	Somatostatin (ng/mg of protein) Hypothalamus	Striatum	n
Saline	19.65 ± 2.14	3.38 ± 0.36	6
Nicotine	160.76 ± 16.86 *	3.13 ± 0.38	6
Mecamylamine plus nicotine	17.55 ± 2.06	---	6
Mecamylamine plus saline	18.98 ± 1.54	---	6

Determinations were made in duplicate for each experiment. The results are expressed as ng somatostatin/mg protein and as the means ± S.E.M. Statistical comparison versus saline: * $p<0.001$; n: number of animals.

Hypothalamic and striatal membrane preparations of both control and nicotine treated rats bound 125I-Tyr11-SS in a time-dependent process, an apparent equilibrium being observed between 50-180 min at 30°C (data not shown). All subsequent binding experiments were therefore conducted at 30°C for 60 min. Membranes from both brain regions showed a similar peptide degradation capacity and the values varied by no more than 10% in all the experimental groups.

Increasing concentrations of unlabelled SS competitively inhibited the specific binding of 125I-Tyr11-SS to hypothalamic and striatal membrane preparations in both control and nicotine-treated rats (Fig. 1, left panel). The specific binding of the tracer to membranes prepared from the hypothalamus was significantly higher in the nicotine-treated rats than in the control animals, both in the absence and in the presence of unlabelled SS throughout the whole range of concentrations studied. However, the striatum presented no differences in tracer binding.

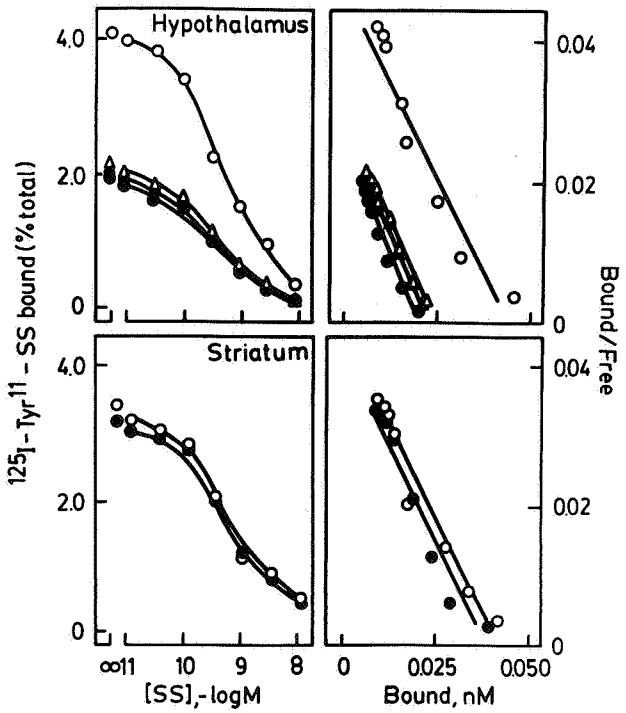

FIG.1

Effect of acute treatment with nicotine or mecamylamine plus nicotine on somatostatin binding to hypothalamic and striatal membranes. Left panel: membranes (0.15 mg protein/ml) were incubated for 60 min at 30°C in the presence of 250 pM 125I-Tyr11-SS and increasing concentrations of native peptide. Points correspond to values for animals in the control (saline) (●), nicotine-treated (○), mecamylamine plus nicotine-treated (▲) and mecamylamine plus saline-treated (△) groups. The animals were killed 4 min after the nicotine injection. Each point is the mean of six replicate experiments. For the sake of clarity, S.E.M. are not represented, but were always below 9% of the mean values. Right panel: Scatchard analysis of the same data.

To determine whether enhanced SS binding in hypothalamus of nicotine-treated rats results from an increased number of receptors or from a change in receptor site affinity we performed a Scatchard analysis (25) of the stoichiometric data. These studies revealed that there was an increase in the number of receptors (Bmax) rather than a change in receptor affinity (Kd) (Fig. 1, right panel and Table II).

Pretreatment with mecamylamine completely blocked the nicotine-induced changes in the number of SS receptors (Fig. 1 and Table II). Mecamylamine alone did not influence the SS receptors (Table II).

TABLE II

Effect of acute treatment with nicotine and mecamylamine plus nicotine on equilibrium parameters of somatostatin binding to hypothalamic and striatal membranes.

Groups	Hypothalamus	Striatum	n
Saline			
Kd	0.97 ± 0.13	1.03 ± 0.07	6
Bmax	152 ± 34	272 ± 38	
Nicotine			
Kd	0.95 ± 0.20	1.06 ± 0.12	6
Bmax	347 ± 26 *	288 ± 36	
Mecamylamine plus nicotine			
Kd	0.94 ± 0.17	—	6
Bmax	163 ± 14		
Mecamylamine plus saline			
Kd	1.04 ± 0.07	—	6
Bmax	173 ± 10		

The maximum number of binding sites (Bmax) and dissociation constant (Kd) were determined by least-squares linear regression. Units for Kd are nM and units for Bmax are fmol of somatostatin bound per mg of protein. The results are presented as the means ± S.E.M. Statistical comparison versus saline: * $p<0.01$; n: number of animals.

Discussion

A single acute intravenous injection of nicotine was followed by an increase in SS content and binding in the hypothalamus but not in the striatum. Pretreatment with mecamylamine prevented the nicotine-induced changes in SS content and binding, while mecamylamine alone had no observable effect on the somatostatinergic system.

The content of SSLI in both brain regions and the binding parameter of brain SS receptors in the control animals were similar to those previously reported by others (9, 21, 26). It should be mentioned that Scatchard analysis demonstrated the existence of only one type of SS receptor. This finding agrees with some reports (8, 21, 27), but not with other previously reported data (28, 29). It is conceivable that the use of reduced SS analogs (28) or receptor labelling with very different isotopes (29) might explain this discrepancy.

The rapid changes in SS content and binding observed after nicotine injection are in keeping with the modifications in this neuropeptide which were provoked by TRH (30) or cysteamine (31). The rapid increases described in hypothalamic SS receptors after nicotine administration are of the same order of magnitude as those reported for other central neurotransmitter receptors altered by various pharmacological manipulations (32).

The effects are clearly limited to hypothalamus. The apparently high degree of selectivity makes the involvement of unspecific factors very unlikely.

The molecular mechanism by which nicotine causes an increase in SS content and binding in the hypothalamus is unknown. However, nicotinic cholinergic receptors seem to mediate the action of nicotine, since the changes induced by nicotine on the somatostatinergic system were prevented by pretreatment with the nicotinic cholinergic blocking agent mecamylamine. In addition, mecamylamine alone had no demonstrable effect on these parameters.

The fact that the changes in the somatostatinergic system occur in the hypothalamus but not in the striatum may be a result of regional differences in the number of nicotinic cholinergic receptors, since the specific binding of nicotine to synaptosomes is higher in the hypothalamus (2-4).

Although such an increase in hypothalamic SSLI levels could conceivably reflect decreased release of the peptide, this seems unlikely. A significant calcium influx can occur through the nicotinic cholinergic receptor channel (33). Although the consequences of this Ca2+ influx cannot be assessed as yet, it may increase the release of SS since SS release is calcium-dependent in the rat hypothalamus (34). On the other hand, intravenous injections of nicotine induce very rapid reductions of hypothalamic catecholamine levels, suggesting increased release of norepinephrine from hypothalamus (13). Since SS co-localizes with tyrosine hydroxylase in the nerve cells of discrete hypothalamic regions in rats (15), it seems likely that SS might also be released from these neurons within the hypothalamus.

These results could also be explained by the hypothesis that the nicotine-induced activation of the SSLI neurons, possibly via increased neuronal activity, involved an enhanced processing of the SS precursor peptide. The processing of precursor peptides by cleavage enzymes has been suggested to play an important role in the regulation of synaptic function in peptide neurons. It seems possible that the cleavage enzymes may have become activated by the increased impulse traffic in the SS neurons (35, 36). However, a direct effect on the synthesis of the SS precursor peptide in the cell bodies cannot be excluded.

Although it is generally accepted that short-term stimuli mainly affect secretion, whereas long-term stimuli regulate synthesis, there is now evidence indicating that short-time exposure can also regulate peptide synthesis (37). A related mechanism by which hypothalamic SS levels might be increased after acute nicotine exposure could be through increased peptide release and the compensatory increase in post-translational processing of an SS prohormone to SS; thus resulting in an increase in both intracellular and extracellular SS. It is tempting to speculate that if the release of hypothalamic SS increases in nicotine-treated rats, it could be responsible for the inhibition of the GH release

via the hypothalamus-hypophysial portal vessels that is caused by a single acute dosis (0.3 mg/Kg) of nicotine (11).

The rapid increase in hypothalamic SSLI content found in the present study is similar to that found with luteinizing hormone releasing hormone-like immunoreactivity 5 min after nicotine injection (38). Andersson et al (38) have not found changes in brain SSLI following acute treatment with nicotine. Several differences exist between both studies: a) these authors assayed the median eminence and paraventricular hypothalamic nucleus, whereas we studied the whole hypothalamus, and, b) the studies involved a different dose and administration route of nicotine.

At present, we do not know the mechanism by which nicotine increases the number of SS receptors in the hypothalamus. Hitzemann et al (39) have shown that nicotine acutely affects polyphosphoinositide synthesis and/or metabolism in the rat brainstem microsomal fraction, which is known to be rich in putative nicotinic cholinergic receptors (40). These effects of nicotine might alter membrane properties and thus expose the receptors normally sequestered within the plasma membrane, although they do not preclude the possibility that other factors may be involved as well. However, to date this effect of nicotine not has been described in the hypothalamus.

Whether the increased number of hypothalamic SS receptors demonstrated in the present experiments is reflected in an altered physiologic function has not been determined. However, it is tempting to speculate that some of the acute effects of nicotine such as increased hypothalamic catecholamine turnover (11, 12) may, at least in part, depend on an increase in the number of SS receptors in the hypothalamus, since this neurotransmitter increases hypothalamic catecholamine turnover (13). Therefore, the present results suggest that the rat hypothalamic somatostatinergic system can be regulated by nicotine-like acetylcholine receptors.

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