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Effects of acute nicotine and mecamylamine administration on somatostatin concentration and binding in the rat brain

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Since nicotine and somatostatin have regulatory effects on locomotor activity it was of interest to determine whether the receptors for somatostatin are modulated by the cholinergic nicotine-like effects. An i.v. dose of 0.3 mg/kg nicotine induced an increase in the concentrations of somatostatin-like immunoreactvity at 4 min in the parietal cortex and at 15 min in the hippocampus. These changes were associated with a significant increase in the total number of specific somatostatin receptors in the parietal cortex at 15 min and in the hippocampus at 30 min following injection. To determine if the above mentioned changes are related to the nicotine activation of central nicotine-like acetylcholine receptors, a cholinergic nicotinic blocking agent, mecamylamine, was administered before the nicotine injection. Pretreatment with mecamylamine (5.0 mg/kg i.v.) prevented the nicotine-induced changes in somatostatin level and binding in both brain areas. Mecamylamine alone had no observable effect on the somatostatinergic system. These results suggest that the somatostatinergic system can be regulated by nicotine-like acetylcholine receptors and may be involved in some of the behavioral central effects of nicotine.

Nicotine; Mecamylamine; Somatostatin receptors; Brain; (Rat)

1. Introduction

Nicotine, a cholinergic drug, influences motor activity (Morrison and Stephenson, 1972). Mecamylamine, a centrally acting antagonist of nicotine acetylcholine receptors, has previously been shown to block the effects of nicotine in the rat brain (Morrison et al., 1969). The tetradecapeptide, somatostatin, which is distributed throughout the mammalian central nervous system (Brownstein et al., 1977), also influences locomotor activity (Rezek et al., 1977). Specific high affinity receptors for somatostatin have been characterized in the rat brain and there is strong evidence that these receptors mediate the biological effects of the neuropeptide (Czernik and Petrack, 1983; Reubi et al., 1981; Weightman et al., 1985).

Since nicotine and somatostatin have regulatory effects on locomotor activity it was of interest to determine whether the receptors for somatostatin are modulated by the nicotine-like acetylcholine receptors. In view of the rapid onset of nicotine action in the central nervous system (Andersson et al., 1982), it is of substantial interest to analyze whether nicotine can induce very rapid changes in somatostatin receptors in the rat brain following its intravenous injection. On the other hand, since somatostatin neurons in the cerebral cortex and hippocampus may receive a cholinergic input (Delfs et al., 1984), it would be interesting to known the effect of nicotine on the somatostatin concentration. The present study served to analyze the action of nicotine on specific

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somatostatin receptor binding and somatostatin-like immunoreactivity in rat parietal cortex and hippocampus 4, 15, 30 and 60 min after its intravenous injection. Pretreatment with mecamylamine was again used in order to evaluate whether the effects of nicotine involved the activation of nicotine-like acetylcholine receptors.

2. Materials and methods

2.1. Experimental animals

Eighty Wistar rats initially weighing approximately 200 g were divided into four groups. In the first study 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, 15, 30 or 60 min before decapitation, as previously described (Andersson et al., 1982). In the second experiment mecamylamine HCl was administered at a dosage of 5 mg/kg (Morgan and Pfeil, 1979) i.v. 30 min before nicotine administration. Nicotine and mecamylamine were dissolved in saline. In all experiments, the control animals received a saline injection via the same route as used for drugs injected into the experimental animals. In a third study the rats received mecamylamine HCl (5 mg/kg) i.v. 30 min before the saline injections. The rats were decapiated, the brains were removed and the parietal cortex and hippocampus were rapidly dissected according to method of Glowinski and Iversen (Glowinski and Iversen, 1966).

2.2. Tissue extraction and SS radioimmunoassay

For somatostatin-like immunoreactivity measurements, the cerebral cortex and hippocampus were rapidly homogenized in 1 ml 2 M acetic acid using a Brinkman polytron (setting 5, 30 s). The extracts were boiled for 5 min in a water-bath, chilled in ice, and aliquots (100 μl) were removed for protein determination (Lowry et al., 1951). The homogenates were subsequently centrifuged at 15000 $\times g$ for 15 min at 4°C and the supernatant was neutralized with 2 M NaOH. The extracts were stored at −70°C until assay. The somatostatin concentration was determined in tissue extracts by a modified radioimmunoassay method (Patel and Reichlin, 1978), with a sensitivity limit of 10 pg/ml. The possibility that other substances present in the tissue might give rise to erroneous results was eliminated by comparing the resulting changes in hormonal immunoreactivity with those of the diluted standards. In addition, known standard amounts of the hormone were added to varying amounts of the extracts and serial dilutions were again assayed in order to determine if this exogenously added hormonal immunoreactivity could be measured reliably in the presence of tissue extracts. Incubation tubes prepared in duplicate contained 100 μl samples of unknown or of standard solutions of 0-500 pg cyclic somatostatin tetradecapeptide diluted in phosphate buffer (0.05 M, pH 7.2 containing 0.3% bovine serum albumin, 0.01 M EDTA), 200 μl appropriately diluted anti-somatostatin serum, 100 μl freshly prepared ^{125}I-[Tyr^{11}]somatostatin diluted in buffer to give 6000 cpm (equivalent to 5-10 pg), and enough buffer to give a final volume of 0.8 ml. All reagents as well as the assay tubes were kept chilled on ice, before their incubation for 48 h at 4°C. Bound hormone was separated from free by the addition of 1 ml dextran-coated charcoal (dextran: Norit A. 2% w/v Serva, Feinbiochemica, Heidelberg, FRG). Serial dilution curves for the samples were parallel with the standard curve. The intra-assay and inter-assay variation coefficients were 6.5 and 8.1% respectively.

2.3. Binding assay for membrane preparations

Synaptosomal membranes from the parietal cortex and hippocampus were prepared as described by Reubi et al. (1981). Parietal cortex and hippocampus were homogenized in 10 mM HEPES-KOH pH 7.6 (10 w/v) and centrifuged as before. The resultant pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.5). Samples were stored at −70°C until assay.

Specific somatostatin binding was measured according to the modified method of Czernik and Petrack (Czernik and Petrack, 1983). Brain membranes (about 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}]somatostatin in the absence or in the presence of 0.01-10 nM unlabelled somatostatin. After 60 min incubation at 30 °C, the free radioligand was separated from the bound radioligand by centrifugation at 12000 $\times g$ (Beckman microcentrifuge) for 1.5 min and the resultant pellet was counted in a Beckman gamma counter. Non-specific binding, i.e., binding occurring in the presence of a high concentration (10^{-7} M) of unlabelled somatostatin, represented about 20% of the binding observed in 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}]somatostatin in the incubation medium after exposure to membranes was evaluated from the ability of the peptide to rebind to fresh membranes (Aguilera et al., 1982).

2.4. Statistical analysis

Statistical evaluation was performed using linear regression analysis and the parametric one-way analysis of variance. The significance of differences between experimental groups was evaluated by means of Newman-Keuls multiple comparison procedures. The criterion of statistical significance was $P < 0.05$. Scatchard analysis (Scatchard, 1949) of the stoichiometric binding data was done with the help of the LIGAND computer program (Munson and Rodbard, 1980).

2.5. Chemicals

Synthetic [Tyr^{11}]somatostatin and somatostatin tetradecapeptide were purchased from Universal Biologicals Ltd. (Cambridge, U.K.); bacitracin, mecamylamine hydrochloride, nicotine hydrogen tartrate and bovine serum albumin (fraction V) from Sigma (St. Louis, MO, U.S.A.); and carrier-free Na^{125}I (IMS 30, 100 mCi/ml) from the Radiochemical Centre (Amersham, U.K.). [Tyr^{11}] somatostatin was radioiodinated by the chloramine-T method (Greenwood et al., 1963). The tracer was purified on 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 radioactivity of tracer was about 350 Ci/g. The rabbit antibody used in the radioimmunoassay technique was purchased from the Radiochemical Centre (Amersham, U.K.). This antiserum was raised in rabbits against somatostatin-14 conjugated to bovine serum albumin and is specific for somatostatin, but since somatostatin-14 constitutes the C-terminal portions of both somatostatin-25 and somatostatin-28, the antiserum does not distinguish between these three forms. Somatostatin-14 is a predominant form in all neural tissues (Patel et al., 1981). The binding of somatostatin-14 to this antibody does not depend on an intact disulfide bond in the molecule, as breaking of the disulfide bond by reaction with 0.1% mercaptoethanol (boiling water bath, 5 min) did not change the immunoreactivity of the peptide. Cross-reactivity with other peptides was less than 0.5%. Cross-reaction with several somatostatin analogues demonstrated that neither the N-terminal glycine nor the C-terminal cysteine residue is required for antibody binding, suggesting that the antigen site is directed towards the central part of the molecule containing the tryptophan residue.

3. Results

Nicotine in a dose of 0.3 mg/kg given i.v. produced a significant increase in the somatostatin-like immunoreactivity levels in the parietal cortex at 4 min (table 1). This increase had already disappeared after 15 min. In the hippocampus, nicotine produced a transitory increase of somatostatin-like immunoreactivity levels only at 15 min following its injection (table 1). When the rats were pretreated with mecamylamine (5 mg/kg), the effects of nicotine on the somatostatin-like immunoreactivity levels in both brain areas were completely inhibited (table 1). Mecamylamine treatment alone did not influence the somatostatin-like immunoreactivity levels in the two brain areas analyzed (table 1).

Brain plasma membranes from the experimental groups bound ^{125}I-[Tyr^{11}]somatostatin in a time-dependent fashion; an apparent equilibrium

TABLE 1

Effect of nicotine and mecamylamine on somatostatin-like immunoreactivity concentration in the parietal cortex and hippocampus of the rat. For details on treatment see Material and methods. Determinations were made in duplicate for each experiment. The results are expressed as ng somatostatin/mg protein and as the means ± S.E.M. of five separate experiments. Statistical comparison versus saline: [a] $P < 0.01$.

Time (min)	Time (min)			
	4	15	30	60
Parietal cortex				
Saline	6.10 ± 0.30	5.44 ± 0.38	5.60 ± 0.54	6.02 ± 0.27
Nicotine	11.44 ± 1.13 [a]	6.19 ± 0.13	6.20 ± 0.96	6.21 ± 0.34
Mecamylamine + nicotine	5.41 ± 0.35	4.98 ± 0.34	5.19 ± 0.48	5.37 ± 0.24
Mecamylamine + saline	5.40 ± 0.28	5.48 ± 0.21	5.50 ± 0.86	5.51 ± 0.30
Hippocampus				
Saline	7.00 ± 0.22	5.67 ± 0.26	6.48 ± 0.60	5.98 ± 0.46
Nicotine	6.33 ± 0.77	7.61 ± 0.54 [a]	6.79 ± 0.45	6.06 ± 0.37
Mecamylamine + nicotine	6.21 ± 0.20	5.10 ± 0.43	6.23 ± 0.58	5.75 ± 0.44
Mecamylamine + saline	5.61 ± 0.68	5.81 ± 0.72	6.53 ± 0.42	5.83 ± 0.36

was observed between 50-180 min at 30 °C (data not shown). All subsequent binding experiments were therefore conducted at 30 °C for 60 min. Peptide degradation was determined (Aguilera et al., 1982) to rule out the possibility of different somatostatin degrading activities in the membrane preparations which might have affected the interpretation of the results. Membranes from both brain areas showed a similar peptide degradation capacity and the values varied by no more than 10% in all the experimental groups.

Increasing concentrations of unlabelled somatostatin inhibited competitively the specific binding of ^{125}I-[Tyr^{11}]somatostatin to brain membranes in the preparations from all the experimental groups (figs. 1 and 2, left panels). Specific binding in the parietal cortex peaked at 15 min after nicotine administration while that in the hippocampus peaked at 30 min after administration.

Because the observed differences in somatostatin binding could have been caused by changes in either the affinity or the capacity of somatostatin binding sites, the stoichiometric data were inter-

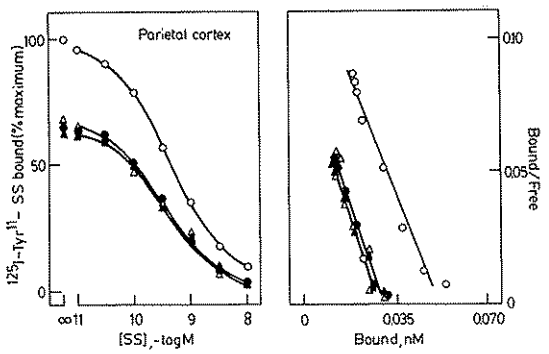

Fig. 1. Left panel: Competitive inhibition of specific ^{125}I-[Tyr^{11}]somatostatin (^{125}I-[Tyr^{11}]SS, 250 pM) binding to membranes of the parietal cortex by unlabelled somatostatin. The membranes (0.15 mg protein/ml) were incubated for 60 min at 30 °C in the presence of 250 pM ^{125}I-[Tyr^{11}]SS and increasing concentrations of native peptide. Points correspond to values for animals in the control (saline) (●), nicotne-treated (○), mecamylamine- and nicotine-treated (△), and mecamylamine- and saline-treated (▲) groups. The animals were killed 15 min after the nicotine injection. Each point is the mean of five replicate experiments. The S.E.M. are not represented but were always well within 10% of the mean values. For the sake of clarity, the other intervals studied are not represented, but the corresponding equilibrium binding parameters are included in table 2. Right panel: Scatchard analysis of the same data.

TABLE 2

Effects of mecamylamine and nicotine on equilibrium parameters of somatostatin binding to parietal cortex membranes. Binding parameters were calculated from Scatchard plots by linear regression. Units for K_d are nM and units for B_{max} are fM of somatostatin bound per mg of protein. The results are presented as the means ± S.E.M. of (five) separate experiments. [a] $P < 0.05$ versus saline.

	Time (min)			
	4	15	30	60
Saline				
K_d	0.33 ± 0.05	0.33 ± 0.06	0.33 ± 0.12	0.41 ± 0.07
B_{max}	286 ± 34	222 ± 32	289 ± 38	312 ± 26
Nicotine				
K_d	0.38 ± 0.05	0.35 ± 0.05	0.33 ± 0.06	0.43 ± 0.10
B_{max}	283 ± 29	365 ± 31 [a]	267 ± 24	292 ± 35
Mecamylamine + nicotine				
K_d	0.37 ± 0.06	0.38 ± 0.09	0.39 ± 0.14	0.40 ± 0.08
B_{max}	261 ± 27	203 ± 20	265 ± 33	285 ± 24
Mecamylamine + saline				
K_d	0.36 ± 0.05	0.34 ± 0.08	0.34 ± 0.07	0.42 ± 0.09
B_{max}	265 ± 28	208 ± 27	251 ± 22	274 ± 33

preted by the method of Scatchard (Scatchard, 1949). This analysis revealed an increase in the number of specific somatostatin receptors in the parietal cortex and hippocampus at 15 and 30 min after nicotine administration, respectively (tables 2 and 3). However, no differences could be established with respect to the affinity values. Pretreatment with mecamylamine completely blocked the nicotine-induced changes in the number of somatostatin receptors. Mecamylamine itself did not influence the somatostatin receptors.

4. Discussion

The present study showed that the injection of a single nicotine dose of 0.3 mg/kg was followed by an increase in the concentrations of soma-

TABLE 3

Effects of mecamylamine and nicotine on equilibrium parameters of somatostatin binding to hippocampus membranes. Binding parameters were calculated from Scatchard plots by linear regression. Units for K_d are nM and units for B_{max} are fM of somatostatin bound per mg of protein. The results are presented as the means ± S.E.M. of five separate experiments. [a] $P < 0.05$ versus saline.

	Time (min)			
	4	15	30	60
Saline				
K_d	0.34 ± 0.06	0.38 ± 0.09	0.42 ± 0.03	0.40 ± 0.05
B_{max}	249 ± 39	229 ± 45	284 ± 15	239 ± 26
Nicotine				
K_d	0.38 ± 0.05	0.39 ± 0.06	0.43 ± 0.05	0.42 ± 0.06
B_{max}	300 ± 53	233 ± 11	408 ± 82 [a]	222 ± 22
Mecamylamine + nicotine				
K_d	0.36 ± 0.05	0.38 ± 0.08	0.37 ± 0.05	0.38 ± 0.05
B_{max}	277 ± 49	215 ± 14	246 ± 32	205 ± 21
Mecamylamine + saline				
K_d	0.32 ± 0.05	0.37 ± 0.09	0.34 ± 0.04	0.37 ± 0.04
B_{max}	285 ± 38	223 ± 32	275 ± 16	232 ± 25

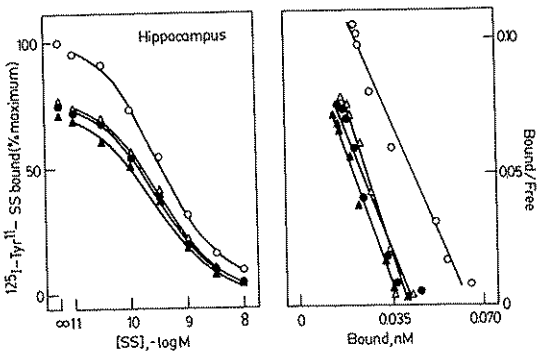

Fig. 2. Left panel: Competitive inhibition of specific ^{125}I-[Tyr^{11}]somatostatin (^{125}I-[Tyr^{11}]SS, 250 pM) binding to membranes of the hippocampus by unlabelled somatostatin. The membranes (0.15 mg protein/ml) were incubated for 60 min at 30 °C in the presence of 250 pM ^{125}I-[Tyr^{11}]SS and increasing concentrations of native peptide. The points correspond to values for animals in the control (saline) (●), nicotine-treated (○), mecamylamine- and nicotine-treated (△) and mecamylamine- and saline-treated (▲) groups. The animals were killed 15 min after the nicotine injection. Each point is the mean of five replicate experiments. The S.E.M. are not represented but were always well within 10% of the mean values. For the sake of clarity, the other intervals studied are not represented, but the corresponding equilibrium binding parameters are included in table 2. Right panel: Scatchard analysis of the same data.

tostatin-like immunoreactivity in the parietal cortex and hippocampus that preceded an increase in the total number of somatostatin receptors in both brain areas. Pretreatment with mecamylamine prevented the nicotine-induced changes in somatostatin level and binding while mecamylamine itself had no observable effect on the somatostatinergic system.

The somatostatin-like immunoreactivity levels in both brain areas as well as the binding parameters of brain somatostatin receptors in the control rats were similar to those previously reported by others (Pitkanen et al., 1986). It should be mentioned that scatchard analysis demonstrated the existence of only one type of somatostatin receptor. This finding agrees with some reports (Czernik and Petrack, 1983) but not with other previously reported data (Reubi, 1984). It is conceivable that use of small somatostatin analogs (Reubi, 1984) might explain this inconsistency.

The mechanism by which nicotine causes an increase in somatostatin concentration and binding is unknown. However, nicotine-like acetylcholine receptors seem to mediate the action of nicotine, since the changes induced by nicotine in the somatostatinergic system were prevented by pretreatment with the nicotine-like acetylcholine blocking agent, mecamylamine. Furthermore, mecamylamine itself had no demonstrable effect on these parameters. These results do not explain the acute action of nicotine to increase ^{125}I-[Tyr^{11}]somatostatin binding and inhibit somatostatin degradation, resulting in an increase in the concentration of ^{125}I-[Tyr^{11}]somatostatin during the binding reaction. A very attractive hypothesis is that nicotine causes a pool of presynthesized somatostatin receptors to become available for ligand binding. If this hypothesis is correct, the acute action of nicotine must involve a pool of receptors either in the plasma membrane or within the cell itself, which would not be used to replenish receptors lost through degradation. In this regard, it has been shown that specific somatostatin receptors exist in secretion vesicles isolates from both pituitary and pancreatic islets (Draznin et al., 1985), and it is likely that the vesicles may be involved in the intracellular (or internalized) part of the receptor somatostatin receptor system between the Golgi complex and cell membranes (Morel et al., 1986). Sequestered membrane receptors have been described by Cuatrecasas (Cuatrecasas, 1971) who reported that phospholipase digestion of isolated fat and liver cells or membranes prepared from these cells leads to a 3- to 6-fold increase in the number of insulin receptors. Nicotine acutely affects polyphosphoinositide synthesis and/or metabolism in the rat brain microsomal fraction (Hitzemann et al., 1978), which is known to be enriched in putative nicotinic acetylcholine receptors (De Blas and Mahler, 1976). These effects of nicotine may represent an overall change in membrane conformation (Aloia et al., 1988) and this change may well result in the exposure of receptors sequestered within the plasma membrane. In addition, it is possible that the acute effects of nicotine to enhance ^{125}I-[Tyr^{11}]somatostatin binding are also related to an effect of nicotine on the internalization or processing steps for the somatostatin receptor.

The rapid changes in somatostatin concentration and binding observed after nicotine injection

are in keeping with the modifications in this neuropeptide which were provoked by TRH (Schonbrunn and Tashjian, 1980) or cysteamine (Srikant and Patel, 1984). The rapid changes observed in cortical and hippocampal somatostatin receptors after nicotine administration are of the same order of magnitude as those reported for other central neurotransmitter receptors altered by various pharmacological manipulations (Burt et al., 1977).

The fact that the changes in the somatostatinergic system in the parietal cortex occur before those in the hippocampus may be a result of regional differences in nicotinic acetylcholine receptors since the specific binding of nicotine to synaptosomes is higher in the cerebral cortex (Clarke et al., 1984). Whether the increase of somatostatin-like immunoreactivity levels in both brain areas of the nicotine-treated rats reflects increased biosynthesis, decreased release, or diminished degradation cannot be answered at this time.

Andersson et al. (1986) did not demonstrate any effect of nicotine on somatostatin concentration in the brain. The discrepancies may be explained in a number of ways: (a) these authors assayed discrete hypothalamic and preoptic nucleic areas, whereas we studied the parietal cortex and hippocampus, and (b) the studies involved different doses and time intervals after nicotine administration. Other authors have shown that acute nicotine administration increases brain cholecystokinin and luteinizing hormone releasing hormone-like immunoreactivity (Andersson et al., 1986).

The consequences of somatostatin receptor modulation on biological responsiveness have not been reported to date. Since the somatostatinergic and cholinergic nicotinic systems are physiological regulators of locomotor activity, heterologous receptor regulation may have important consequences on the control exercised by these two neurotransmitter systems in the intact animal.

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