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Acute effect of nicotine on the striatal dopaminergic system in the rat

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The acute effects of nicotine (1·5 mg kg^{-1} day^{-1}) on the striatal dopaminergic system have been examined in the rat. Twenty-four hours after nicotine treatment, spontaneous locomotor activity in response to apomorphine or (+)-amphetamine and the binding of [^{3}H]spiperone to striatal particulates were determined. Pretreatment of nicotine did not alter the characteristics of [^{3}H]spiperone binding to striatal dopamine receptors. The nicotine treatment did not affect the apomorphine-induced locomotor activity, however, the (+)-amphetamine-stimulated locomotor activity was attenuated. These results suggest that nicotine acutely alters the presynaptic dopaminergic activity without significantly affecting the postsynaptic dopaminergic function.

Some of the central effects of nicotine are suggested to be mediated via the nicotinic receptors located at the corpus striatum (Westfall 1974; Giorguieff-Chesselet et al 1979; Morley 1981; Lichtensteiger et al 1982). Acute administration of nicotine to rodents excites striatal dopaminergic neurons and increases dopamine (DA) turnover in the striatum (Andersson et al 1981), whereas repeated administration of nicotine decreases DA turnover (Fuxe et al 1977; Naquira et al 1978). However, in most of these studies, large doses of nicotine were administered to animals so that large amounts of nicotine would be rapidly accumulated in the central nervous system.

In the present study, nicotine was administered to rats by the subcutaneous implantation of osmotic mini-pumps. We believe this method of continuous nicotine administration will more closely resemble the amount of nicotine intake as with tobacco smoking in humans. The effects of nicotine administration on [^{3}H]spiperone binding in the striatum and on locomotor response of animals to apomorphine and (+)-amphetamine were examined.

Methods

Animals. Male Sprague-Dawley rats (Sasco, Omaha), 200–240 g, were anaesthetized with Equathesin (3 ml kg^{-1}) and were implanted with an Alzer osmotic mini-pump model 2001 containing either physiological saline or nicotine (1·5 mg kg^{-1} day^{-1}). This dosage of nicotine was chosen in an attempt to approximate the dosage pattern of an individual who smokes two packets of cigarettes a day (Russell et al 1975; Turner et al 1977; Hill et al 1983). Before implantation, each pump was primed for 6 h at 37 °C in physiological saline solution. Animals were maintained on a normal 12 h light/12 h dark cycle at 21 ± 1 °C. Behavioural and biochemical studies were conducted 24 h after the pump implantation.

Biochemical assay of DA receptors. Striata from the nicotine-treated or saline-treated control animals were homogenized in 4 ml of 50 mM Tris-HCl buffer (pH 7·4m, 4 °C) containing 0·1% absorbic acid, 0·01 mM pargyline, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. The homogenate was centrifuged at 27 000 g for 20 min at 4 °C. The pellet was washed twice more with the same buffer in the same manner. [^{3}H]Spiperone binding assay was performed as previously described (Lau et al 1984) using (+)-butaclamol (1 μM) for determining the non-specific binding sites and mianserin (1 μM) for masking [^{3}H]spiperone binding to 5-hydroxytryptamine receptors. Protein assay was by the Lowry method. The binding parameters (K_D and B_{max}) of the high affinity [^{3}H]spiperone binding sites were determined by using the non-linear least-squares regression LIGAND analysis (Munson & Rodbard 1980).

Assessment of spontaneous locomotor activity. Locomotor activity of rats was measured with a motor activity cage equipped with photocells and an automatic counter (Lehigh Valley Electronics, Allentown, NJ). Each animal was allowed to adapt to the cage for 1 h before the administration of either saline (s.c.), apomorphine (0·7 mg kg^{-1} s.c.) or (+)-amphetamine (1 mg kg^{-1} s.c.). After the drug administration, the locomotor activity of each animal was determined at 10 min intervals for 90 min.

Results and discussion

In this study animals were implanted with osmotic minipumps containing either saline or nicotine. Nicotine was delivered to the animal at a constant rate of 1·5 mg kg^{-1} day^{-1}. One day of treatment with nicotine in the rat did not affect the postsynaptic DA receptor function in the striatum. This observation was demonstrated by the lack of changes in (i) striatal [^{3}H]spiperone binding characteristics (B_{max} and K_D) (Table 1) and (ii) the locomotor behavioural response to a direct DA receptor agonist, apomorphine (Fig. 1), when saline-treated and nicotine-treated animals were compared. Interestingly, the locomotor response to an indirect DAergic agent (+)-amphetamine was significantly diminished in nicotine-treated rats (Fig. 2).

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Table 1. Effect of nicotine treatment on striatal DA receptors.

Treatment	[^{3}H]spiperone binding K_D (nM)	B_{max} (fmol mg^{-1})
Saline	0·15 ± 0·02	203·8 ± 11·6
Nicotine (1·5 mg kg^{-1} day^{-1})	0·16 ± 0·015	195·5 ± 33·3

Values are presented as mean ± s.e.m. of 4–6 independent experiments. Animal treatment and binding assay are described in the Method section. The values for saline-treated and nicotine-treated animals are not statistically different ($P > 0·05$, Student's t-test).

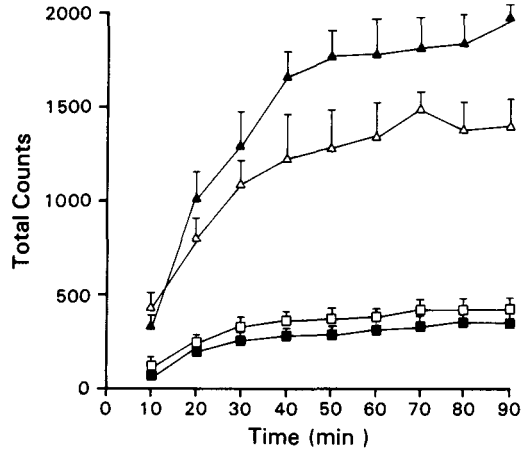

FIG. 1. Rats were implanted with osmotic mini-pumps containing either physiological saline or nicotine (1·5 mg kg^{-1} day^{-1}). Twenty-four hours later, each animal was allowed to adapt to a motor activity cage for 1 h. Saline or apomorphine (0·7 mg kg^{-1} s.c.) was given to all animals. Locomotor activities were monitored for 90 min. Data are presented as mean ± s.e.m. of 5 animals. □—□ Animals were implanted with saline pumps followed by saline injection, ■—■ animals were implanted with nicotine pumps followed by saline injection, △—△ animals were implanted with saline pumps followed by apomorphine injection, ▲—▲ animals were implanted with nicotine pumps followed by apomorphine injection.

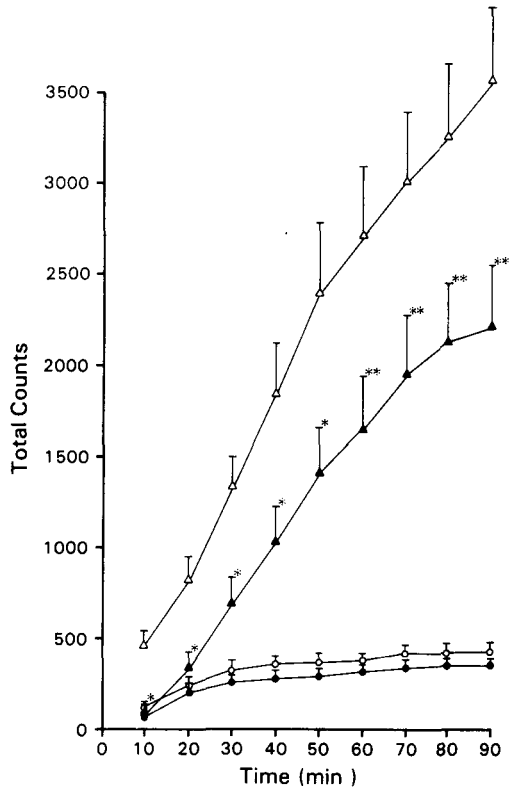

FIG. 2. Rats were implanted with osmotic mini-pumps containing either physiological saline or nicotine (1·5 mg kg^{-1} day^{-1}). Twenty-four hours later, each animal was allowed to adapt to a motor activity cage for 1 h. Saline or (+)-amphetamine (1 mg kg^{-1} s.c.) was given. Locomotor activities were monitored for 90 min. Data are presented as the mean ± s.e.m. of 5 animals. ○—○ Animals were implanted with saline pumps followed by saline injection, ●—● animals were implanted with nicotine pumps followed by saline, △—△ animals were implanted with saline pumps followed by (+)-amphetamine injection, ▲—▲ animals were implanted with nicotine pumps followed by amphetamine injection. $^*P < 0·01$, $^{**}P < 0·05$ (Student's t-test).

One of the possible mechanisms for the above observation is that nicotine pretreatment in the rat could reduce the effect of (+)-amphetamine by preventing its uptake into presynaptic neurons. However, this mechanism is not supported by the results of our previous study which showed that in our system an active neuronal uptake process of (+)-amphetamine has not been identified (Fung & Uretsky 1980).

An alternative explanation for our observation is that nicotine pretreatment could interfere with the synthesis and/or release of DA by (+)-amphetamine (Von Voigtlander & Moore 1973; Fung & Uretsky 1982). The activation of striatal nicotine receptors has been shown to release DA in-vitro and in-vivo (Westfall 1974; Giorguieff-Chesselet et al 1979). Furthermore, continuous administration of nicotine has induced receptor desensitization (Fuxe et al 1977) or has caused a depolarization block of presynaptic neurons (Misgold et al 1980). Either or both of these phenomena could reduce the ability of (+)-amphetamine to release DA and thereby contribute to the attenuation of amphetamine-induced locomotor activity in the rat.

In summary, our results showed that one day pretreatment of rats with nicotine significantly reduced the action of (+)-amphetamine on locomotor responses, whereas the action of apomorphine on locomotor responses and the striatal [^{3}H]spiperone binding characteristics were not affected. These results suggest that

nicotine acts on the presynaptic nerve terminals probably by blocking the amphetamine-stimulated release of DA.

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The effect of sodium deoxycholate given by gavage with heparin on the histology of the intestinal mucosa of the rat

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To gain direct insight into the mechanism of sodium deoxycholate (DOC)-induced enhancement of gastroenteral heparin absorption in rats, we performed light and electron microscopic examination of the mucosa of the small intestine of animals treated orally with DOC, heparin or DOC plus heparin. The sole morphological change observed after DOC and DOC plus heparin administration was a marked reduction in the length and distribution of glycocalyx filaments on the microvilli of epithelial cells. The morphological picture had reverted to normal after 24 h, when the promotion of enteral heparin absorption by DOC is greatly reduced. Thus, we suggest that DOC may promote the enteral absorption of heparin in rats by affecting some as yet unidentified barrier mechanism requiring glycocalyx integrity.

Experimental evidence shows that certain drugs, such as EDTA, salicylic acid derivatives, ionic and non-ionic surfactants, bile acids and others, allow heparin to be absorbed (Windsor & Cronheim 1961; Engel & Riggi 1969; Davis et al 1970; Gibaldi & Feldman 1970; Nishihata et al 1981b; Ziv et al 1983; Guarini & Ferrari 1984, 1985) by the gastrointestinal tract and so produce its typical pharmacological effects, i.e. inhibition of blood coagulation and lipaemic plasma clearing activity (PC). However, the mechanism(s) of action has not, as yet, been identified. Some researchers attribute the effect to morphological disruption of the gastrointestinal mucosa (Davis et al 1970; Whitmore et al 1979; Nishihata et al 1981b), others to more subtle alterations of the biochemical mechanisms responsible for maintaining the impermeability of the mucosa to heparin (Guarini & Ferrari 1985).

Our previous work has shown that the facilitation of heparin absorption by the rat gastrointestinal tract afforded by bile acids and certain non-ionic surfactants of the polyoxyethylene-series is strictly governed by their molecular structure (Guarini & Ferrari 1984). Accordingly, we tend to support the hypothesis of a selective functional interference rather than that of a structural disruption.

To gain direct insight into the mechanism by which sodium deoxycholate (DOC) enhances heparin absorption, we examined the morphological aspect of the intestinal mucosa of rats receiving DOC, heparin, DOC plus heparin, or water, inasmuch as the DOC effect may be of clinical interest. The present paper presents the results of our investigation and shows that the activity of DOC might be attributed to its influence on glycocalyx filaments of intestinal epithelial cells.

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