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Effects of chronic nicotine pretreatment on (+)-amphetamine and nicotine-induced synthesis and release of [^{3}H]dopamine from [^{3}H]tyrosine in rat nucleus accumbens

YIU K. FUNG, *University of Nebraska Medical Center, Department of Oral Biology, College of Dentistry, 40th and Holdrege, Lincoln, NE 68583-0740, USA*

Abstract—The effects of chronic (14 day) administration of nicotine (1·5 mg kg^{-1} day^{-1}) on the rat nucleus accumbens have been examined. Pretreatment of animals with nicotine increased the endogenous level of dopamine. The ability of (+)-amphetamine to stimulate formation and release of [^{3}H]dopamine from [^{3}H]tyrosine was greatly potentiated in tissue slices from the nucleus accumbens of rats pretreated with nicotine. Furthermore, nicotine was effective in stimulating the formation and release of from [^{3}H]dopamine from [^{3}H]tyrosine in tissue slices from chronic nicotine-treated animals.

Some of the central nervous system effects of nicotine are suggested to be mediated via the nicotinic receptors located at the nigrostriatal (striatum) and mesolimbic (nucleus accumbens) regions (Giorguieff-Chesselet et al 1979; Clarke & Kumar 1983; Fung & Lau 1986). Autoradiographic and receptor binding studies have confirmed the presence of nicotinic receptors located at nigrostriatal and mesolimbic dopaminergic (DAergic) neurons (Clark & Pert 1985; Marks et al 1986; Kellar et al 1987; Martino-Barrows & Kellar 1987). Activation of nicotinic receptors is effective in stimulating the release of newly taken up [^{3}H]dopamine (DA) in the striatum and nucleus accumbens of rodents (Giorguieff-Chesselet et al 1979; Balfour 1982; Westfall et al 1983; Rowell et al 1987). Furthermore, this stimulatory effect of nicotine on DA release is greater at the nucleus accumbens than the caudate-putamen region (Imperato et al 1986). However, no information is available regarding the ability of nicotine to stimulate formation and release of DA in the nucleus accumbens.

This study was undertaken to investigate the effects of chronic (14 day) nicotine administration on the ability of tissue slices from the nucleus accumbens to synthesize and release [^{3}H]DA from [^{3}H]tyrosine. Nicotine was administered to rats by the subcutaneous implantation of osmotic minipumps containing nicotine (1·5 mg kg^{-1} day^{-1}) to each animal. This method of nicotine administration resembles the amount of nicotine intake via cigarette smoking in humans (Russell et al 1975; Benowitz 1986; Murrin et al 1987). The effect of continuous nicotine administration on the levels of DA in the nucleus accumbens of rats was also examined.

Methods

Animals. Male Sprague-Dawley rats (Sasco, Omaha, NE), 200–230 g were used. They were housed in groups of 3 per cage in a temperature (23 ± 1°C) and light controlled room (12/12 h light/dark cycle; lights on at 0700 h) and allowed free access to food (Purina Laboratory Chow) and water.

Animals were anaesthetized with a mixture of halothane and oxygen and were implanted subcutaneously posterior to the shoulder with an Alzet osmotic minipump (model 2002). The incision was closed with wound clips and covered with an antibiotic cream and 5% lidocaine ointment. The pumps were filled with either sterile physiological saline or nicotine (1·5 mg kg^{-1} day^{-1}). The dose of nicotine administered was calculated as the free base using nicotine tartrate dissolved in physiological saline solution. This dose of nicotine was chosen since it has been shown to produce a plasma level of nicotine in rats (20 ± 2 ng mL^{-1}) similar to the plasma level of nicotine found in humans smoking one pack of cigarettes per day (Russell et al 1975; Hill et al 1983; Murrin et al 1987). Before implantation, each pump was primed for 6 h at 37°C in physiological saline solution. Biochemical studies were conducted 14 days after pump implantation.

Determination of [^{3}H]DA synthesis and release. This study was conducted according to the method as described by Fung & Uretsky (1980). Rats were killed and their nucleus accumbens were dissected, weighed and sliced in 0·25 mm × 0·25 mm square sections using a McIlwain tissue chopper. The tissue slices were dispersed in ice cold normal medium containing (mM) NaCl 118·4, KCl 4·73, KH_2PO_4 1·2, $MgSO_4.7H_2O$ 1·18, $CaCl_2.2H_2O$ 1·25 and HEPES with dextrose 2 mg mL^{-1}. The solution was bubbled with 95% O_2 and 5% CO_2 for 30 min and adjusted to pH 7·2 with 1 M NaOH. The slices were then centrifuged at 500 *g* for 5 min. The supernatant fluid containing amino acids released from the preparation of the slices was discarded (Balcar & Johnston 1975). The slices were then resuspended in a volume of cold normal medium such that 0·25 mL samples of this suspension would contain 25 mg of the tissue slices. (+)-Amphetamine (10^{-6} or 10^{-5} M) or L-nicotine (10^{-6} or 10^{-5} M) was added and the final volume of the suspension was 3 mL. The slices were incubated under an atmosphere of 95% O_2 and 5% CO_2 in a shaking water bath at 37°C for 8 min. [^{3}H]Tyrosine was then added to a final concentration of 10 μM. The incubation was continued for an additional 20 min and the reaction was stopped by cooling the flasks on ice. Replicate slices that were kept on ice throughout the experiment served as blanks. Tissues were separated from the medium by centrifugation and both fractions were assayed for [^{3}H]DA. [^{3}H]DA was separated from [^{3}H]tyrosine by alumina absorption and ion exchange (Amberlite CG 50) chromatographics. The radioactivity present was determined by liquid scintillation counting. The total [^{3}H]DA formation was obtained by adding the activity from both tissue and medium fractions. The release of newly synthesized [^{3}H]DA from the tissue was calculated by dividing the amount of [^{3}H]DA in the medium by the total amount of [^{3}H]DA formed.

Determination of DA levels. Nucleus accumbens from nicotine-treated or saline-treated rats were killed and suspended in 0·2 mL of 0·2N perchloric acid. The sample was sonicated and centrifuged at 11,000 *g* for 5 min at 4°C. The supernatant was filtered through a nylon syringe filter unit (0·45 micron). An aliquot of the filtrate was injected into a high performance liquid chromatography (HPLC) (Waters, Milford, MA) in a mobile phase consisting of 100 mM sodium acetate, 20 mM citric acid, 100 mg L^{-1} sodium octyl sulfate (Eastman Organic Chemicals, Rochester, NY). 50 mg L^{-1} EDTA and 4% (v/v) methanol, pH 4·1. The sample was chromatographed by uBondapak C_{18} reversed phase column (3·9 × 150 mm, Waters, Milford, MA) at a constant flow rate of 2 mL min^{-1}. The concentration of DA in each sample was determined by electrochemical detection at a potential of 0·6 V.

Drugs. [^{3}H]tyrosine (55 Ci mmol^{-1}) was purchased from Amersham (Chicago, IL). Nicotine tartrate and (+)-amphetamine sulphate were purchased from Sigma Chemical Co. (St. Louis,

MO). Alzet osmotic minipumps (model 2002) were purchased from Alza Corp. (Palo Alto, CA).

Statistical analysis. All statistical comparisons were made using analysis of variance followed by Newman-Keuls for comparison between and within groups and the two-tailed student's *t*-test for independent means.

Results and discussion

Rats which were pretreated with nicotine for 14 days showed a significant increase in the endogenous levels of DA in the nucleus accumbens compared with saline-treated controls (15·4 ± 0·2 vs 11·6 ± 1·1 $\mu g\ g^{-1}$ tissue, n = 5–6 $P < 0{\cdot}01$). Analysis of variance (ANOVA) revealed a significant drug treatment effect on dopamine formation F (9, 50) = 78·4, and dopamine release F (9, 50) = 61·5, $P < 0{\cdot}01$. In a dose-dependent manner, (+)-amphetamine stimulated the formation and release of [^{3}H]DA from [^{3}H]tyrosine in tissue slices from rats which were pretreated with saline (Table 1). Furthermore, this stimulatory effect of (+)-amphetamine was greatly potentiated in animals which were pretreated with nicotine for 14 days. Nicotine alone had no stimulatory effect on DA formation and release from tissue slices of saline-treated animals. Surprisingly, nicotine was found to be effective in stimulating the formation and release of [^{3}H]DA in rats which were pretreated with nicotine for 14 days.

Table 1. Effect of chronic nicotine (14 days) pretreatment on nicotine and (+)-amphetamine-stimulated formation and release of [^{3}H]DA from [^{3}H]tyrosine rat nucleus accumbens.

Pretreatment	Drug added to medium	[^{3}H]DA formed (nmol g^{-1} 20 min)	[^{3}H]DA released (%)
Saline	saline	1·3 ± 0·1	32 ± 1·5
Nicotine	saline	1·5 ± 0·2	37 ± 0·8
Saline	Amph. (10^{-6}M)	3·4 ± 0·3	54 ± 3·0
Nicotine	Amph. (10^{-6}M)	4·3 ± 0·1*	69 ± 0·7*
Saline	Amph. (10^{-5}M)	3·9 ± 0·2	70 ± 1·2
Nicotine	Amph. (10^{-5}M)	5·8 ± 0·2*	80 ± 0·2*
Saline	Nicotine (10^{-6}M)	1·2 ± 0·1	31 ± 2·8
Nicotine	Nicotine (10^{-6}M)	2·4 ± 0·1*	48 ± 0·4*
Saline	Nicotine (10^{-5}M)	1·4 ± 0·2	48 ± 4·5
Nicotine	Nicotine (10^{-5}M)	2·6 ± 0·1*	50 ± 0·8

Tissue slices (25 mg) from nucleus accumbens of saline or nicotine-treated (1·5 mg kg^{-1} day^{-1}) animals were incubated in normal medium containing saline, (+)-amphetamine (10^{-6} or 10^{-5}M) or nicotine (10^{-6} or 10^{-5}M). The slices were preincubated for 8 min, [^{3}H]tyrosine was added and the reaction was continued for 20 min, at which time [^{3}H] DA formation and release were determined. Data are mean ± s.e.m. of 5 determinations.

* Significantly different from saline-pretreated group ($P < 0{\cdot}01$, Newman-Keuls test).

In this study, nicotine was delivered at a constant rate of 1·5 mg kg^{-1} day^{-1} to rats via implantation of osmotic minipumps. This method of nicotine administration will circumvent any instantaneous burst of nicotine to the brain as in the case of systemic injection (Murrin et al 1987). Consequently, changes in neurochemistry in the brain with continuous administration of nicotine may provide important information regarding the effects of nicotine on the nucleus accumbens.

This study demonstrated a stimulatory effect of nicotine on both formation and release of [^{3}H]DA in chronic nicotine-treated rats and a potentiation of the biochemical effect of (+)-amphetamine. This biochemical effect of nicotine on the rat nucleus accumbens may also account for an increase in (+)-amphetamine-stimulated locomotor activity in these chronic nicotine-treated animals (unpublished data).

The mechanisms by which nicotine may act to enhance the effect of (+)-amphetamine and to elicit an effect of its own in stimulating the formation and release of DA in the nicotine-pretreated animals remain unclear. Since continuous administration of nicotine increases the endogenous level of DA, it is possible that the activity of tyrosine hydroxylase, the rate-limiting enzyme in the formation of DA is increased upon nicotine administration. The (+)-amphetamine-stimulated formation and release of [^{3}H]DA is a calcium-dependent process (Schwarz et al 1980; Fung & Uretsky 1982). Thus, it is possible that chronic administration of nicotine may enhance the influx of extracellular calcium leading to an enhancement of the effect of (+)-amphetamine. The biochemical effects of nicotine on the nucleus accumbens appear to be rather complex and warrant further investigation.

In our previous studies, we have found that pretreatment of rats with nicotine for 14 days via the osmotic minipumps did not potentiate the stimulatory effect of (+)-amphetamine on [^{3}H]DA synthesis and release in tissue slices from rat striatum (unpublished data). Therefore, in comparison to the present study, nicotine appears to exert a differential biochemical effect on these two different areas of DAergic systems. The effect of nicotine on the nucleus accumbens is important in light of the involvement of this brain region in the regulation of locomotor activity.

In conclusion, this study shows that continuous administration of nicotine enhanced the stimulatory effects of (+)-amphetamine on DA synthesis and release. Furthermore, nicotine is effective in stimulating the formation and release of DA from chronic nicotine-treated animals. The endogenous level of DA in the rat nucleus accumbens is increased upon continuous administration of nicotine.

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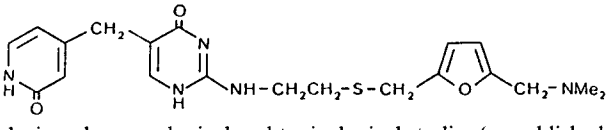
SK&F 93574, a histamine H_2-receptor antagonist, releases histamine in the dog

C. A. JONES, G. CONYBEARE, A. MORTENSEN, L. C. CATTO, C. K. ATTERWILL, *Smith Kline & French Research Ltd., The Frythe, Welwyn, Herts. AL6 9AR, UK*

Abstract—This study was designed to establish whether SK&F 93574 releases histamine in dogs. Three female beagle dogs each received single infusions (on separate days) of each of SK&F 93574 (2·5 mg kg^{-1}), polyvinylpyrrolidone (PVP, 20 mg kg^{-1}) and sterile saline. The treatments were given at 14 day intervals by rapid intravenous infusion at 0·5 mL kg^{-1} min^{-1} for 2 minutes. Dogs showed clinical signs of histamine release such as vasodilation, licking lips, head drooping and increased gut movement after treatment with the known histamine releaser PVP or the test compound SK&F 93574. These signs were of similar severity and duration for the two compounds. No such changes were observed when the dogs received vehicle alone. Treatment with PVP or SK&F 93574 also resulted in markedly elevated plasma histamine concentrations (> 10-fold increase over control). It is concluded that intravenous administration of SK&F 93574 to dogs is associated with histamine release.

SK&F 93574 (I) is a potent and long acting histamine H_2-receptor antagonist (Blakemore et al 1985). It was observed



during pharmacological and toxicological studies (unpublished data) that some dogs treated with SK&F 93574 collapsed and showed clinical signs which were consistent with histamine release.

A wide variety of compounds is known to release histamine (Paton 1957). Xenobiotics and endogenous substances containing one or more amine group are thought to act partly by displacing histamine from its binding sites and partly by facilitating exocytotic release of the storage granules (see Bowman & Rand 1980). It seems that the histamine H_1-receptor antagonists may act directly in this way. The effect of H_2-receptor antagonists (blockade of histamine-induced inhibition of histamine release) may be related to the existence of histamine H_2-receptors on basophils (Lichtenstein & Gillespie 1975). Large molecules such as compound 48/80, dextran and polyvinylpyrrolidone are also able to release histamine (see Paton 1957).

Correspondence to: C. A. Jones, Smith Kline & French Research Ltd., The Frythe, Welwyn, Herts. AL6 9AR, UK.

SK&F 93574 is a highly basic drug with several amine groups and consequently may have the potential for this effect. The present study was designed to establish whether, in beagle dogs, this was the case.

Methods

Animals. Purebred beagle dogs between 2 and 4 years old, 10–16 kg, were housed singly in pens and allowed access to SDS dog diet (Special Diet Services Ltd., Witham, Essex, UK) for 1 h a day and free access to drinking water.

Clinical procedure. The dogs each received single infusions of each of saline, SK&F 93574 (2·5 mg kg^{-1}) and positive control compound PVP (20 mg kg^{-1}). The treatments were given in a 'Latin square'-type order at 14 day intervals. Preliminary experiments (not reported) had shown that if a second dose of a histamine releaser was given 7 days after a single dose then a diminished response was seen after the second dose, but at a 14 day interval the responses were of the same magnitude.

The infusions were made with the dogs conscious but restrained in a Pavlov sling. Indwelling cannulae (21 gauge intravenous infusion set, Portex, Hythe, Kent) were placed into both cephalic veins 30 min before treatment. Drug was infused via the cannula in the right cephalic vein. Ten minutes after the infusion (or earlier if there were marked clinical changes) a blood sample was withdrawn from the cannula in the left cephalic vein. Clinical observations were recorded during and after each treatment.

To accustom the dogs to the procedure, and so minimize the effects of stress (which may alter histamine concentrations), sham experiments were carried out on two occasions (3 weeks and 2 weeks) before the first treatment day. On these occasions the cephalic veins were cannulated, 2 mL of saline was injected, and a blood sample was obtained.

Preparation of plasma. The blood samples were taken into heparinized syringes (final concentration approx 15 iu heparin per mL blood), immediately centrifuged (1600*g*, 4°C), and the plasma stored below −40°C until assayed for histamine. The blood was handled gently to minimize disruption of basophils.