Br. J. Pharmacol. (1992), **105**, 849–856

The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity

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1 The effects of acute and subchronic nicotine and (+)-amphetamine on the extracellular levels of dopamine and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in nucleus accumbens (NAc) have been studied in conscious, freely-moving rats by use of *in vivo* microdialysis.

2 In rats which had been habituated to the test apparatus for approximately 80 min, the acute subcutaneous (s.c.) administration of nicotine (0.1 or 0.4 mg kg^{-1}) caused a dose-dependent increase ($P<0.01$) in spontaneous activity and evoked significant increases ($P<0.05$) in the extracellular levels of DOPAC and HVA.

3 Measurements made 24 h after the last injection of nicotine showed that pretreatment with the higher doses tested (0.4 mg kg^{-1}) resulted in increased basal levels of dopamine ($P<0.01$) and decreased basal levels of DOPAC ($P<0.05$) in the NAc dialysates.

4 Pretreatment with nicotine (0.1 or 0.4 mg kg^{-1} daily for 5 days) enhanced the effects of the drug on spontaneous locomotor activity and enhanced the effects of the drug on extracellular levels of dopamine to the extent that the response became significant ($P<0.05$).

5 If a dopamine uptake inhibitor, nomifensine, was added to the Ringer solution used to dialyse the probe, the s.c. administration of both acute and subchronic nicotine (0.4 mg kg^{-1}) resulted in significant increases ($P<0.05$) in the dopamine concentration in the dialysate. Under these conditions, pretreatment with nicotine prior to the test day prolonged ($P<0.05$) the dopamine response to a challenge dose of nicotine.

6 Subcutaneous injections of (+)-amphetamine (0.2 or 0.5 mg kg^{-1}) evoked dose-dependent increases in both spontaneous activity and the concentration of dopamine in NAc dialysates. These responses were unaffected by 5 days pretreatment with the drug.

7 The results of this study support the conclusion that the enhanced locomotor response to nicotine observed in animals pretreated with the drug prior to the test day is associated with potentiation of its effects on dopamine secretion in the NAc.

Keywords: Nicotine; amphetamine; mesolimbic dopamine; sensitization; locomotor activity

Introduction

There is substantial evidence to suggest that nicotine plays a pivotal role in maintaining the tobacco smoking habit and that many habitual smokers become dependent upon the drug (Balfour, 1990). Nicotine self-administration has also been demonstrated in infra-human species, (Goldberg *et al.*, 1981; Cox *et al.*, 1984; Hutchinson & Emley, 1985; Corrigal & Coen, 1991) although it appears to be a relatively weak substrate when compared with drugs such as (+)-amphetamine and cocaine (Pickens *et al.*, 1978; Collins *et al.*, 1984). Wise & Bozarth (1987) have hypothesized that the neural substrate which underlies drug self-administration is the mesolimbic dopamine system which arises in the ventral tegmental area and terminates on target structures within the nucleus accumbens and olfactory tubercle (Fallon & Moore, 1978). There is convincing evidence for the involvement of this system as a mediator of the self-administration and locomotor stimulant (Creese & Iversen, 1975; Kelly *et al.*, 1975; Lyness *et al.*, 1979) properties of (+)-amphetamine. The lesion studies of Singer *et al.* (1982) indicated that this system also appears to mediate nicotine self-administration. This conclusion is supported by more recent studies which suggest that exposure to both nicotine (Andersson *et al.*, 1981a,b; Imperato *et al.*, 1986; Di Chiara & Imperato, 1988; Damsma *et al.*, 1989) and tobacco smoke (Fuxe *et al.*, 1989) can evoke a preferential increase in dopamine secretion in the mesolimbic structures. In addition, there is increasing evidence to suggest that the locomotor stimulant properties of nicotine may also be mediated by this system (Clarke *et al.*, 1988; Vale & Balfour, 1988; Reavill & Stolerman, 1990). Other studies have shown that if rats are pretreated with nicotine for some days before the test day, the locomotor response to the drug is significantly enhanced (Stolerman *et al.*, 1973; Clarke & Kumar, 1983; Vale & Balfour, 1989). The purpose of the present study, therefore, was to employ the technique of *in vivo* microdialysis, which allows the simultaneous monitoring of extracellular neurotransmitter levels and behaviour, to test the hypothesis that the enhanced locomotor responses observed in rats given repeated injections of nicotine are associated with an increased mesolimbic dopamine response to the drug.

Methods

Animals and drug pretreatments

Male Sprague Dawley rats, bred in the Animal Services Unit, Ninewells Hospital and Medical School, from stock purchased from Interforna Ltd and weighing 250–350 g at the start of the experiments, were used throughout. The animals, which had free access to food and water, were housed in pairs prior to surgery and then individually following surgery. In the experiments involving the systemic administration of saline or drug solution on the test day, the rats were pretreated with 5 consecutive daily subcutaneous injections of sterile isotonic saline, (+)-amphetamine sulphate or nicotine hydrogen tartrate. The drugs were dissolved in saline and, when necessary, the pH was adjusted to 7.4 by the

addition of a small quantity of NaOH. Drug doses were expressed in terms of the free base.

Implantation of the microdialysis probe

The animals were anaesthetised with Avertin (2,2,2, tribromoethanol: isoamyl alcohol: saline: ethanol in a ratio of 5:5:250:20) injected in a volume of 1 ml 100 g^{-1}, i.p. This procedure was carried out at least 3 h after the fifth daily injection. Dialysis loops (Figure 1) were implanted in the nucleus accumbens using the coordinates of 1.7 mm rostral and 1.5 mm lateral to bregma and 7.5 mm vertically from the surface of the brain according to Paxinos & Watson (1986). At the end of the experiment the position of the probes was routinely determined histologically from sections prepared at *postmortem* from tissue fixed in formalin.

Microdialysis and measurement of locomotor activity

Eighteen to twenty four hours following implantation of the dialysis probe, the animals were placed in an activity box (40 cm square × 25 cm high) in which locomotor activity was assessed by photocells mounted 3 cm high on adjacent sides of the box, each infrared beam crossing being recorded as an activity count (Vale & Balfour, 1989). At this time, the dialysis loop was also connected via a liquid swivel to a syringe pump containing a Ringer solution (NaCl 147 mM, KCl 4 mM and $CaCl_2$ 1.25 mM) which was perfused at a constant rate of 1.7 μl min^{-1}. Samples were collected in small plastic tubes mounted above the outlet tubing. Samples were removed at 20 min intervals for 80 min in order to establish stable baseline levels of dopamine and its metabolites. At this time, treatments were administered either subcutaneously or directly into the nucleus accumbens via the dialysis loop. In the experiments designed to investigate the effects of KCl on dopamine release into the probe, the Ringer solution was changed to one containing KCl (100 mM), the osmolarity of the solution being maintained by an appropriate adjustment in the concentration of NaCl. For some of the experiments, the Ringer solution contained the dopamine uptake inhibitor, nomifensine (10 μM).

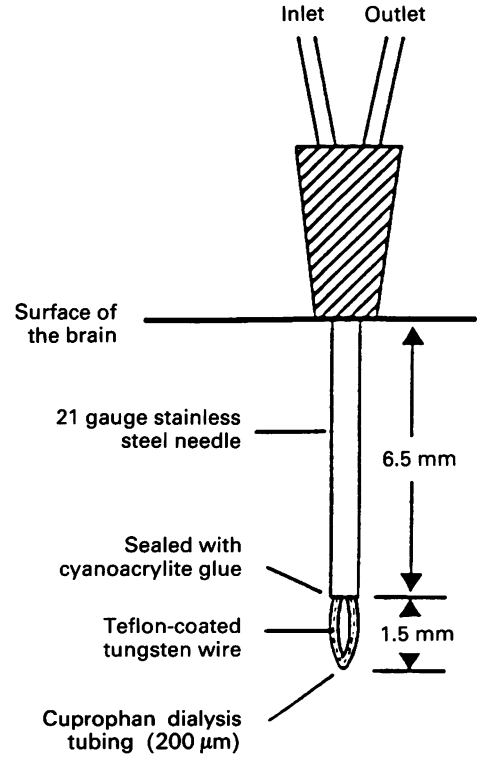

Figure 1 The dialysis loop was constructed, in house, by feeding plastic-coated tungsten wire (0.075 mm o.d., Clarke Electromedical) through the lumen of cuprophan dialysis tubing (0.200 mm o.d., Medicell international). A 6 cm length of this was cut, folded loosely and fed through a 21 gauge stainless steel needle which had been cut to the appropriate length. The loop was sealed into the needle housing with cyanoacrylite glue (Loctite). One cm lengths of 21 gauge steel tubing were pushed down over the projecting ends of the dialysis tubing and into the needle cap. Loctite glue was introduced into the top of the needle cap to seal the dialysis fibre within this tubing by capillary action and fix the inlet and outlet tubes to the needle cap, the purpose of which was to facilitate the attachment of the perfusion tubing and the sample tubing.

Tissue levels of dopamine, dihyroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured in ultrafiltered supernatants prepared from homogenates of NAc in 0.1 N perchloric acid (100 mg of tissue per ml) by high performance liquid chromatography (h.p.l.c.) with electrochemical detection. The concentrations of dopamine, DOPAC and HVA in brain dialysates were assayed by injecting the dialysate samples directly into the h.p.l.c. The chromatography was performed on an ODS2 column with a solvent solution system composed of disodium orthophophosphate (0.1 M), EDTA (0.1 mM), sodium octane sulphonic acid (0.06 mM) and methanol (12% v/v). The pH was adjusted to 3.9 with citric acid. Dopamine and its metabolites were detected with a Coulochem Electrochemical (ESA) detector. DOPAC and HVA were measured by oxidation using the first detector set at 1 = + 0.35V. Dopamine was measured by reduction of the product of dopamine oxidation at detector 1 using detector 2 set at − 0.2V. The limit of detection of dopamine was approximately 0.02 pmol. Samples of modified Ringer solutions (containing 0.1 mM EDTA or 10 μM nomifensine) and standard solutions containing nicotine or amphetamine at a concentration of 10 μM, concentrations well in excess of those which could be expected to occur in the nucleus accumbens following systemic injections of these compounds at the highest doses employed in this study, were tested on the h.p.l.c. system and found to cause no extra or interfering peaks.

Data analysis

In each experiment the mean concentrations of dopamine and its metabolites for the three samples collected before the test treatment were calculated and defined as 100%. The concentrations of dopamine and the metabolites in each fraction collected were then expressed as percentages of these mean values. The statistical analysis of the behavioural and biochemical data was performed by analysis of variance for repeated measures with treatment and time as the independent factors analysed. *Post hoc* analyses were performed with Duncan's test.

Drug sources

Nomifensine hydrogen maleate was a generous gift from Hoescht Pharmaceuticals. Nicotine hydrogen tartrate, (+)-amphetamine sulphate and tetrodotoxin (TTX) were purchased from The Sigma Chemical Company. EDTA and sodium octane sulphonic acid were purchased from Fisons PLC; h.p.l.c. grade methanol was supplied by Rathburn Chemical Ltd. All other laboratory and h.p.l.c. grade reagents used were obtained from British Drug Houses.

Results

Characterization of the release of dopamine and monamine metabolites into the dialysis probe

The recoveries of dopamine, DOPAC and HVA, through the probe when determined *in vitro* before implantation, were 10 ± 3, 14 ± 3 and 13 ± 2% respectively. The basal levels of

dopamine and the monoamine metabolites in the dialysate samples, collected from rats which had received no injections, were unaffected by 5 daily injections of saline. The addition of tetrodotoxin (1 μM) to the dialysis fluid caused a rapid and marked ($F(1,7) = 40.4$; $P < 0.001$) reduction in the concentration of dopamine in the dialysate (Figure 2a) The dopamine concentration in the dialysate was also reduced substantially ($F(1,5) = 49.4$; $P < 0.001$) if the Ringer solution was changed to one containing 12 mM $MgCl_2$ (Figure 2b). If the Ringer solution was changed to one containing a depolarizing concentration of KCl (100 mM), the concentration of dopamine in the dialysate was increased significantly ($F(1,9) = 12.4$; $P < 0.01$) (Figure 2c). The response was rapidly reversed by changing the Ringer solution back to one containing 4 mM KCl. The increase in dialysate dopamine levels evoked by K^+ was attenuated significantly (F treatment × time (7,42) = 3.0; $P < 0.05$) if the experiment was performed with a $CaCl_2$-free Ringer solution containing EDTA (0.1 mM).

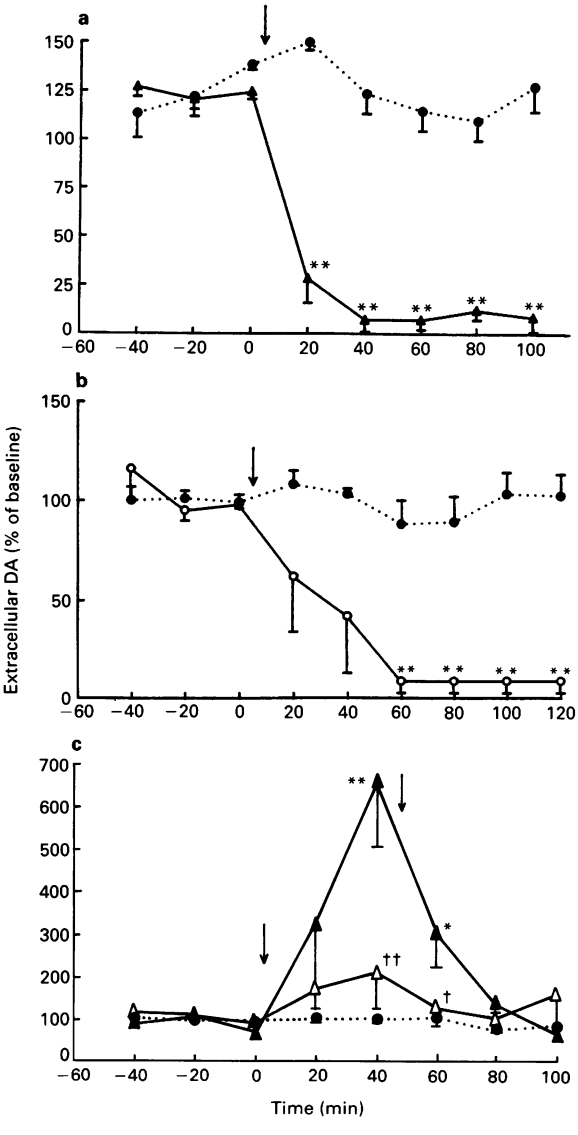

Figure 2 The effects on extracellular dopamine (DA) of changing from a perfusion syringe containing normal Ringer to a second syringe containing normal Ringer (●) or (a) 1 μM tetrodotoxin (▲): (b) Ringer + 12 mM $MgCl_2$ (○) or (c) Ringer containing 100 mM KCl (▲) or Ca^{2+}-free Ringer containing 100 mM KCl and 100 μM EDTA (△) at the time = 0 min. In the experiments represented in (c), the solutions were changed back to normal Ringer after 40 min, at the time indicated by the second arrow. The results are means ($n = 5$ for figure (a); $n = 3$ for figure (b); $n = 4$ or 5 for (c)) expressed as % of the mean pretreatment levels measured in the three samples collected prior to time 0; vertical bars show s.e.mean. Significantly different from control: $*P < 0.05$, $**P < 0.01$; significantly different from KCl group: $\dagger P < 0.05$, $\dagger\dagger P < 0.01$.

The effect of acute and subchronic nicotine

Under the conditions used, the acute administration of nicotine caused a dose-dependent increase ($F(2,19) = 7.02$; $P < 0.01$) in activity (Figure 3a). Acute nicotine (0.4 mg kg^{-1}) also appeared to increase the extracellular levels of dopamine in the dialysate (Figure 3b) although this effect was not statistically significant. The increases in extracellular DOPAC and HVA levels evoked by acute nicotine (Figure 3c and 3d) were significant ($F(2,19) = 3.52$; $P < 0.05$ and $F(2,19) = 5.45$; $P < 0.01$ for DOPAC and HVA respectively).

Pretreatment with nicotine (0.4 mg kg^{-1}) for 5 days before the test day resulted in a significant increase ($P < 0.01$) in the basal levels of dopamine in the dialysate measured 27 h after the last injection of nicotine (Table 1). Pretreatment with nicotine (0.1 and 0.4 mg kg^{-1}) also caused significant decreases in the basal concentrations ($P < 0.05$) of DOPAC in the NAc dialysates. The concentrations of HVA were not altered significantly by pretreatment with nicotine. The systemic administration of nicotine to rats pretreated with the compound resulted in significant increases ($F(2,22) = 8.22$; $P < 0.01$) in activity (Figure 4a) which were significantly greater (F treatment by time (12,270) = 2.47; $P < 0.05$) than those observed in response to acute nicotine. Subchronic nicotine also evoked statistically significant increases (F treatment by time (12,108) = 2.03; $P < 0.05$) in the concentrations of dopamine in the NAc dialysates (Figure 4b). Interestingly, under the conditions of the present study, neither the locomotor nor the mesolimbic dopamine responses to subchronic nicotine appeared to be dose-dependent. The concentrations of DOPAC and HVA were also increased significantly ($F(2,22) = 3.52$; $P < 0.05$ and $F(2,22) = 6.14$; $P < 0.05$ for DOPAC and HVA respectively) (Figure 4c and d).

Tissue levels of dopamine, dihydroxyphenylacetic acid and homovanillic acid

The concentration of dopamine, DOPAC and HVA in the nucleus accumbens, 24 h after the last of 5 consecutive daily injections of nicotine remained unchanged when compared with those measured in saline-treated controls (Table 2).

Effect of nomifensine on responses to nicotine

The inclusion of nomifensine (10 μM) in the perfusate caused a significant (F treatment (1,8) = 46, $P < 0.01$) and sustained elevation in extracellular dopamine concentrations in the NAc (Figure 5). The intra-probe administration of nomifensine, however, had no significant effects on the concentrations of DOPAC or HVA in the dialysates. When nomifensine was present in the Ringer solution perfusing the dialysis probe both the acute and chronic administration of nicotine (0.4 mg kg^{-1}) resulted in a significant increase in extracellular dopamine (F acute nicotine (1,9) = 8.9; $P < 0.05$; F chronic nicotine (1,15) = 5.1; $P < 0.05$) (Figure 6). In the presence of nomifensine the peak dopamine response to nicotine did not appear to be enhanced by pretreatment with the drug. The duration of the response to nicotine, however, was prolonged (F treatment × time (8,80) = 2.8; $P < 0.01$) when compared with that to acute nicotine if the animals were pretreated with the alkaloid. In addition, the increase in basal dopamine levels evoked by 5 days pretreatment with nicotine was still observed when the experiment was performed with nomifensine (Table 3). However, the increase in activity evoked by either acute or chronic nicotine was not altered significantly by the addition of nomifensine to the probe.

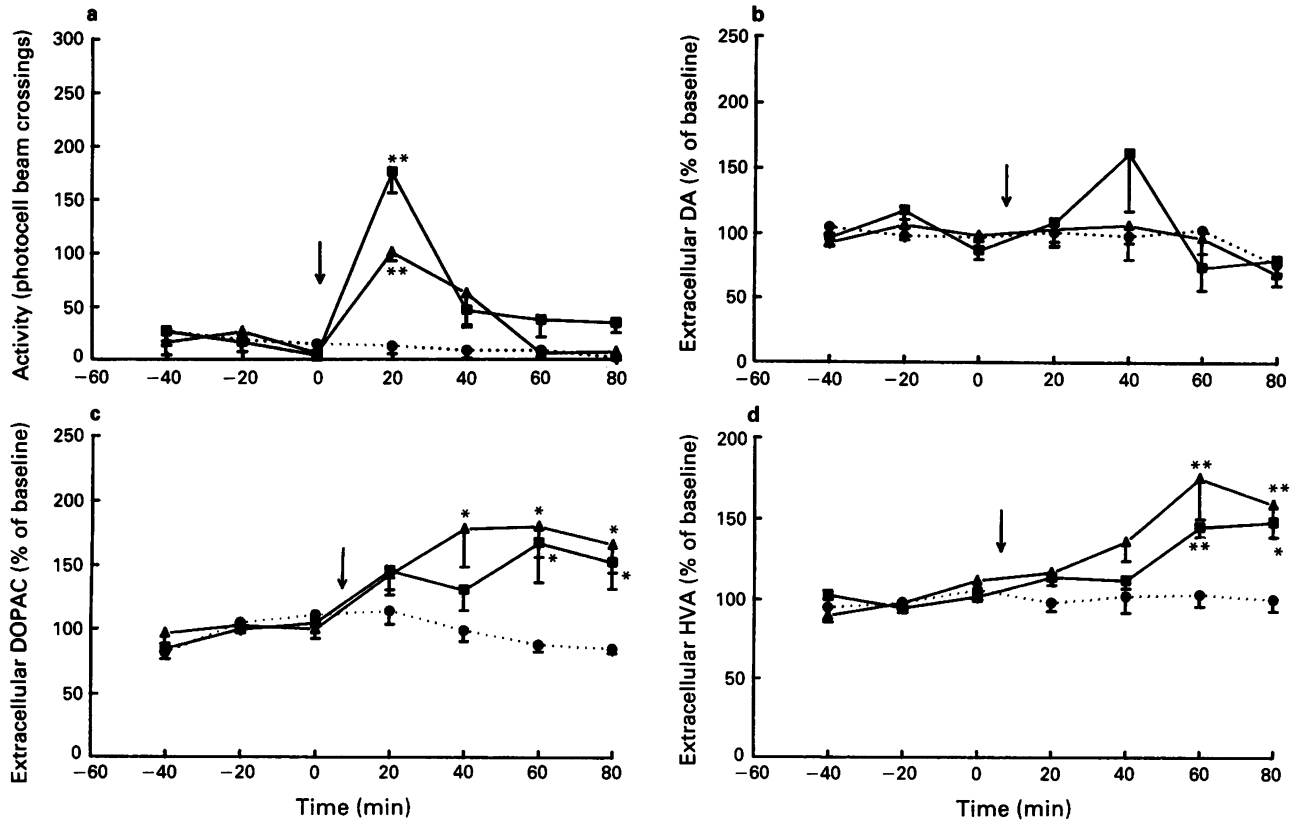

Figure 3 The effects of acute subcutaneous injections of nicotine on spontaneous activity and the concentrations of dopamine (DA) and its metabolites in nucleus accumbens dialysates. Subcutaneous injections of saline (●, $n = 6$), 0.1 mg kg^{-1} nicotine (▲, $n = 6$) or 0.4 mg kg^{-1} nicotine (■, $n = 8$) were given at the point indicated by the arrow (time 0). The results are expressed as means of the numbers of observations shown above in parentheses; vertical bars show s.e.mean. The data for DA, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) are expressed as percentages of the mean pretreatment value. Significantly different from control: *$P<0.05$, **$P<0.01$.

Table 1 The influence of pretreatment with nicotine or (+)-amphetamine on the concentrations of dopamine and its metabolites in nucleus accumbens dialysates

		Dialysate DA and metabolite concentrations		
		DA	*DOPAC*	*HVA*
Pretreatment	(n)	(fmol 20 µl^{-1})	(pmol 20 µl^{-1})	(pmol 20 µl^{-1})
Saline	(14)	86.7 ± 12.4	12.84 ± 2.05	8.46 ± 1.32
Nic (0.1)	(7)	98.6 ± 24.9	5.20 ± 1.40*	8.78 ± 1.67
Nic (0.4)	(10)	365.9 ± 85.1**	6.16 ± 1.68*	5.46 ± 0.56
Amphet (0.5)	(4)	70.1 ± 14.1	13.69 ± 1.68	7.68 ± 1.11

Pretreatment protocols consisted of 5 consecutive daily injections of saline, 0.1 or 0.4 mg kg^{-1} nicotine (Nic 0.1; Nic 0.4) or 0.5 mg kg^{-1} (+)-amphetamine (Amphet 0.5). The basal levels were measured in dialysate samples collected approximately 24 h after the last injection of the pretreatment protocol. Basal levels were calculated from the 3 samples collected before the administration of the challenge drug on the final experimental day and represent the mean ± s.e.mean of the numbers of observations in parentheses. Significantly different from rats receiving daily injections of saline: *$P<0.05$; **$P<0.01$.

Effects of acute and subchronic (+)-amphetamine

The subcutaneous administration of amphetamine induced a significant and dose-dependent ($F(2,13) = 6.5$; $P<0.01$) increase in the extracellular levels of dopamine in the NAc (Figure 7b) which peaked during the third 20 min period following amphetamine treatment. The time course for the increase in the dialysate dopamine content paralleled, very closely, the increase in locomotor activity observed in these animals ($F(2,13) = 12.05$; $P<0.01$) (Figure 7a). Statistical analysis of the data obtained from rats pretreated with (+)-amphetamine (0.5 mg kg^{-1}) daily for 5 days before the test day showed that the pretreatment regimen had no effects on the basal levels of dopamine and its metabolites in the NAc dialysates (Table 1) or on the locomotor or NAc dopamine responses to a challenge dose of (+)-amphetamine (0.5 mg kg^{-1}) (Figure 7a and b).

Discussion

The preliminary results showed that the extracellular levels of dopamine in the dialysate were substantially reduced if tetrodotoxin or excess Mg^{2+} ion were added to the perfusion fluid, data which suggest that under the conditions used, most of the basal dopamine sampled from the extracellular space by the dialysis probe had been secreted from dopaminergic nerve terminals via impulse-dependent mechanisms (Westerink & De Vries, 1988; Di Chiara, 1990). In addition it

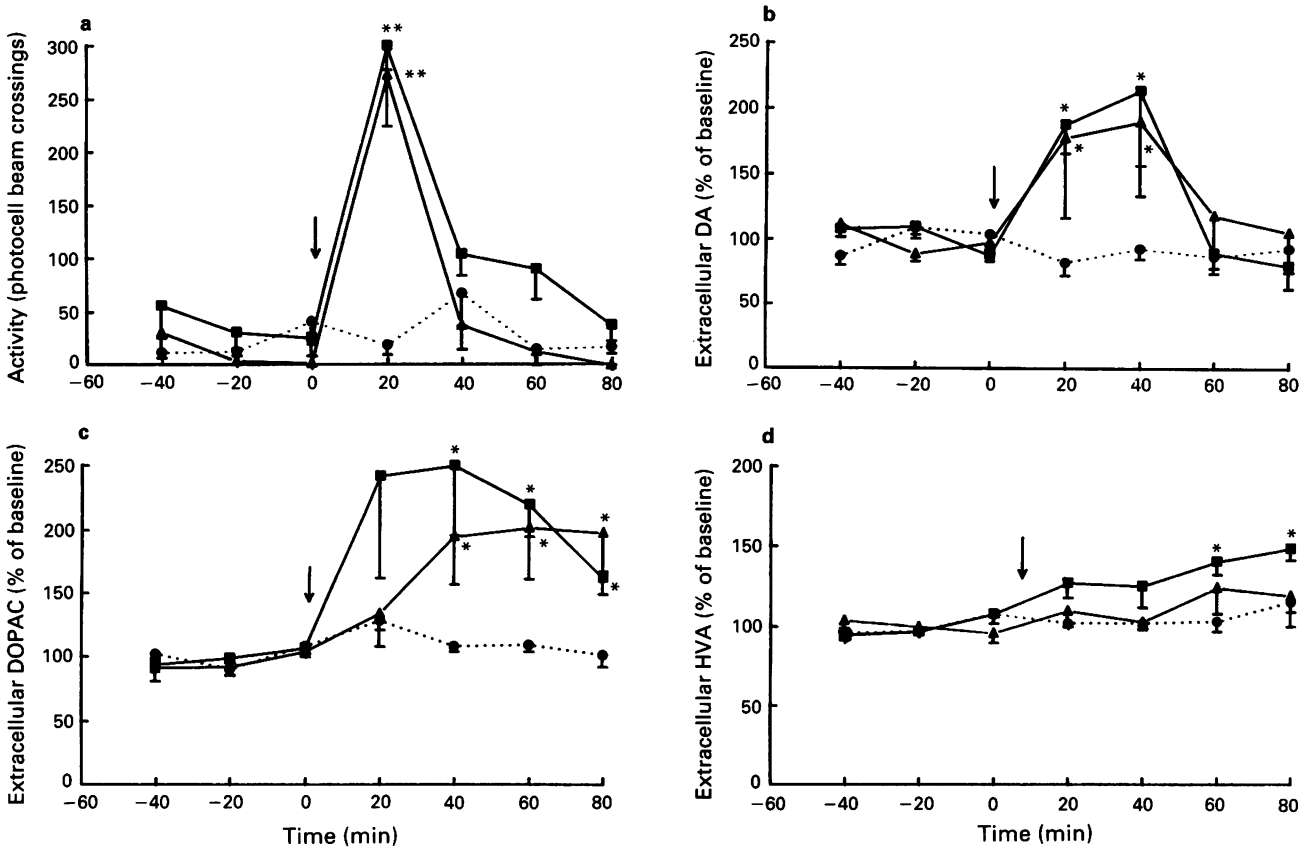

Figure 4 The effects of subchronic nicotine on spontaneous activity and the concentrations of dopamine (DA) and its metabolites in nucleus accumbens dialysates. The rats were pretreated with daily subcutaneous injections of saline (●, $n = 6$), 0.1 mg kg^{-1} nicotine (▲, $n = 6$) or 0.4 mg kg^{-1} nicotine (■, $n = 10$) for 5 days before the test day. On the test day the animals were given injections of saline or nicotine (0.1 or 0.4 mg kg^{-1}) respectively and the time indicated by the arrow (time 0). The results are expressed as means of the numbers of observations shown above in parentheses; vertical bars show s.e.mean. The data for DA, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) are expressed as percentages of the mean pretreatment value. Significantly different from control: $*P<0.05$, $**P<0.01$. Peak levels at time = 40 min were 84.9 ± 4.2, 187.3 ± 55.2 and 789.0 ± 208.0 fmol 20 µl^{-1} for rats challenged with saline, 0.1 or 0.4 mg kg^{-1} nicotine respectively.

Table 2 Tissue concentrations of dopamine (DA) and its metabolites in the nucleus accumbens of rats pretreated subchronically with nicotine

	Tissue DA and metabolite concentrations (pmol mg^{-1} wet tissue)		
Pretreatment	*DA*	*DOPAC*	*HVA*
Saline	31.6 ± 5.1	9.5 ± 1.4	4.4 ± 1.9
Nic (0.1)	37.5 ± 5.4	8.8 ± 1.7	5.0 ± 3.5
Nic (0.4)	32.9 ± 3.4	7.8 ± 0.8	3.5 ± 1.7

Results are the means ± s.e.mean of 6 observations. Rats received 5 consecutive daily injections of saline or nicotine (Nic 0.1 or 0.4 mg kg^{-1}) and were killed 24 h following their last treatment.

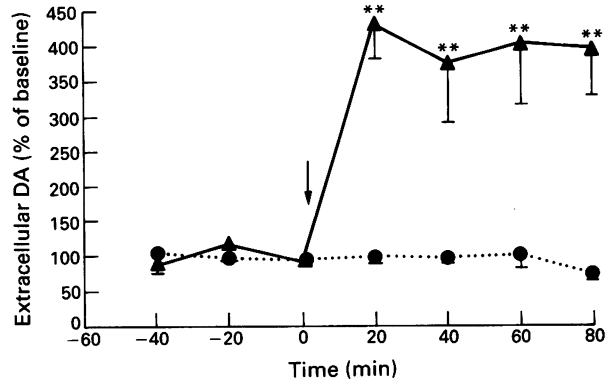

Figure 5 The effect of nomifensine on dopamine (DA) levels in the nucleus accumbens dialysates. At the time indicated by the arrow (time 0), the Ringer solution used to perfuse the dialysis probe was changed to one containing nomifensine (10 µM) (▲) or the same Ringer solution (●). The results are the means of at lest 4 experiments and are expressed as a percentage of the mean pretreatment value; vertical bars show s.e.mean. Significantly different from control: $**P<0.01$.

was found that the increase in extracellular dopamine levels evoked by perfusing a depolarizing concentration of KCl through the probe was attenuated to a significant extent if a Ca^{2+}-free solution containing a small quantity of EDTA was used in place of the normal Ringer solution. Results very similar to these have been taken by others (Kalivas & Duffy, 1990) as evidence that the increase in dopamine secretion evoked by depolarization of the nerve terminals is calcium-dependent. In addition the studies which showed that the inclusion of the dopamine uptake inhibitor, nomifensine, in the Ringer solution used to perfuse the probe greatly increased the concentration of dopamine in the dialysate, suggest that much of the dopamine released in the NAc is normally rapidly recaptured by the dopaminergic terminals in the structure.

In contrast to results reported by some other groups (Imperato *et al.*, 1986; Di Chiara & Imperato, 1988; Damsma *et al.*, 1989), the present study failed to demonstrate a

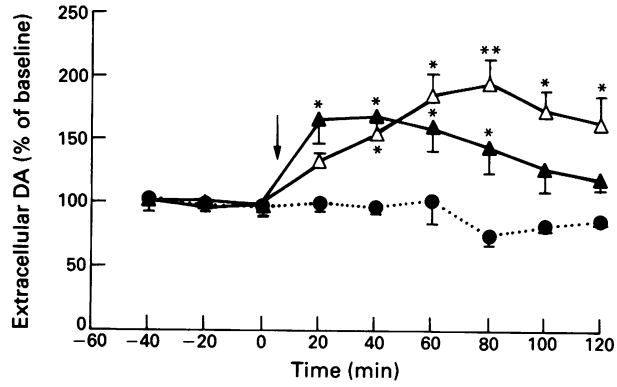

Figure 6 The effects of nomifensine on the responses to acute and subchronic nicotine. The rats were given daily injections of saline or nicotine (0.4 mg kg^{-1}) for 5 days before the test day. On the test day the animals pretreated with saline were given saline (●) or an acute injection of nicotine (0.4 mg kg^{-1}, ▲) at the point indicated by the arrow (time 0). At this time the rats pretreated with nicotine were also given an injection of nicotine (△). The results are the means of at least 4 experiments and are expressed as a percentage of the mean pretreatment value; vertical bars show s.e.mean. Significantly different from control: $^{**}P<0.01$.

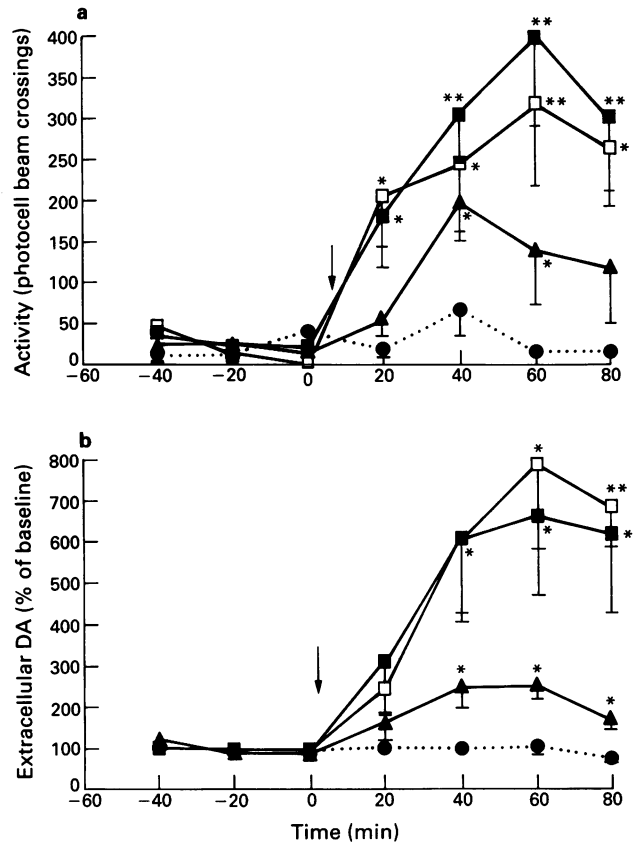

Figure 7 The effects of (+)-amphetamine on spontaneous activity and the concentration of dopamine (DA) in nucleus accumbens dialysates. The rats were pretreated with daily subcutaneous injections of saline or (+)-amphetamine (0.5 mg kg^{-1}) for 5 days. On the test day the rats pretreated with saline were given saline (●, $n=6$) 0.2 mg kg^{-1} (+)-amphetamine (▲, $n=6$) or 0.5 mg kg^{-1} (+)-amphetamine (■, $n=5$) at the point indicated by the arrow (time 0). At this time the rats pretreated with 0.5 mg kg^{-1} (+)-amphetamine were given 0.5 mg kg^{-1} (+)-amphetamine (□, $n=6$). The results are the means of the numbers of observations shown in parentheses; vertical bars show s.e.mean. The data for dopamine are expressed as a percentage of the mean pretreatment value. Significantly different from control: $^{*}P<0.05$; $^{**}P<0.01$.

significant increase in extracellular dopamine levels in the NAc in response to the acute administration of nicotine when compared with the control response observed in rats given saline although the apparent increase in extracellular dopamine levels observed in rats given the higher dose of nicotine tested (0.4 mg kg^{-1}) was of a similar magnitude to that reported by Damsma and colleagues (1989). Recent studies have shown that the dopaminergic innervation of the NAc is heterogeneous to the extent that the fibres which innervate the caudal NAc also contain cholecystokinin whereas those which innervate the rostral NAc do not (Hokfelt *et al.*, 1980). It is possible, therefore, that acute nicotine acts preferentially on one of the pathways which supply the NAc and that, in this study, the probes which were located in the rostral rather than the caudal NAc, were not located in the terminal field of this pathway. Acute nicotine, however, did increase the concentrations of DOPAC and HVA in the dialysate, data which support the conclusion that acute nicotine did increase dopamine turnover in the area of the brain sampled by the dialysis probe. Although increased dopamine turnover does not necessarily reflect increased dopamine release, the results suggest this probably was the case in these experiments because, when neuronal dopamine uptake was antagonized with nomifensine, acute nicotine administration did result in increased extracellular levels of dopamine. Therefore, these data imply that in the area of NAc sampled by the dialysis probe in this study, dopamine uptake is probably fairly rapid and that in the absence of an uptake inhibitor, the effects of acute nicotine were not sufficiently great to result in substantial leakage from the synaptic cleft into the extracellular space, the compartment actually sampled by microdialysis (Benveniste, 1989; Di Chiara, 1990).

In contrast to the results obtained with rats treated acutely with nicotine, subchronic administration of the drug did result in a significant increase in extracellular dopamine. For the rats pretreated with 0.4 mg kg^{-1} nicotine this represented a substantial enhancement of the response to the drug since pretreatment with this dose also caused increased basal

Table 3 The concentrations of dopamine (DA) and its metabolites in dialysates of nucleus accumbens measured in the presence of nomifensine

		Dialysate DA and metabolite concentrations		
Pretreatment	(n)	*DA* (fmol 20 µl^{-1})	*DOPAC* (pmol 20 µl^{-1})	*HVA* (pmol 20 µl^{-1})
Saline	(10)	263 ± 32	10.47 ± 2.34	5.25 ± 0.48
Nicotine (0.4 mg kg^{-1})	(10)	386 ± 87*	9.21 ± 0.96	4.58 ± 0.75

Rats received 5 daily injections of saline or nicotine before implantation of the dialysis probe. The dialysate samples were collected 27 h after the last injection of the pretreatment protocol using a Ringer solution containing nomifensine (10 µM). Basal levels were calculated from the 3 samples obtained on the final experimental day prior to the administration of the challenge drug and represent the means ± s.e.mean of the numbers of observations in parentheses. Significantly different from the saline-pretreated group: $^{*}P<0.05$.

dopamine levels prior to the injection of the challenge dose. These results are consistent with the potentiation of mesolimbic dopamine responses observed in tissue slices prepared from the NAc of nicotine-pretreated rats (Fung, 1989) but do not entirely agree with the results obtained by Damsma and colleagues (1989) who obtained no significant change in the mesolimbic dopamine response to s.c. nicotine, as measured by microdialysis, following a period of chronic treatment. The reason for the difference between the two studies remains to be established although they could reflect differences in the experimental procedures such as the use of transcerebral cannulae by Damsma's group as opposed to the loop probe employed in the present study or, as has been suggested above, the area of the NAc sampled by the probe. However, Damsma *et al.* (1989) did note a tendency for the basal dopamine levels to be raised in nicotine-treated rats. Elevated endogenous levels of NAc dopamine have previously been reported after 5 (Fung & Lau, 1988) and 14 (Fung, 1989) days of nicotine infusion. However, the enhanced basal extracellular dopamine, observed in the present study, was not accompanied by increased tissue levels of dopamine since the more moderate dosing regimen used here had no significant influence on the concentration of dopamime or its metabolites measured *post-mortem*. This finding confirms that *post mortem* tissue levels do not adequately reflect the activity of dopamine systems *in vivo*. Recent studies, however, suggest that nicotine at low doses can act as a non-competitive antagonist of the neuronal dopamine transporter (Izenwasser *et al.*, 1991) and it is possible, therefore, that the increased dopamine response observed in the animals pretreated with nicotine could be the result of a nicotine-induced attenuation of neuronal dopamine reuptake which persists for at least 24 h after the last nicotine injection. For the higher dose of nicotine at least, this hypothesis is consistent with the fact that pretreatment results in increased basal levels of dopamine and decreased levels of DOPAC in the NAc dialysates prior to the administration of the challenge dose of nicotine and with the observation that sub-chronic nicotine elevates NAc dopamine to a lesser extent in nomifensine-treated rats than it does in untreated animals. Furthermore, the studies using the Ringer solution containing nomifensine suggested that pretreatment with nicotine increases the duration of the response to a challenge dose of the alkaloid on the mesolimbic dopaminergic system although, clearly, further experiments are necessary to confirm this conclusion.

Studies in other laboratories suggest that the locomotor stimulant properties of both nicotine and amphetamine are mediated by their effects on dopamine secretion in the mesolimbic dopamine system (Kelly *et al.*, 1975; Imperato *et al.*, 1986; Clarke *et al.*, 1988). In the present study the effects of (+)-amphetamine on the extracellular levels of dopamine in the NAc paralleled both quantitatively and temporally the changes in locomotor activity evoked by the drug, data which are clearly consistent with the results of the earlier studies. The finding that the enhanced effects of nicotine on NAc dopamine, seen in animals pretreated with the drug, were accompanied by enhanced locomotor responses to the drug suggests that the behavioural sensitization could be mediated by potentiation of its effects on mesolimbic dopamine secretion. Interestingly Lapin *et al.* (1987) have reported that nicotine-induced circling behaviour in rats with unilateral lesions of the nigrostriatal system is only observed in animals which have been pretreated with the drug for 5 days, results which suggest that pretreatment with nicotine over a period of time similar to that used in the present studies may also result in sensitization of its effects on the nigrostriatal dopamine system.

The mesolimbic dopaminergic system is also thought to mediate the reinforcing properties of nicotine (Singer *et al.*, 1982; Wise & Bozarth, 1987) and, if this is the case, the present data suggest that pre-exposure to nicotine may enhance the reinforcing properties of a subsequent injection. This interesting possibility is supported by the results of recent studies which suggest that pretreatment with nicotine, for 7 days, enhances its ability to evoke a conditioned place preference response (M. Shoaib & I.P. Stolerman, personal communication). In the present study, the short period of pretreatment with a relatively low dose of (+)-amphetamine did not influence its effects on either extracellular dopamine levels in the NAc or locomotor activity. Studies in other laboratories, however, have shown that pretreatment with other psychostimulant drugs can enhance their effects on the secretion of dopamine in the mesolimbic system (Robinson *et al.*, 1988; Kalivas & Duffy, 1980; Pettit & Justice, 1991) although, in the case of amphetamine, this appears to occur in animals which are tested some weeks after cessation of treatment with relatively high doses of the drug (Robinson *et al.*, 1988). Thus, sensitization of the mesolimbic dopamine response following chronic treatment may be a property which is common to most, if not all, psychostimulant drugs of dependence although it seems likely that different mechanisms mediate sensitization to the different groups of drugs since pretreatment with nicotine does not appear to result in sensitization of the responses to other psychostimulant drugs (Schenk *et al.*, 1991).

In conclusion, these results suggest that the mesolimbic dopaminergic system does not develop tolerance to nicotine and indeed may become sensitized following repeated exposure to the alkaloid. In addition, the evidence presented suggests that this neural pathway may subserve, to some extent at least, the enhanced behavioural effects seen in these animals.

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(Received September 12, 1991
Revised December 5, 1991
Accepted December 10, 1991)