Desensitization of the nicotine-induced mesolimbic dopamine responses during constant infusion with nicotine

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1 The effects of constant nicotine infusions (0.25, 1.0 and 4.0 mg kg⁻¹ day⁻¹) on extracellular dopamine levels in the nucleus accumbens (NAc) and on locomotor activity have been compared with the changes evoked by repeated daily injections (0.4 mg kg⁻¹ day⁻¹ for 5 days) of the drug.

2 The extracellular dopamine concentration in the NAc was significantly increased ($P < 0.05$) following a challenge dose of nicotine (0.4 mg kg⁻¹, s.c.) in animals which had been pretreated with daily injections of the drug. This effect was accompanied by an enhanced locomotor response to nicotine.

3 The stimulant effects of nicotine on mesolimbic dopamine secretion and on locomotor activity were significantly inhibited ($P < 0.01$) by the prior administration of mecamylamine (2.0 mg kg⁻¹, s.c.) but not by hexamethonium (2.0 mg kg⁻¹, s.c.).

4 The constant infusion of nicotine at a rate of 1 and 4 but not 0.25 mg kg⁻¹ day⁻¹ abolished the sensitized dopamine response in the NAc to an injection of nicotine in animals pretreated with the drug. The locomotor responses to nicotine in the nicotine-pretreated rats were significantly attenuated by the infusion of nicotine at all 3 doses, although the nicotine induced locomotor activity, in the rats infused with 0.25 mg kg⁻¹ day⁻¹ was also significantly ($P < 0.05$) higher than that observed in the rats treated acutely with nicotine.

5 Significantly ($P < 0.01$) enhanced mesolimbic dopamine responses, to a challenge injection of nicotine (0.4 mg kg⁻¹, s.c.), were observed 2 and 7 days after termination of the infusion of nicotine (4 mg kg⁻¹ day⁻¹ for 14 days); locomotor responses were enhanced ($P < 0.01$) 1, 2 and 7 days after termination of the infusion.

6 The results suggest that sensitized mesolimbic dopamine responses to nicotine occur as a result of stimulation of centrally located nicotinic receptors but that these receptors may be desensitized during periods of chronic exposure to nicotine at doses which may be relevant to smoking.

Keywords: Nicotine; repeated injection; constant infusion; mesolimbic dopamine; locomotor activity; sensitization; desensitization

Introduction

A considerable body of evidence indicates that the mesolimbic dopamine system is involved in mediating the reinforcing properties of many psychostimulant drugs of abuse (Wise & Bozarth, 1987). It is now clear that this system is also stimulated by injections of nicotine (Clarke *et al.*, 1988; Di Chiara & Imperato, 1988; Damsma *et al.*, 1989; Benwell & Balfour, 1992) and that the rewarding (Singer *et al.*, 1982; Corrigan *et al.*, 1992) and locomotor stimulant (Clarke *et al.*, 1988; Reavill & Stolerman, 1990; Benwell & Balfour, 1992) effects of this drug may be dependent upon its ability to stimulate mesolimbic dopamine secretion. Moreover, repetitive daily injections of the drug appear to sensitize the mesolimbic system to a subsequent challenge with the drug (Benwell & Balfour, 1992). Intermittent exposure to tobacco smoke also increases the turnover of dopamine in the mesolimbic dopamine system, an effect which has been shown to be due to the nicotine content of the tobacco (Fuxe *et al.*, 1987). Therefore, it is thought that activation of mesolimbic dopamine systems may be responsible for the 'pleasurable' effects of smoking (Balfour, 1990). However, in all of these studies, the effects of nicotine were tested at a time when the plasma concentration of the alkaloid was either very low or negligible. In contrast, the profile of plasma nicotine in smokers often results in significant trough levels being maintained between cigarettes for much of the day (Russell, 1990; Benowitz *et al.*, 1990).

In one of the few studies to date which have considered the effect of constant exposure to nicotine on brain dopamine systems, the results have indicated that chronic nicotine may decrease dopamine turnover in the brain, suggesting that desensitization of the stimulant responses to nicotine may occur (Kirch *et al.*, 1987). It has long been recognised that peripheral nicotinic cholinergic receptors, especially those present on autonomic ganglia, are susceptible to depolarization block following prolonged exposure to nicotine (Rang & Dale, 1992). This phenomenon is thought to be responsible for the acute tolerance which develops to some of the effects of nicotine, such as nicotine-induced increases in heart rate, during periods of continuous exposure (Russell & Feyereabend, 1978; West & Russell, 1987). The up-regulation of central nicotinic receptors which occurs with smoking (Benwell *et al.*, 1988) or prolonged exposure to nicotine (Marks *et al.*, 1985; Schwartz & Kellar, 1985) suggests that central nicotinic receptors may also desensitize. It is presumed that the nicotinic receptors which mediate the stimulatory effects of nicotine on mesolimbic dopamine systems are those reported to be located on the dopaminergic neurones themselves (Clarke *et al.*, 1988). However, little attention has been paid to the possibility that the effects of nicotine on mesolimbic dopamine activity can be desensitized when the drug is present in concentrations which are regularly maintained in the plasma of habitual smokers for much of the day. The purpose of this study was to investigate this possibility by comparing the effects of constant nicotine infusions on mesolimbic dopamine activity and nicotine-

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induced locomotor stimulation with the responses to nicotine administered as an injection. In addition, the study has also investigated the extent to which the constant infusion of nicotine, at doses which achieve plasma levels comparable to those found in smokers, influences the responses to nicotine when administered as an acute injection both during and at various intervals following cessation of nicotine infusions.

Methods

Animals and drug pretreatments

Male Sprague–Dawley rats, bred in the Biomedical Services Unit, Ninewells Hospital and Medical School, from stock purchased from Interforna Ltd and weighing 250–350g at the start of the experiments were used throughout. The animals had free access to food and water and were housed in pairs prior to and singly following surgery. In the experiments where the influence of mecamylamine and hexamethonium on nicotine-induced responses were investigated, all animals were pretreated for 5 days with daily subcutaneous injections of (–)-nicotine hydrogen tartrate (0.4 mg kg^{-1} , free base dissolved in isotonic saline and adjusted to pH 7.0 by the addition of a small quantity of NaOH). In the experiments involving the constant infusion of nicotine, all animals were implanted subcutaneously, while anaesthetized with halothane (3%), with Alzet minipumps (2ml2) containing either saline or (–)-nicotine hydrogen tartrate dissolved in saline and delivering 0.25, 1.0 or $4.0 \text{ mg kg}^{-1} \text{ day}^{-1}$ of (–)-nicotine free base concentration for 14 days. On days, 9–13, half of the saline and half of each of the nicotine-infused groups also received daily subcutaneous (s.c.) injections of saline while the remainder of the animals received daily injections of nicotine (0.4 mg kg^{-1} , s.c.). In the experiments in which the effects of nicotine were investigated following removal of the infusion pumps, rats were infused with either saline or nicotine ($4 \text{ mg kg}^{-1} \text{ day}^{-1}$) and received daily injections of saline on days 9–13 as described and the pumps were removed, under halothane anaesthesia, on day 14. Measurements were then made on days 15, 16 or 21.

Stability of nicotine in the Alzet minipumps and plasma nicotine estimation

These parameters were assessed by adding [^3H](–)-nicotine hydrogen tartrate to the nicotine in the osmotic minipumps. In the experiments designed to measure the concentration of nicotine in the plasma, the final specific activity of the [^3H](–)-nicotine was 1.305, 0.326 and $0.081 \text{ mCi mmol}^{-1}$ for the doses of 0.25, 1.0 and $4.0 \text{ mg kg}^{-1} \text{ day}^{-1}$, respectively. The rats were killed on day 14 of the infusion and blood collected by cardiac puncture, centrifuged to obtain the plasma and stored at -70°C until assayed. The radioactivity was extracted into chloroform from plasma made alkaline with the addition of NaOH and samples of the chloroform extract spotted onto alkylated silica gel thin layer chromatography (t.l.c.) plates and run in a system of methanol:ammonia (100:1.5). The plasma concentration of nicotine was estimated by counting the radioactivity which co-chromatographed with unlabelled nicotine carrier followed by back calculation. The stability of the (–)-nicotine in the pumps was assessed by spotting samples of the solutions remaining in the pumps at day 14 on alkylated silica gel t.l.c. plates and run in the system described above.

Implantation of microdialysis probe

This procedure was carried out at least 3 h after the last of the pretreatments, i.e., on day 5 in the mecamylamine/hexamethonium experiments and on day 13 in the infusion experiments. In the case of the withdrawal experiments, the

probes were implanted on days 14, 15 and 20 to investigate the effects of nicotine 1, 2 and 7 days following pump removal respectively. The dialysis probe and its implantation into the nucleus accumbens were carried out as described previously (Benwell & Balfour, 1992). The position of the probes was routinely determined histologically from sections prepared at postmortem from tissue fixed in formalin.

Microdialysis and estimation of locomotor activity

Eighteen to 24 h following implantation of the dialysis probe, the animals were placed individually in a black perspex activity box (40 cm square $\times$ 25 cm high) where the locomotor activity is assessed by 4 photocells located on adjacent sides of the box as described by Vale & Balfour (1989). At this time, the dialysis loop was connected, via a liquid swivel, to a syringe pump containing Ringer solution composition mM (NaCl 147 KCl 4.0 and CaCl_2 1.25) which was perfused at a constant rate of $1.7 \mu\text{l min}^{-1}$. Following a 40–60 min equilibration period, dialysate samples were removed at 20 min intervals for 60 min to establish a stable baseline level of dopamine and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). At this time, in the experiments investigating the effects of mecamylamine and hexamethonium, a third of the animals received injections of saline (s.c.), a third received mecamylamine (2.0 mg kg^{-1} s.c.) and the remainder received hexamethonium (2.0 mg kg^{-1} , s.c.) and another $3 \times 20 \text{ min}$ samples were taken. At this time, the rats were challenged with nicotine (0.4 mg kg^{-1} s.c.) and at least 5 more 20 min samples were taken. In the experiments investigating the effects of nicotine infusions, the response of all animals to a

Figure 1 The effect of mecamylamine and hexamethonium on nicotine-induced changes in NAC dopamine and locomotion. All rats were pretreated with daily injections of nicotine (0.4 mg kg^{-1} s.c.) for 5 days. On day 6, the rats received either saline (●, $n=4$), mecamylamine (◆, 2.0 mg kg^{-1} s.c., $n=5$) or hexamethonium (■, 2.0 mg kg^{-1} s.c., $n=4$) at the time indicated by the first arrow (1). All animals received an injection of nicotine (0.4 mg kg^{-1} , s.c.) at the time indicated by the second arrow (Nic). The results in (a) are the extracellular dopamine (DA) levels in the NAc expressed as percentages of the means $\pm$ s.e.mean of the 3 samples removed before the nicotine injection. The results in (b) are the means $\pm$ s.e.mean of the locomotor activity in these animals. * $P<0.05$, ** $P<0.01$; significantly different when compared to the saline-injected group.

saline injection (s.c.) was tested once a stable baseline had been obtained and a further 3×20 min samples taken before all animals received a challenge dose of nicotine (0.4 mg kg^{-1} s.c.) and at least 5 more 20 min samples taken. The concentrations of dopamine, DOPAC and HVA in the dialysates were assayed by injecting the samples directly onto a high performance liquid chromatography (h.p.l.c.) system as described elsewhere (Benwell & Balfour, 1992).

Data analysis

In each experiment, the mean concentrations of dopamine and its metabolites, for the 3 samples collected after the saline, mecamlamine or hexamethonium injection and before the administration of nicotine, were estimated and defined as 100%. The concentration of dopamine and its metabolites in each sample collected during the experimental day were then expressed as percentages of these mean values. Statistical analysis of the locomotor activity and the dialysis data was performed by analysis of variance for repeated measures with infusion and pre-injection as independent factors in the infusion experiments and pretreatment with saline, mecamlamine and hexamethonium as independent factors in the mecamlamine/hexamethonium experiments. *Post hoc* analyses were performed, where appropriate, using Duncan's test or a paired *t* test.

Drugs

(-)-Nicotine hydrogen tartrate, mecamlamine hydrochloride and hexamethonium bromide were obtained commercially from Sigma Chemical Company; [^3H]-(-)-nicotine from New England Nuclear; EDTA from Fisons PLC; h.p.l.c. grade methanol from Rathburn Chemical Ltd and all other h.p.l.c. grade reagents from British Drug Houses.

Results

Stability of nicotine in the Alzet minipump and plasma concentrations

Fourteen days after implantation, $93 \pm 3\%$ of the tritium labelling remaining in the pumps co-chromatographed with authentic nicotine. The concentrations of nicotine achieved in the plasma, at this time, were 9 ± 4 , 24 ± 5 and $87 \pm 12 \text{ ng ml}^{-1}$ during the infusion of nicotine at rates of 0.25, 1.00 and $4.00 \text{ mg kg}^{-1} \text{ day}^{-1}$ respectively. There was a significant correlation ($r = 0.92$, $n = 4/\text{concentration}$) between the doses of nicotine administered and the plasma nicotine concentration achieved.

Pretreatments and basal dopamine and metabolite levels

The basal levels of dopamine, DOPAC and HVA in the dialysates obtained from the nucleus accumbens of the saline-infused/saline-injected rats were 0.142 ± 0.032 , 12.23 ± 3.00 and $5.72 \pm 1.12 \text{ pmol } 20 \mu\text{l}^{-1}$ respectively. Although there was a tendency for the basal dopamine levels to be lower (0.081 ± 0.012) in the nicotine-infused rats and higher (0.201 ± 0.053) in those pretreated with saline infusions and daily injections of nicotine, these did not prove significantly different in comparison with those obtained from the saline-infused/saline-injected control rats.

The effect of mecamlamine and hexamethonium on nicotine-induced stimulation of mesolimbic dopamine secretion and locomotion

Injection of saline, mecamlamine or hexamethonium had no significant effect on the extracellular concentration of dopamine in the NAc (Figure 1a) or on locomotor activity (Figure 1b) during the 60 min following these treatments.

However, the injection of nicotine was associated with a significant (F time (7,21) = 3.05, $P < 0.05$) increase in the extracellular level of dopamine in the NAc of the saline-injected animals, an effect which was completely abolished (F mecamlamine $\times$ time (7,49) = 4.26, $P < 0.01$) when the rats had received mecamlamine 60 min before being challenged with nicotine. The nicotine-induced locomotor response (F time (7,21) = 8.42, $P < 0.01$) was also completely inhibited (F mecamlamine $\times$ time (7,49) = 5.72, $P < 0.01$) in these animals. There were no significant changes in the nicotine-induced effects on NAc dopamine secretion and locomotor activity which could be attributed to the prior administration of hexamethonium.

The influence of nicotine infusions on mesolimbic dopamine and locomotor responses to nicotine bolus-dopamine

There was no significant effect on the extracellular levels of dopamine in the NAc which could be attributed to the infusion of nicotine nor the prior exposure to nicotine as daily injections when the rats were injected with saline on the test day. With respect to the responses to a challenge injection of nicotine, administered on the test day, statistical analysis of the overall data revealed a significant interaction (F infusion $\times$ pre-injection $\times$ time (21,231) = 1.61, $P < 0.05$) between the infusion of nicotine, the pre-injections and the injection of nicotine on the test day. In rats which had been pre-injected daily with saline, the increase in the extracellular level of dopamine in the NAc, evoked by a test injection of nicotine to saline-infused rats, did not reach statistical significance (Figure 2a). However, a test injection of nicotine did produce an increase in this parameter in the animals

Figure 2 The influence of nicotine on extracellular dopamine in the NAc. The rats were implanted with osmotic minipumps delivering either saline (●) or nicotine at rates of 0.25 (□), 1.0 (Δ) and $4.0 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 14 days. On days 9–13, the animals also received daily injections of saline (a) or nicotine (0.4 mg kg^{-1} s.c., b). On day 14, the responses of all animals to test injections of saline (Sal) and nicotine (Nic, 0.4 mg kg^{-1} s.c.) were tested. The results are the extracellular dopamine (DA) levels in the NAc expressed as percentages of the means $\pm$ s.e.mean of the 3 samples removed before the nicotine injection. There are 4–8 observations in each group. * $P < 0.05$, ** $P < 0.01$; significantly different when compared to the saline-infused group.

receiving a constant infusion of the lowest dose of nicotine ($0.25 \text{ mg kg}^{-1} \text{ day}^{-1}$) which was significant ($F(7,63) = 2.92$, $P < 0.01$) in comparison with the saline-infused/saline pre-injected controls. No significant changes were observed when nicotine was injected during the infusion of nicotine at rates of 1 or $4 \text{ mg kg}^{-1} \text{ day}^{-1}$. In agreement with results of previous studies (Benwell & Balfour, 1992), the administration of nicotine, to saline-infused rats pre-injected with daily injections of the drug, was associated with a significant enhancement ($F \text{ pre-injection} \times \text{time} (7,63) = 2.71$, $P < 0.05$) of the increase in extracellular dopamine in the NAc seen in the saline pre-injected animals (Figure 2b). In these nicotine pre-injected animals, this enhanced response was completely abolished ($P < 0.01$) in the animals receiving constant infusions of nicotine at rates of 1 and $4 \text{ mg kg}^{-1} \text{ day}^{-1}$. Although there appeared to be an attenuation of the mesolimbic dopamine response to a bolus dose of nicotine, during the constant infusion of the lowest dose used in this study ($0.25 \text{ mg kg}^{-1} \text{ day}^{-1}$), this did not attain statistical significance. In fact, the NAc dopamine levels were significantly increased ($F(7,21) = 2.65$, $P < 0.05$) following a bolus injection of nicotine in these rats receiving this low dose infusion.

The injection of saline had no influence on the extracellular levels of DOPAC or HVA in any of the treatment groups studied. The injection of nicotine, on the test day, was associated with a significant increase in the extracellular concentration of DOPAC ($F(7,70) = 6.95$, $P < 0.01$) and HVA ($F(7,70) = 5.44$, $P < 0.01$) in the NAc of the saline-infused animals, an effect which was not influenced by pre-injection with nicotine. However, the infusions did affect the nicotine-induced increases in DOPAC ($F \text{ infusion} \times \text{time} (21,231) = 2.79$, $P < 0.01$) and HVA ($F \text{ infusion} \times \text{time} (21,231) = 2.4$, $P < 0.01$).

The levels of DOPAC were reduced from a peak level of

$155 \pm 12\%$ of the pre-nicotine injection levels between 60 and 80 min post-nicotine injection, in the saline-infused rats, to 119 ± 4 , 113 ± 15 and $114 \pm 8\%$ during the infusion of 0.25, 1.0 and $4.0 \text{ mg kg}^{-1} \text{ day}^{-1}$ respectively. The level of HVA in the extracellular fluid at this time was significantly ($P < 0.05$) reduced from $128 \pm 10\%$ in the saline-infused rats to $97 \pm 6\%$ during the constant infusion of the highest dose of nicotine only. The reductions during the constant infusions of 0.25 and $1.0 \text{ mg kg}^{-1} \text{ day}^{-1}$ to 121 ± 7 and $108 \pm 7\%$ respectively, did not attain significance.

The injection of saline had no significant effects on locomotion in any of the treatment groups. However, the injection of nicotine on the test day stimulated ($F(7,231) = 72.43$, $P < 0.001$) locomotor activity in all groups of animals and statistical analysis of the overall data revealed a significant interaction ($F \text{ infusion} \times \text{pre-injection} \times \text{time} (21,231) = 1.94$, $P < 0.01$) between the infusion of nicotine, the pre-injections and the stimulant response evoked by an injection of nicotine on the test day. In rats which had been pre-injected daily with saline, the increase in the locomotor activity, evoked by a test injection of nicotine to the animals being constantly infused with the lowest dose of nicotine ($0.25 \text{ mg kg}^{-1} \text{ day}^{-1}$), was significantly greater ($F(7,70) = 3.96$, $P < 0.01$) than the behavioural response in the saline-infused controls (Figure 3a). However, the locomotor responses elicited during the infusion of 1 and $4 \text{ mg kg}^{-1} \text{ day}^{-1}$ were not significantly different from the saline-infused controls. The administration of nicotine to saline-infused rats pre-injected with daily injections of the drug, was associated with a significant enhancement ($F \text{ pre-injection} \times \text{time} (7,63) = 7.73$, $P < 0.001$) of the increase in the locomotor activity seen in the animals pre-injected with saline. In the

Figure 3 The influence of nicotine on locomotor activity. The rats were implanted with osmotic minipumps delivering either saline (●) or nicotine at rates of 0.25 (□), 1.0 (Δ) and $4.0 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 14 days. On days 9–13, the animals also received daily injections of saline (a) or nicotine ($0.4 \text{ mg kg}^{-1} \text{ s.c.}$). On day 14, the responses of all animals to test injections of saline (Sal) and nicotine (Nic, $0.4 \text{ mg kg}^{-1} \text{ s.c.}$) were tested. The results are the means $\pm$ s.e.mean of 4–8 observations in each group. * $P < 0.05$, ** $P < 0.01$; significantly different when compared to the saline-infused group.

Figure 4 The influence of nicotine on extracellular dopamine in the NAc and locomotor activity following cessation of the nicotine infusions. The rats were implanted with osmotic minipumps delivering nicotine at a rate of $4.0 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 14 days. The effects of a bolus injection of saline (Sal) and nicotine (Nic, $0.4 \text{ mg kg}^{-1} \text{ s.c.}$) were tested during the infusion (●, $n = 4$) and at 1 (□, $n = 6$), 2 (Δ, $n = 4$) and 7 (◆, $n = 6$) days after removal of the infusion pumps. The results in (a) are the extracellular dopamine (DA) levels in the NAc expressed as percentages of the means $\pm$ s.e.mean of the 3 samples removed before the nicotine injection. The results in (b) show the locomotor activity. The results are the means $\pm$ s.e.mean of 4–6 observations in each group. * $P < 0.05$, ** $P < 0.01$; significantly different when compared to the response obtained during the infusion of nicotine.

nicotine pre-injected animals (Figure 3b), the enhanced behavioural effect was significantly (F infusion $\times$ pre-injection $\times$ time (7,63) = 2.76, $P < 0.05$) attenuated by the constant infusion of nicotine at a rate of $0.25 \text{ mg kg}^{-1} \text{ day}^{-1}$ (Figure 3b) and was completely abolished in the animals receiving constant infusions of nicotine at rates of 1 (F (7,70) = 4.58, $P < 0.01$) and 4 (F (7,63) = 8.12, $P < 0.01$) $\text{mg kg}^{-1} \text{ day}^{-1}$.

The effects of nicotine boli following termination of the constant infusion of nicotine

As shown in Figure 4a, there were no significant changes in the extracellular dopamine levels in the NAc which could be attributed to the acute injection of saline during or at 1, 2 or 7 days after termination of the infusion of nicotine ($4.0 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 14 days). The extracellular dopamine levels in the NAc of rats, receiving a constant infusion of nicotine at a rate of $4 \text{ mg kg}^{-1} \text{ day}^{-1}$, remained unchanged following an acute challenge with nicotine. However, within 24 h of cessation of this infusion, the extracellular dopamine levels were increased by an acute nicotine injection, an effect which achieved significance 2 (F infusion $\times$ time (7,49) = 2.27, $P < 0.05$) and 7 (F infusion $\times$ time (7,49) = 3.03, $P < 0.01$) days after removal of the osmotic minipumps. Figure 4b shows the locomotor responses in these animals. The stimulation (F (7,91) = 32.27, $P < 0.001$) of locomotor activity, induced by acute nicotine, was significantly enhanced at 1 (F infusion $\times$ time (7,42) = 4.39, $P < 0.01$), 2 (F infusion $\times$ time (7,49) = 7.01, $P < 0.01$) and 7 (F infusion $\times$ time (7,49) = 5.24, $P < 0.01$) days after cessation of the nicotine infusion when compared with the responses obtained when nicotine was administered during the infusion.

Discussion

This study has established that over 90% of the nicotine in the osmotic minipumps remained as nicotine over the 14 day period and allows the conclusion that any effects observed are likely to be due to the nicotine released from the pumps. Moreover, the plasma concentrations of nicotine which were achieved are consistent with those reported by Jansson *et al.* (1989) and Neil Grunberg (personal communication). They also fall within the range which has been reported to occur in smokers in that the lowest dose ($0.25 \text{ mg kg}^{-1} \text{ day}^{-1}$) gave a plasma level ($\sim 9 \text{ ng ml}^{-1}$) which lies at the lower end of the range and also a level which is often found following overnight abstinence and the $1 \text{ mg kg}^{-1} \text{ day}^{-1}$ dose resulted in levels ($\sim 24 \text{ ng ml}^{-1}$) commonly reported in smokers (Russell, 1990; Benowitz *et al.*, 1990; Henningfield *et al.*, 1993). The highest dose ($4.0 \text{ mg kg}^{-1} \text{ day}^{-1}$) resulted in nicotine levels ($\sim 87 \text{ ng ml}^{-1}$) similar to those found in arterial blood following the inhalation of smoke from cigarettes (Henningfield *et al.*, 1993).

It has previously been reported that the stimulant effects of acute nicotine administration on mesolimbic dopamine secretion is centrally mediated since it is blocked by the nicotinic antagonist, mecamylamine, but not by hexamethonium, which does not readily cross the blood brain barrier (Imperato *et al.*, 1986). The present study has demonstrated that the sensitized stimulant effects of nicotine on NAc dopamine secretion, seen in animals which have received prior intermittent treatment with the drug, is also completely inhibited by mecamylamine, but not by hexamethonium. Furthermore, the locomotor stimulant effects of nicotine, in these animals, were completely abolished by mecamylamine but not hexamethonium. Therefore, it would appear that the stimulant effects exerted by nicotine on both mesolimbic dopamine activity and locomotion, in animals with prior experience of the drug, are mediated by central nicotinic receptors.

As reported previously (Benwell & Balfour, 1992), the acute injection of nicotine to drug naive animals appeared to cause a modest increase in the extracellular level of dopamine in the NAc which was close to significance. However, nicotine evoked a mesolimbic dopamine response in saline pre-injected animals receiving a constant infusion of the lowest dose of nicotine tested ($0.25 \text{ mg kg}^{-1} \text{ day}^{-1}$) which was significantly greater than that seen in the saline-infused controls. Therefore prolonged exposure to this low level of nicotine is, itself, capable of priming the mesolimbic dopamine system to be more responsive to a bolus dose of nicotine. This did not, however, occur when saline pre-injected rats were challenged with an acute injection of the drug during the infusion of 1 or $4 \text{ mg kg}^{-1} \text{ day}^{-1}$. In agreement with previous studies (Benwell & Balfour, 1992), there was an enhanced mesolimbic dopamine response, seen when nicotine was injected into rats which had been previously exposed to the drug with daily pre-injections of nicotine (0.4 mg kg^{-1} , s.c.). One of the principal findings of the present study is that this enhanced response was attenuated or abolished when nicotine was maintained in the plasma at concentrations which are commonly found in habitual smokers. For instance, when nicotine is infused at a rate of $1.0 \text{ mg kg}^{-1} \text{ day}^{-1}$, which produces a plasma concentration of approximately 24 ng ml^{-1} , there is complete inhibition of the increase in extracellular dopamine and attenuation of the elevation of DOPAC within the NAc, which are evoked by an injection of nicotine. The HVA response is also reduced when the infusion dose is increased to $4.0 \text{ mg kg}^{-1} \text{ day}^{-1}$. In addition, the infusions of nicotine at doses of $1 \text{ mg kg}^{-1} \text{ day}^{-1}$ or greater abolished the enhanced locomotor stimulant response to nicotine in these rats, data which support the conclusion that the enhanced behavioural response to the drug may be related to its effects on mesolimbic dopamine (Benwell & Balfour, 1992). The nature of this reduced sensitivity to nicotine remains to be established. However, exhaustion of the dopamine stores available for release is unlikely since the constant infusion of nicotine has been reported to evoke, if anything, an increase in the tissue levels of dopamine in the NAc and enhance amphetamine-induced release of [^3H]-dopamine from slices prepared from the NAc (Fung, 1989). Previous studies have indicated that smoking may be associated with an increase in the density of nicotinic receptors in the human brain (Benwell *et al.*, 1988) which is very likely to be attributable to the nicotine content of tobacco since this up-regulation is also observed in experimental animals following chronic treatment with nicotine (Marks *et al.*, 1985; Schwartz & Kellar, 1985). These changes have been interpreted as evidence that desensitization of central nicotinic receptors occurs. Indeed, it has been proposed that the apparent neuroprotective effect of smoking in Parkinson's disease may be due to desensitization of the nicotinic receptors located on nigrostriatal dopamine systems which results in reduced firing rates of these neurones and thus reduced energy demands (Jansson *et al.*, 1986; 1988; 1989). Therefore, the most likely explanation for the reduced sensitivity of the mesolimbic dopamine system to nicotine injections, observed when the plasma nicotine levels are maintained at 24 ng ml^{-1} and above, is that the receptors which mediate the effects of nicotine on mesolimbic dopamine secretion are desensitized with prolonged exposure to nicotine at this level. Clarke & Pert (1985) demonstrated the presence of nicotinic receptors on the membranes of both the nerve terminal and somatodendritic regions of this neural pathway. Recent results (Benwell *et al.*, 1993; Nisell *et al.*, 1994; Reavill & Stolerman, 1990) suggest that the postsynaptic, somatodendritic receptors in the Ventral Tegmental area are the best candidates for mediating the effects of nicotine on mesolimbic dopamine secretion and the enhanced locomotor responses, at least at the dose of nicotine (0.4 mg kg^{-1} , s.c.) which was used in the present study. Therefore, it seems reasonable to suggest that these receptors are desensitized during the constant infusion of nicotine.

Previous studies have suggested that the locomotor stimulant effects of nicotine are related to its ability to stimulate mesolimbic dopaminergic neurones (Clarke *et al.*, 1988; Balfour *et al.*, 1990). The present study suggests that there is a clear dose-relationship between the ability of the infusions to attenuate both the nicotine-induced increases in the concentration of dopamine in the extracellular space of the NAc and locomotor stimulation. However, the temporal correlation between these two parameters is not particularly close since the peak extracellular level of dopamine in the NAc appears to lag behind the peak locomotor effect and to be of longer duration. Although the nicotine-induced locomotor stimulation was completely inhibited by mecamylamine, this parameter was only attenuated and not abolished during the infusion of nicotine at a rate of $1 \text{ mg kg}^{-1} \text{ day}^{-1}$ and this was despite the apparent total inhibition of the dopamine overflow in the NAc in these animals. It is unlikely that this was due simply to a submaximal desensitizing dose since a locomotor stimulant effect, of similar magnitude, was still evident when the infusion dose was increased four fold. The explanation for this remains unclear although it is consistent with the possibility that the locomotor responses to the drug may be mediated by heterogeneous nicotinic receptors and that the same subtype is responsible for the enhanced locomotor effect and activation of mesolimbic dopaminergic neurones. Activation of a different subtype may mediate the residual locomotor stimulation and this may be less susceptible to desensitization but equally sensitive to mecamylamine.

The desensitization of the central nicotinic receptors, associated with the mesolimbic dopamine system, is clearly a reversible phenomenon since enhanced mesolimbic and locomotor responses are evoked, in response to a bolus dose of nicotine, 24 h after abstinence from nicotine. This sensitization to nicotine was still apparent 7 days after cessation of the nicotine infusion. Therefore, prolonged desensitization also leads to sensitized responses to nicotine administered as a bolus during period of abstinence. However, it remains to be established whether the mechanisms involved in the development of sensitization, induced by low level chronic exposure to nicotine, and the sensitization seen during periods of abstinence from desensitizing doses of the drug are the same as those involved in the increased responsiveness seen following intermittent exposure to nicotine.

There is a widely held view that stimulation of mesolimbic dopaminergic neurones plays a central role in the rewarding and addictive properties of psychostimulant drugs, including nicotine (Wise & Bozarth, 1987; Corrigan *et al.*, 1992; Singer *et al.*, 1982). The data described here are of relevance in this regard. The results imply that plasma nicotine levels of 10 ng ml^{-1} and above may attenuate the mesolimbic dopamine response to a nicotine bolus. Therefore, if increased dopamine secretion in the mesolimbic system is the

principal mediator of the rewarding properties of nicotine, the results suggest that this will be attenuated or abolished in people who smoke or use nicotine preparations in a way which maintains levels significantly above 10 ng ml^{-1} . Acute tolerance to many of the effects of nicotine develops with repeated exposure during a single day (Russell & Feyereabend, 1978; West & Russell, 1987; Perkins *et al.*, 1993), observations which when taken in conjunction with the increased density of nicotinic receptors in human brain tissue taken from habitual smokers (Benwell *et al.*, 1988) provides evidence that repetitive or prolonged periods of receptor desensitization may, indeed, occur with smoking. Therefore, it seems reasonable to speculate that, although stimulation of mesolimbic dopamine systems may be an important factor in the development of nicotine addiction, for heavy smokers who smoke in a way which maintains their nicotinic receptors in a desensitized state for much of the day, other neural mechanisms may also be important in contributing to the reinforcing effects of the drug. It is also possible that people who smoke most heavily and consequently are likely to maintain the highest plasma levels of nicotine and be most addicted to the drug, are actually smoking to maintain nicotinic receptors in a desensitized state. If this is the case, it is possible that re-sensitization of the central nicotinic receptors following periods of desensitization is aversive to the heavy smoker. It is particularly interesting that both sensitization and desensitization of responses to nicotine appear to be achieved over the plasma nicotine ranges which smokers could be expected to experience. It has been suggested that smokers can vary their pattern of nicotine intake to help them stay alert and concentrate or to relax depending upon the situation (Warburton, 1990). Therefore, it is tempting to speculate that smokers learn to titrate their nicotine intake to achieve either sensitization or desensitization depending on whether they wish to be alert or to relax.

In conclusion, the data presented here suggest that the nicotinic receptors, responsible for the stimulant effects of nicotine on NAc dopamine secretion and enhanced locomotor responses, may be susceptible to desensitization when nicotine is administered at a rate which maintains the plasma concentration at levels commonly found in smokers. The results clearly support the hypothesis that the up-regulation of nicotinic receptors, observed in brain tissue taken at post mortem from habitual smokers, is caused by prolonged periods of receptor desensitization. In addition, the data also cast doubt on hypotheses for nicotine dependence which assumes that the administration of the compound invariably causes stimulation of mesolimbic dopamine secreting neurones.

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