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Pre- and postsynaptic dopamine mechanisms after repeated nicotine: effects of adrenalectomy

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Abstract

The reinforcing properties of nicotine may be related to its ability to release dopamine in the nucleus accumbens and to increase locomotor activity in experimental animals. Both these effects are sensitized following repeated drug exposure, a phenomenon that may underlie important aspects of addiction. Adrenal steroids may be involved both in positive reinforcement and in sensitization. Adrenalectomy hampers, e.g., the induction of locomotor sensitization to nicotine, and cross-sensitization between stress and psychostimulants may develop. Here, the effect of adrenalectomy on postsynaptic and presynaptic changes of the mesolimbic dopamine system in association with nicotine sensitization was examined. Adrenalectomy or sham-operated rats received daily nicotine (0.4 mg/kg s.c.) or vehicle for 15 days, after which the locomotor responses to nicotine (0.2 mg/kg s.c.) and the dopamine D1/D2 receptor agonist apomorphine (1.0 mg/kg s.c. or 100 μM in the nucleus accumbens by reversed microdialysis) were recorded. In addition, accumbal dopamine output was monitored by in vivo microdialysis after nicotine challenge. Sham/nicotine animals showed a sensitized locomotor response to systemic and local apomorphine compared to all other groups, including the adrenalectomized/nicotine group. Nicotine increased accumbal dopamine output in all animals. In contrast, nicotine induced a pronounced increase in locomotor activity in the sham/nicotine animals compared to the other vehicle group and the adrenalectomized animals. These results indicate that adrenal steroids are involved in the induction of the postsynaptic component of nicotine sensitization, whereas their involvement in tentative presynaptic changes remains unclear.

Keywords: Adrenal steroid; Dopamine; Microdialysis; Nicotine; Nucleus accumbens; Sensitization

1. Introduction

The abuse potential of nicotine is suggested to be linked to its rewarding and reinforcing properties, involving activation of the brain mesocorticolimbic dopamine system (Clarke, 1990), which is also hypothesized to be the neural substrate underlying drug self-administration in experimental animals. The cell bodies of this system are located in the ventral tegmental area and the axons project mainly to the limbic forebrain, including the nucleus accumbens, the frontal cortex, the amygdala and the septal area (Dahlström and Fuxe, 1964).

Systemic administration of nicotine has repeatedly been shown to increase both the regular and the burst firing activity of dopamine neurons (Grenhoff et al., 1986; Mereu et al., 1987), resulting in increased dopamine output in the nucleus accumbens (Benwell and Balfour, 1992; Benwell et al., 1994; Imperato et al., 1986; Nisell et al., 1994b). Stimulation of nicotinergic acetylcholine receptors in the ventral tegmental area (Nisell et al., 1994a) is critically involved in mediating this nicotine-induced enhancement of dopamine neural activity, even though nicotinergic acetylcholine receptors are also found on dopamine terminals, e.g., in the nucleus accumbens and the amygdala (Clarke and Pert, 1985; Schwartz, 1986; Schwartz et al., 1984).

Several studies have examined the influence of repeated nicotine administration on accumbal dopamine output. The results are contradictory, with some studies showing unaltered (Damsma et al., 1989a) and others enhanced dopamine output (Benwell and Balfour, 1992; Olausson et al., 2000; Wonnacott et al., 1990) after repeated nicotine administration. However, repeated nicotine administration is most

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commonly suggested to enhance dopamine output and is further proposed to induce a transformation of the mesocorticolimbic dopamine system into a sensitized "state", where administration of the same nicotine dose results in an increased response (Robinson and Berridge, 1993). The reinforcing and behavioral stimulant responses are also sensitized after repeated nicotine administration, and in addition to the presynaptic changes (increase in dopamine output), also postsynaptic alterations (Henry and White, 1995; Johnson et al., 1995; Pierce and Kalivas, 1997), such as increased dopamine receptor function (Fung and Lau, 1988) and enhanced locomotor response to the dopamine D1/D2 receptor agonist apomorphine (Suemaru et al., 1993), appear to be involved.

Substantial evidence indicates that the reinforcing and behavioral stimulant effects of nicotine and other drugs of abuse are influenced by environmental factors, in particular by stress and stress hormones. Thus, corticosterone, the principal corticosteroid hormone in the rat, facilitates the locomotor response to psychostimulant drugs, and the hypothalamic–pituitary–adrenal axis has been implicated in sensitization phenomena (Cador et al., 1993). Indeed, amphetamine, cocaine and nicotine stimulate the hypothalamic–pituitary–adrenal axis (Knych and Eisenberg, 1979; Morse, 1989; Swerdlow et al., 1993), and suppression of corticosterone secretion via bilateral adrenalectomy (Marinelli et al., 1994; Swerdlow et al., 1993) or pretreatment with the corticosterone synthesis inhibitor methyrapone (Piazza et al., 1994) reduces the locomotor response to cocaine. Conversely, corticosterone administration to adrenalectomized animals dose dependently increases the locomotor response to cocaine (Marinelli et al., 1997). Moreover, repeated administration of corticosterone, in the range of stress-induced concentrations, increases the locomotor stimulatory effects of drugs of abuse (Deroche et al., 1992; Piazza et al., 1991). In addition, adrenalectomy hampers the development of behavioral sensitization to amphetamine (Rivet et al., 1989) and nicotine (Johnson et al., 1995), effects that are prevented by replacement treatment with corticosterone or the glucocorticoid (type II) receptor agonist dexamethasone but not with the mineralocorticoid (type I) agonist aldosterone.

In this report, we further examined the role of the adrenal glands in induction of the presynaptic (accumbal dopamine release) and/or postsynaptic (functional dopamine receptor sensitivity) components of nicotine sensitization. To this end, locomotor activity and accumbal dopamine output after systemic nicotine (0.2 mg/kg s.c.) were studied in four groups of animals: adrenalectomized/nicotine, adrenalectomized/vehicle, sham/nicotine and sham/vehicle (i.e. adrenalectomized or sham-operated animals previously treated with nicotine (0.4 mg/kg s.c.) or vehicle once daily for 15 days). In addition, locomotor activity, after local (100 μM) as well as systemic (1 mg/kg s.c.) administration of apomorphine, was measured in all four groups of rats.

2. Material and methods

2.1. Animals

Male adult Sprague–Dawley rats weighing between 250 and 300 g were supplied by Beekay (Stockholm, Sweden). The animals were housed in groups of four at a constant cage temperature (25 °C) and humidity (65 °C). The animals were allowed to adapt for 1 week to the novel environment before any experiment was performed. They were kept under regular light–dark conditions (light on at 07:00 a.m. and off at 19:00 p.m.) with free access to standard rat feed (Beekay feed) and tap water. This study was approved by the Ethics Committee for Animal Experiments, Göteborg, Sweden.

2.2. Drugs

(−)-Nicotine ditartrate salt (Sigma, St. Louis, MO) was dissolved in 0.9% NaCl (2 ml/kg), neutralized with $NaHCO_3$ (E. Merck, Darmstadt, Germany) and administered in a dose of 0.4 mg/kg (during sensitization) or 0.2 mg/kg (during experiment) nicotine base (1 or 0.5 mg/kg of the salt). NaCl 0.9% with $NaHCO_3$ (2 ml/kg) was used as the control solution (vehicle). In all experiments, nicotine or vehicle was given s.c. daily for various periods of time. Before and after microdialysis surgery, adrenalectomized and sham-operated rats were treated with corticosterone (Sigma) dissolved in a drop of Tween 80 and 0.9% NaCl (2.5 mg/kg s.c., 2 ml/kg). Apomorphine chloridum (Apoteksbolaget, Göteborg, Sweden) was dissolved in Ringer solution (in mmol/l: NaCl, 140; $CaCl_2$, 1.2; KCl, 3.0; and $MgCl_2$, 1.0) to a concentration of 100 μM or in 0.9% NaCl with a few grains of ascorbate. The apomorphine solution was administered in the perfusate into the nucleus accumbens or systemically (s.c.).

2.3. In vivo microdialysis

Brain microdialysis experiments were performed in awake freely moving animals as described by Waters et al. (1993). Rats were anesthetized with a mixture of Ketalar (Parker-Davis, Solna, Sverige (ketamine 50 mg)) 67 mg/kg i.p. and Rompun®vet (Bayer, Leverkusen) 13 mg/kg i.p. Before positioning of the microprobe, the animals were mounted in a Kopf stereotaxis instrument. The probes were lowered into the nucleus accumbens monolaterally according to co-ordinates obtained from Paxinos and Watson (1986) (A/P +1.85, L/M −1.4, V/D −7.8, relative to the bregma) (). The probes were then fixed to the skull with Phosphatine cement (Dentalhuset, Sweden). After surgery, the rats were allowed to recover for 2 days before the dialysis experiments. On the day of the dialysis experiment, the rats were connected to a microperfusionpump (CMA/100, Carnegie Medicine, Sweden) and placed in locomotor activity boxes (Electrone mobility meter, equipped with 64

photoconductive sensors) where they could move freely. The pump was set to 2 μl/min. Samples (40 μl) were collected every 20 min and injected into the chromatographic system. The perfusion medium was Ringer solution. To analyze dopamine in the fractions, a high-performance liquid chromatography system with electrochemical detection was used (Waters et al., 1993). After completion of the experiment, the animals were killed by decapitation and the location of the probes was determined by sectioning of the brain. Only animals with correctly placed probes were included in the statistical analyses.

2.4. Adrenalectomy

The rats were anesthetized with a mixture of Ketalar (Parker-Davis (ketamine 50 mg)) 67 mg/kg i.p. and Rompun®vet (Bayer) 13 mg/kg i.p. Bilateral adrenalectomy was performed between 8:00 and 11:00 a.m. by exposing the kidneys and removing the adrenal glands. Sham-operated animals underwent the same surgical procedure except for the removal of the adrenal glands. To allow the rats to compensate for salt loss due to the lack of the adrenal gland hormone aldosterone, the rat cages were provided with salt-stones. All animals were allowed to recover from surgery for 1 week before being subjected to further experimentation. The absence of the adrenal glands in adrenalectomized animals was verified by visual inspection after completion of the experiment.

2.5. Locomotor activity recording during chronic treatment with nicotine or vehicle

Locomotor activity was measured by photocell recordings. The animals were allowed a 30-min habituation period, after which they were taken out, injected with nicotine or vehicle and replaced into the boxes. Locomotor activity was recorded for 60 min after drug injection. The counts obtained during the first 5-min period after injection was excluded in order to avoid the influence of unspecific injection-induced hypermotility. During the dialysis experiments, the animals were habituated to the test apparatus for 60 min in parallel with baseline sampling of dopamine.

2.6. Experimental procedure

On day 1, bilateral adrenalectomy or sham operations were performed on male Sprague–Dawley rats. The animals were divided into four groups and allowed to recover for 1 week (groups: sham/nicotine, adrenalectomized/nicotine, sham/vehicle, adrenalectomized/vehicle). All animals recovered and neither baseline locomotor activity nor the acute locomotor response after nicotine on day 1 was altered. All adrenalectomized animals had continuous access to salt-stones and were able to maintain their salt balance.

Nicotine or vehicle was daily administered to the animals from day 1 to 15, and locomotor activity was registered on days 1, 9 and 15. Insertion of a microdialysis probe into the nucleus accumbens was carried out in rats used in the combined dialysis and locomotor activity experiment. The operation was followed by a 2-day recovery period. During the entire dialysis experiment, the animals were placed in activity boxes in which locomotor activity was recorded. The dialysis experiment consisted of Ringer perfusion and sample collection during adaptation (60 min), followed by Ringer perfusion and sample collection for 60 min after s.c. administration of 0.2 mg/kg of nicotine (2 ml/kg s.c.) and, finally, accumbal perfusion of apomorphine in Ringer (100 μM) for 60 min. The systemic dose of nicotine was chosen based on studies indicating that this is a submaximal dopamine-releasing dose, thus allowing for detection of enhanced dopamine release after subchronic nicotine treatment. Rats used for the evaluation of locomotor activity after systemic administration of apomorphine (1 mg/kg s.c.) were first allowed to habituate to the new environment in the locomotor activity boxes for 30 min. All rats were challenged with 1 mg/kg of apomorphine or vehicle s.c. and locomotor activity was recorded for an additional 60 min.

2.7. Statistics

In the dialysis, combined dialysis/locomotor and locomotor experiments data were statistically analyzed using analysis of variance (ANOVA) for repeated measures (dialysis) or factorial measures (locomotor) followed by post hoc analysis by means of the Fisher's Protected Least Significant Difference (PLSD) test. A probability value (P) less than 0.05 was considered statistically significant. All values are expressed as means ± S.E.M.

3. Results

3.1. Nicotine-induced changes in extracellular concentrations of dopamine in the nucleus accumbens

All animals in the different groups were challenged with nicotine (0.2 mg/kg s.c.) directly after time-point 0 (arrow). Accumbal dopamine output was significantly increased after nicotine (0.2 mg/kg s.c.) administration in all groups [time factor for 0–60 min, $F(4,88) = 8.161$, $P \leq 0.0001$]. Neither adrenalectomy nor subchronic nicotine (0.4 mg/kg s.c.) pretreatment significantly altered the nicotine-induced response 20–60 min after nicotine challenge [$F(3,22) = 0.253$, $P = 0.8582$] [interaction factor, $F(6,44) = 0.236$, $P = 0.9625$] (Fig. 1A). Fig. 1B illustrates accumbal dopamine output 20–60 min (time-point 20 + 40 + 60/3, average dopamine output) after nicotine challenge for each group [$F(3,22) = 0.253$, $P = 0.8582$].

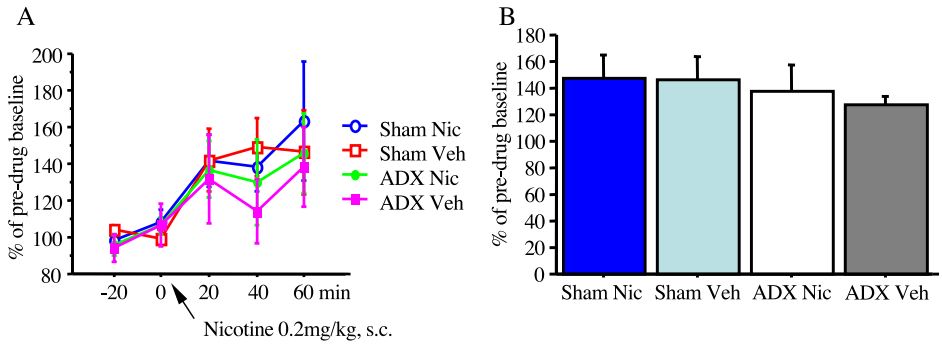

Fig. 1. (A, B) Effects of subchronic nicotine (0.4 mg/kg s.c.) or vehicle pretreatment on accumbal dopamine output after acute nicotine challenge (0.2 mg/kg s.c.). Rats pretreated with nicotine (0.4 mg/kg s.c.) or vehicle for 15 days were challenged with a low dose of nicotine (0.2 mg/kg s.c.) on the experimental day (day 17). Shown are the means ± S.E.M., $n = 5–9$. Statistics: ANOVA for repeated measures (A) and ANOVA for factorial measures, total dopamine output 20–60 min after nicotine challenge (B) followed by Fisher's PLSD.

3.2. Effects of nicotine on locomotor activity

The locomotor activity of adrenalectomized animals did not significantly differ from that of sham-operated controls after acute injection of vehicle. Acute nicotine (0.4 mg/kg s.c.) treatment on day 1 increased locomotor activity in both adrenalectomized and sham-operated animals, and this nicotine-induced response did not differ between the two groups. The nicotine-induced (0.4 mg/kg s.c.) locomotor response was significantly increased in sham-operated rats on days 9 and 14 compared to that observed on day 1 (data not shown).

After a 60-min period of habituation to the locomotor activity boxes and simultaneous baseline sampling of dialysates, all rats were challenged with a low dose (0.2 mg/kg s.c.) of nicotine. As shown in Fig. 2A, the locomotor stimulatory response was elevated in all rats after nicotine challenge compared to baseline values [time factor for 0–60 min, $F(6.186) = 5.541$, $P = 0.0024$]. Nicotine (0.2 mg/kg s.c.) produced a significantly elevated locomotor response in sham/nicotine rats compared to sham/vehicle rats. Sham/nicotine rats also responded with an elevated locomotor response compared to adrenalectomized/nicotine rats during 5–30 min [$F(3,31) = 3.393$, $P = 0.0309$] [interaction factor, $F(15,155) = 0.447$ $P = .9621$] (Fig. 2A). Fig. 2B illustrates total locomotor activity during 5–30 min after nicotine challenge for each group [$F(3,31) = 3.393$ $P = 0.0301$].

3.3. Effects of apomorphine on locomotor activity

The combined dialysis and locomotor activity experiment including the sham/nicotine, adrenalectomized/nicotine, sham/vehicle and adrenalectomized/vehicle groups was completed by perfusion of the dopamine D1/D2 receptor agonist apomorphine (100 μM) into the nucleus accumbens of all animals for 1 h, starting 1 h after the nicotine challenge. As illustrated in Fig. 3, apomorphine increased locomotor activity at all time points (5, 10, 15, 20, 25, 30 and 35 min) after apomorphine perfusion in the sham/nicotine group only [group effect, $F(3,36) = 3.435$ $P = 0.0269$; interaction factor, $F(18,216) = 0.645$ $P = 0.8611$; time factor, $F(6,216) = 0.750$

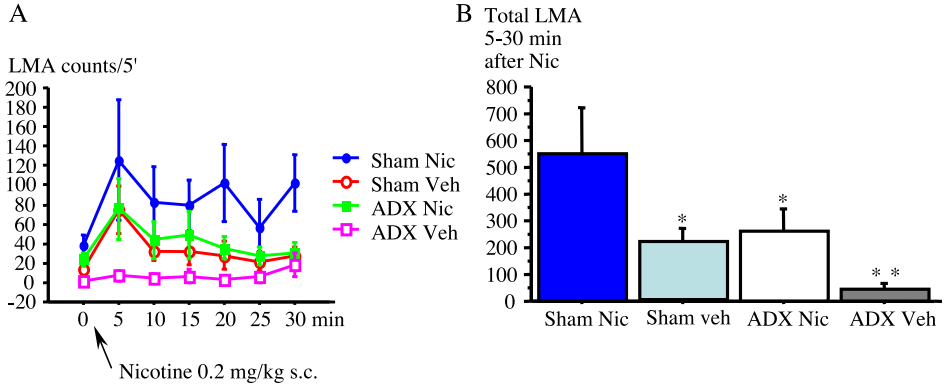

Fig. 2. (A, B) Effects of adrenalectomy or sham operation and subchronic nicotine (0.4 mg/kg s.c.) or vehicle pretreatment on locomotor activity after a low dose of nicotine (0.2 mg/kg s.c.). Shown are the means ± S.E.M., $n = 5–12$. Statistics: ANOVA for repeated measures followed by Fisher's PLSD on data collected during nicotine treatment 5–30 min (A) and ANOVA for factorial measures followed by Fisher's PLSD on total data collected over 5–30 min (B): sham/nicotine vs. sham/vehicle, $P = 0.0202$; sham/nicotine vs. adrenalectomy/nicotine, $P = 0.0458$; sham/nicotine vs. adrenalectomy/vehicle, $P = 0.0052$; sham/vehicle vs. adrenalectomy/nicotine, $P = 0.7076$; sham/vehicle vs. adrenalectomy/vehicle, $P = 0.2717$; and adrenalectomy/nicotine vs. adrenalectomy/vehicle, $P = 0.1719$, $*P \leq 0.05$, $**P \leq 0.01$.

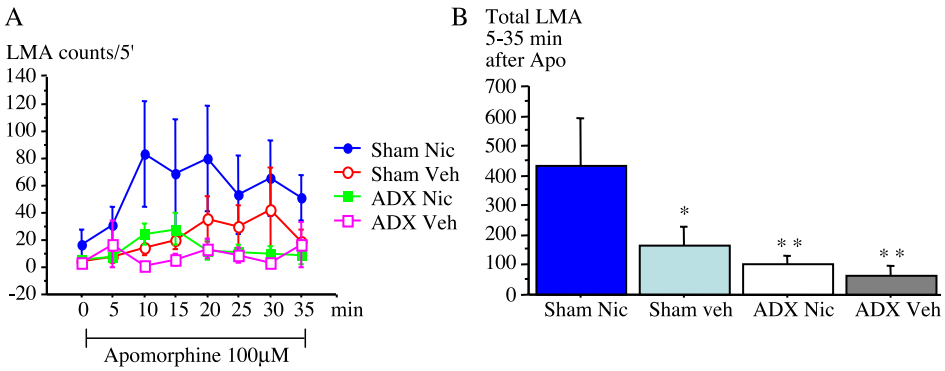

Fig. 3. (A, B) Effects of adrenalectomy or sham operation and subchronic nicotine (0.4 mg/kg s.c.) or vehicle pretreatment on locomotor activity response after local apomorphine (100 µM) challenge. Shown are the mean ± S.E.M., $n = 7–13$ (A, B). Statistics: ANOVA for repeated measures for data collected over 5–35 min followed by Fisher's PLSD (A) and factorial measures for total data collected over 5–35 min followed by Fisher's PLSD (B): sham/nicotine vs. sham/vehicle, $P = 0.0272$; sham/nicotine vs. adrenalectomy/nicotine, $P = 0.0090$; sham/nicotine vs. adrenalectomy/vehicle, $P = 0.0095$; sham/vehicle vs. adrenalectomy/nicotine, $P = 0.5578$; sham/vehicle vs. adrenalectomy/vehicle, $P = 0.4201$; and adrenalectomy/nicotine vs. adrenalectomy/vehicle, $P = 0.7738$, $*P \leq 0.05$, $**P \leq 0.01$.

$P = 0.6096$] (Fig. 3A). Fig. 3B illustrates locomotor activity during 5–35 min after local apomorphine administration for each group [$F(3,36) = 3.435$ $P = 0.0269$].

3.4. Effects of systemically administered apomorphine on locomotor activity

Rats used for the evaluation of locomotor activity after systemic apomorphine, including a sham/nicotine, adrenalectomized/nicotine, sham/vehicle and a adrenalectomized/vehicle group, were challenged with apomorphine, 1 mg/kg s.c., or vehicle solution after a 30-min period of habituation in the locomotor activity boxes. Fig. 4 illustrates locomotor activity 5–60 min after systemic apomorphine administration. Apomorphine induced an increased locomotor response in the sham/nicotine group compared to the adrenalectomized/nicotine and sham/vehicle rats [$F(3,28) = 3.506$ $P = 0.0282$].

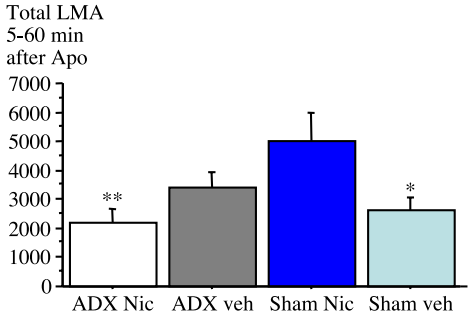

Fig. 4. Effects of adrenalectomy or sham operation and subchronic nicotine (0.4 mg/kg s.c.) or vehicle pretreatment on locomotor activity response after systemic apomorphine (1 mg/kg s.c.). Shown are the means ± S.E.M., $n = 8$. Statistics: ANOVA for repeated measures for data for 5–60 min followed by Fisher's PLSD: sham/nicotine vs. sham/vehicle, $P = 0.0164$; sham/nicotine vs. adrenalectomy/nicotine, $P = 0.0056$; sham/nicotine vs. adrenalectomy/vehicle, $P = 0.0962$; sham/vehicle vs. adrenalectomy/nicotine, $P = 0.6570$; sham/vehicle vs. adrenalectomy/vehicle, $P = 0.4122$; and adrenalectomy/nicotine vs. adrenalectomy/vehicle, $P = 0.2106$, $*P \leq 0.05$, $**P \leq 0.01$.

4. Discussion

In accordance with previous studies, acute nicotine (0.4 mg/kg s.c.) administration to animals habituated to the testing apparatus increased locomotor activity, and this behavioral stimulatory effect was sensitized after daily nicotine (0.4 mg/kg s.c.) pretreatment for 15 days (data not shown). Also, an intermediate dose of nicotine (0.2 mg/kg s.c.) increased the locomotor response in nicotine-sensitized animals compared to control animals on day 15.

Previous investigations indicate that the locomotor stimulatory effect of nicotine is due to dopamine receptor activation in the nucleus accumbens secondary to nicotine-induced liberation of dopamine in this brain area. Sensitization of this stimulant effect has been suggested to involve an enhancement of the dopamine-releasing effect of nicotine as well as an sensitivity of postsynaptic dopamine receptors. In the present study, nicotine increased accumbal dopamine levels but there was no enhancement of this effect after repeated treatment despite the fact that behavioral sensitization was observed after the same dose. After repeated nicotine treatment, there was clear-cut sensitization of the locomotor stimulatory effect of the dopamine D1/D2 receptor agonist apomorphine after both systemic and accumbal administration. Increased dopamine D1 as well as D2 receptor responses have previously been demonstrated not only after repeated nicotine (Suemaru et al., 1993) but also after other drugs of abuse, e.g., amphetamine and cocaine (Henry and White, 1991; Levy et al., 1988). These results thus indicate that the expression of locomotor sensitization to an intermediate dose of nicotine (0.2 mg/kg s.c.) may derive from postsynaptic rather than presynaptic hyperreactivity of the mesocorticolimbic dopamine system.

The lack of enhancement of the dopamine-liberating effect of nicotine after repeated treatment is in accordance with findings of some workers (Damsma et al., 1989b) but in contrast to those of, e.g., Benwell and Balfour (1992) as well as to recent findings from our own group (Olausson et al., 2001). One possible explanation for these discrepancies could be that the facilitation of dopamine release after repeated nicotine is observed mainly in the core but not in the shell of the nucleus accumbens (Birrell and Balfour, 1998). In the present study, the dialysis probe was placed in such a way that sampling was performed simultaneously from the core and the shell. Thus, with a comparatively low challenge dose the enhanced output from the core might have been too small to be noticed when concomitantly sampling from the shell, a region in which no enhancement is to be expected. The reason why we previously observed an increased nicotine-induced dopamine output using the same probe coordinates might have been that the larger challenge dose applied (0.4 mg/kg s.c.) produced a larger dopamine response and thereby possible spillover also to the shell region.

Repeated nicotine administration, as compared to repeated vehicle pretreatment, produced no statistically significant sensitization of the locomotor response to an intermediate dose of nicotine in adrenalectomized animals. Thus, the locomotor response to nicotine was significantly higher in sham-compared to adrenalectomized nicotine-treated rats. These results demonstrate that sensitization to the locomotor stimulatory effect of the psychostimulant nicotine is hampered by adrenalectomy, a conclusion that is consistent with previous results from our research group (Johnson et al., 1995) and with experimental studies on amphetamine-induced sensitization (Rivet et al., 1989). These previous investigations demonstrated that the effect of adrenalectomy most likely is due to a shortage of corticosterone, and, more specifically, to a lack of steroid type II receptor (glucocorticoid) activation. It is reasonable to assume that similar mechanisms were operating here, and in the following discussion, adrenalectomy will therefore be equated with a lack of corticosterone.

Numerous studies has demonstrated that corticosteroids play a critical role in mediating the effects of psychostimulant drugs and that exposure to a stressful environmental stimulus, such as repeated tail-pinch (Piazza et al., 1991) or daily exposure to uncontrollable electric foot-shock (Goeders and Guerin, 1994), enhances individual sensitivity to dopamine-related behavioral (Antelman et al., 1980; MacLennan and Maier, 1983) and neurochemical (Sorg and Kalivas, 1991) effects of psychostimulant drugs. In addition, other studies have demonstrated that glucocorticoids are able to increase dopamine synthesis by acting on tyrosine hydroxylase (Markey et al., 1982; Ortiz et al., 1995) and that glucocorticoids might decrease monoamine oxidase activity (Ho-Van-Hap et al., 1967) as well as dopamine reuptake (Gilad et al., 1987). Stress-induced dopamine neuronal firing (Overton et al., 1996) and accumbal dopamine release also appear to be facilitated by corticosteroids and corticosteroid receptors have been identified in dopamine neurons in the ventral tegmental area (Harfstrand et al., 1986).

In the present study, the enhancement of accumbal dopamine levels after nicotine challenge (0.2 mg/kg s.c.) was, however, not altered by adrenalectomy, indicating that endogenous corticosterone is not involved in mediating the dopamine-liberating effect of a submaximal dose of nicotine. It should be noted that these results do not exclude the possible involvement of corticosterone in modulating spontaneous or nicotine-induced dopamine release under other conditions. Thus, previous studies suggest that corticosterone might determine the higher and longer dopamine responses to stress (Rouge-Pont et al., 1998), and it is likely that plasma corticosterone concentrations need to be elevated above a certain threshold level to promote an increase in dopamine release. Corticosterone in concentrations comparable to those after stress have also been shown to increase dopamine concentrations in rats with a high dopamine tonus, such as during the dark period of the circadian cycle or in so-called high responder rats (Piazza et al., 1996). As mentioned above, the rats in this study were not exposed to any particular stressor and the experiments were performed during the light phase of the circadian cycle, when dopamine tonus is low in rats. These circumstances could have influenced the results obtained.

Since adrenalectomy did not alter nicotine-induced dopamine release but did prevent the behavioral sensitization to nicotine, the reason for this prevention may be looked for on the postsynaptic side of the dopamine system or beyond. Indeed, similar to the results obtained after nicotine challenge, enhanced locomotor response to the dopamine D1/D2 receptor agonist apomorphine, after both systemic and accumbal application, was observed in nicotine-treated sham-operated rats only but not in, e.g., nicotine-treated adrenalectomized rats. These results further tie a tentative up-regulation of postsynaptic accumbal dopamine receptors to behavioral sensitization to nicotine and suggest that corticosteroids are required for this phenomenon. Adrenalectomy has previously been shown to reduce the number of dopamine D1 and D2 receptors in the brain, an effect that is reversed and, in the case of dopamine D1 receptors, even potentiated by chronic dexamethasone treatment (type II receptor agonist). The latter observation is interesting when considering the fact that in the study by Rivet et al. (1989), dexamethasone substitution to adrenalectomized animals potentiated the behavioral sensitization to amphetamine.

In conclusion, the present study indicates that behavioral sensitization to nicotine is associated with concomitant cross-sensitization to the locomotor stimulatory effects of the dopamine D1/D2 receptor agonist apomorphine, and that the latter phenomenon is likely to involve dopamine receptors in the nucleus accumbens. Moreover, the expression of behavioral sensitization to nicotine, at least after an intermediate challenge dose, may be more dependent on

postsynaptic than presynaptic hyperreactivity of the mesolimbic dopamine system. Furthermore, the adrenal glands, i.e., most likely the corticosteroids secreted from the adrenal cortex, appear to promote postsynaptic but not presynaptic sensitization of the mesolimbic dopamine system in response to repeated nicotine exposure. Whether the corticosteroids are involved in the induction and/or the expression of behavioral sensitization to these drugs cannot be determined from the present study but previous results indicate an involvement in the inductive phase (Johnson et al., 1995).

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