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Induction but not expression of behavioural sensitization to nicotine in the rat is dependent on glucocorticoids

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Abstract

Behavioural sensitization has been implicated in the development of addictive behaviour, and several studies suggest that corticosteroids may be involved in this phenomenon. In the present study, the effects of adrenalectomy and steroid replacement treatments on the behavioural sensitization observed after daily injections of nicotine (0.4 mg/kg s.c.) were investigated in the rat. Adrenalectomy completely prevented sensitization to the locomotor stimulating effect of nicotine after repeated injections but did not influence the acute locomotor activating effect of the drug or an already established sensitization to nicotine. In adrenalectomized animals receiving replacement treatment with corticosterone or dexamethasone, but not aldosterone, repeated administration of nicotine produced behavioural sensitization. Repeated dexamethasone treatment per se failed, however, to sensitize rats to nicotine. Post mortem neurochemical studies showed that repeated administration of nicotine significantly increased homovanillic acid (HVA) levels, as well as the dihydroxyphenylacetic acid (DOPAC)/dopamine quotient, in the limbic forebrain. Adrenalectomy per se significantly increased HVA levels and tended to elevate the DOPAC/dopamine quotient. When repeatedly treated with nicotine, adrenalectomized rats displayed a higher DOPAC/dopamine quotient, but no significant difference in HVA levels, compared to nicotine-treated sham-operated controls. In the striatum and the cortex, no significant effects of nicotine treatment or adrenalectomy were observed on any of the neurochemical measures. The present results suggest that glucocorticoid (type II) receptor activation is required for induction of sensitization to the locomotor stimulatory effect of nicotine, whereas corticosteroids are not required for the expression of the behavioural sensitization once established. Provided that HVA levels and the DOPAC/dopamine quotient relatively well reflect the presynaptic dopamine activating effect of nicotine, it may be suggested that corticosteroid-related mechanisms associated with behavioural sensitization to nicotine are post- rather than presynaptically located in relation to mesolimbic dopamine neurons.

Keywords: Adrenalectomy; Corticosteroid; Dopamine; Limbic forebrain; Locomotor activity; Nicotine; Sensitization; (Rat)

1. Introduction

Nicotine is a widely abused drug causing enormous health problems. The abuse liability of nicotine has been suggested to be linked to its rewarding properties (for review see Clarke, 1990) although other hypotheses, e.g. that implicating the anti-anxiety actions of the drug, have been advanced (cf. Pomerleau and Pomerleau, 1990). An increasing amount of evidence suggests that the reinforcing and associated locomotor stimulatory effects of nicotine, as well as of other psychostimulants, are exerted by activation of the mesocorticolimbic dopamine system (e.g. Wise and Bozarth, 1987). For instance, nicotinic acetylcholine receptors are present on nigrostriatal and mesolimbic dopaminergic cell bodies and terminals (Schwartz et al., 1984; Clarke and Pert, 1985), and nicotine increases both the firing of dopaminergic neurons (Lichtensteiger et al., 1982) and dopamine release, especially in the nucleus accumbens (e.g. Imperato et al., 1986). Moreover, microinjection of nicotine into the nucleus accumbens or the ventral tegmemtal area causes hyperlocomotion (Museo and Wise, 1990a,b), and nicotine-induced stimulation of locomotor activity is suppressed by depletion of accumbal dopamine (Clarke et al., 1988) or by microinjections of dopamine receptor antagonists into the nucleus accumbens (Museo and Wise, 1990a; Kita et al., 1992).

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Tolerance development to several effects of nicotine has been observed after repeated administration. However, as for other psychostimulants, the locomotor stimulating effect of nicotine is enhanced after repeated treatment (e.g. Morrison and Stephenson, 1972). This behavioural sensitization is a long-lasting phenomenon and has been implicated in the development of addictive behaviour (Kalivas et al., 1993, for review). For instance, repeated prior drug experience may increase the probability to self-administer amphetamine (Piazza et al., 1990) or cocaine (Horger et al., 1990) in laboratory animals. It is also interesting to note that cross-sensitization to the reinforcing (e.g. Woolverton et al., 1984) and locomotor activating effects (Kalivas et al., 1985; Schenk et al., 1991) of psychostimulants may develop. As regards nicotine, conflicting results have, however, been reported. Thus, repeated nicotine predisposes rats to self-administer a low dose of cocaine (Horger et al., 1992), increases the locomotor response to apomorphine and methamphetamine in the rat (Suemaro et al., 1993), and enhances ethanol intake and preference in the rat (Söderpalm et al., 1993) as well as ethanol-induced locomotion in the mouse (Johnson et al., 1995). On the other hand, repeated nicotine treatment in rats fails to increase the locomotor activating effect of morphine (Vezina et al., 1992) and cocaine (Schenk et al., 1991). In addition, and in contrast to the case of other psychostimulants (e.g. Vezina et al., 1987), sensitization to nicotine can be induced not only after injections into the ventral tegmental area but also after application in the nucleus accumbens (Kita et al., 1992). Taken together, these findings indicate that the neurochemical mechanisms involved in sensitization to nicotine may not be identical to those mediating sensitization to amphetamine and cocaine.

The neurochemical mechanisms underlying behavioural sensitization to nicotine are not fully understood. Studies on dopamine neurochemistry in the nucleus accumbens after repeated treatment with nicotine have shown increased (Clarke et al., 1988), unaltered (Damsma et al., 1989) or decreased dopamine turnover (e.g. Lapin et al., 1989) and are thus contradictory. Both in vivo and in vitro studies have, however, indicated that nicotine-induced dopamine release in the nucleus accumbens may increase in animals repeatedly exposed to nicotinic agonists (Wonnacott et al., 1990; Benwell and Balfour, 1992; see however Damsma et al., 1989). In addition, the locomotor response to both an indirect and a direct dopamine receptor agonist is enhanced in nicotine-sensitized rats (Suemaro et al., 1993), suggesting that both pre- and a post-synaptic alterations of the dopaminergic system may be involved in behavioural sensitization to nicotine. A nicotine-induced up-regulation of central nicotinic acetylcholine receptors could also be of importance for both the behavioural and the neurochemical consequences of repeated nicotine administration (Ksir et al., 1985).

An increasing amount of data implicates the hypothalamo-pituitary-adrenal axis in sensitization phenomena (Koob and Cador, 1993), and amphetamine, cocaine and nicotine are all stimulators of this axis (Knych and Eisenberg, 1979; Morse, 1989; Swerdlow et al., 1993). Interestingly, stress and amphetamine may be interchangeable in their ability to produce sensitization (Antelman et al., 1980), and stressor-induced sensitization to morphine and amphetamine is prevented in animals in which stress-induced corticosterone release is suppressed (Deroche et al., 1992b). Furthermore, repeated corticosterone administration (Deroche et al., 1992a) or implantation of pellets releasing corticosterone in levels similar to those produced by chronic stress causes sensitization to the locomotor stimulatory effects of amphetamine (Cador et al., 1993a). In addition, adrenalectomy prevents behavioural sensitization to amphetamine, unless the animals are treated with the glucocorticoid (type II) receptor agonist dexamethasone (Rivet et al., 1989). It has also been demonstrated that blockade of the hypothalamo-pituitary-adrenal axis by means of corticotropin-releasing factor (CRF) antiserum pretreatment attenuates *d*-amphetamine-induced sensitization (Cole et al., 1990b), and recent experiments suggest that brain CRF systems unrelated to the hypothalamo-pituitary-adrenal axis may also be involved in stressor-induced sensitization and cross-sensitization phenomena (Cador et al., 1993b; Cole et al., 1990a).

In the present study, the effects of adrenalectomy and different corticosteroid replacement treatments on the locomotor sensitizing effect of nicotine were investigated in the rat. Furthermore, alterations of brain dopamine neurochemistry in the limbic forebrain, the striatum and the cortex after sensitization to nicotine in adrenalectomized or sham-operated animals were studied ex vivo by means of high performance liquid chromatography with electrochemical detection (HPLC-ED).

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats supplied by Beekay (Stockholm, Sweden), weighing between 300 and 420 g, were housed in single cages and maintained at a constant cage temperature (25°C) and humidity (65%). The animals were kept under regular light-dark conditions (light on at 5:00 a.m. and off at 7:00 p.m.) and had free access to rat standard feed (Beekay Feeds) and tap water. In all experiments drug-naive animals were used. An adaptation period of at least 7 days to

the animal maintenance facilities of the department was allowed prior to the start of the experiments. This study was approved by the Ethics Committee for Animal Experiments, Göteborg, Sweden.

2.2. Adrenalectomy

Animals were anaesthetized with a mixture of ketamine, 65 mg/kg (Parke-Davies, Barcelona, Spain) and xylazin, 15 mg/kg (Bayer, Leverkusen, Germany). Bilateral adrenalectomy was performed between 9 a.m. and 2 p.m. by exposing the kidneys and removing the adrenal glands. Sham-operated rats were submitted to the same surgical procedure except for removal of the adrenal glands. To permit the rats to compensate for the salt loss caused by the adrenalectomy, these animals were supplied with water containing 0.5% NaCl. In all experiments animals were allowed one week of recovery after surgery. The rats were weighed at least once a week during the course of chronic drug treatment. The absence of the adrenal glands was verified by ocular inspection after the experiments.

2.3. Drugs

Corticosterone (Sigma Chemical Co., St. Louis, MO, USA), when administered chronically, was dissolved in the drinking fluid (25 mg/1000 ml tapwater) presented to the animal (approximately 2.6 mg/kg/day; see Akana et al., 1985). When given acutely, corticosterone was suspended in distilled water with Tween 80 (0.5%) and administered per os (p.o.) in a dose of 2.5 mg/kg (2 ml/kg). Aldosterone (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in 0.5% ethanol, gently warmed and diluted in 0.9% NaCl and administered subcutaneously (s.c.) in a dose of 0.25 mg/kg. Dexamethasone (MSD, Haarlem, Netherlands) 0.5 mg/kg (s.c.) was diluted in 0.9% NaCl. Aldosterone and dexamethasone were administered once daily between 4 and 7 p.m. in volumes of 2 ml/kg. Steroid substitutions were started one day post-surgery and were administered to the animals in their home cages. (−)-Nicotine ditartrate salt (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% NaCl (2 ml/kg) and administered s.c. in a dose of 0.4 mg/kg nicotine base (1.0 mg/kg of the salt). In all experiments, starting from the eighth post-operative day, nicotine or vehicle was given once daily for various periods of time, and, when applicable, contingent with steroid substitution treatments. The rats received all nicotine injections in their home cages except on locomotor activity testing days.

2.4. Locomotor activity studies

Locomotor activity was measured by photocell recordings as previously described (Söderpalm et al., 1991). The instrument (M/P 40 Electronic Mobility Meter, Motron Products, Stockholm, Sweden) was equipped with 40 photoconductive sensors (5 rows × 8, centre/centre distance 40 mm) covered by a translucent floor, upon which a Plexiglas test cage (21 × 32 × 35 cm) was placed. The number of counts, representing all light beam interruptions of any of the sensors, was printed by external timer-controlled counters every 5 min.

Untreated animals exposed to a novel environment display an initially high (exploratory) motor activity followed by a decline (Ahlenius et al., 1973), thus reflecting a habituation procedure. Consequently, animals respond differently to drug treatment depending upon their baseline activity (i.e. hyperactivity is more easily detected in habituated rats, as is sedation in nonhabituated animals).

Animals were allowed a 60-min habituation period, after which they were taken out, injected whith the drugs concerned and replaced into the boxes. Locomotor activity was recorded for 65 min after drug injection. The counts obtained during the first 5-min period after injection were excluded in order to avoid influence of unspecific injection-induced hypermotility. All experiments were run between 9 a.m. and 5 p.m in a randomized order.

2.5. Experimental design

Experiment No. 1: Twenty-one animals were divided into one sham-operated group and one group subjected to adrenalectomy. One half of the animals in each group received daily injections of vehicle for 10 days and the other half nicotine, thus forming the groups: Sham/Vehicle, Sham/Nicotine, Adrenalectomy/Vehicle and Adrenalectomy/Nicotine ($n = 4$–6 all groups). Locomotor activity was measured on day 1, 5 and 10. On day 10, after locomotor activity testing, the animals were killed and their brains were collected for determination of monoamine levels.

Experiment No. 2: Adrenalectomized animals receiving corticosterone replacement treatment in the drinking water were subjected to daily injections of vehicle or nicotine for 10 days, thus forming the groups: Adrenalectomy + Corticosterone/Vehicle and Adrenalectomy + Corticosterone/Nicotine ($n = 6$ both groups). Locomotor activity was measured on day 1 and 10.

Experiment No. 3: Adrenalectomized animals receiving daily injections of aldosterone, dexamethasone or vehicle were treated with nicotine once daily for 10 days, thus forming the groups: Adrenalectomy + Vehicle/Nicotine, Adrenalectomy + Aldosterone/Nicotine, Adrenalectomy + Dexamethasone/Nicotine ($n = 7$–8 all groups). Locomotor activity was recorded on day 1 and 10.

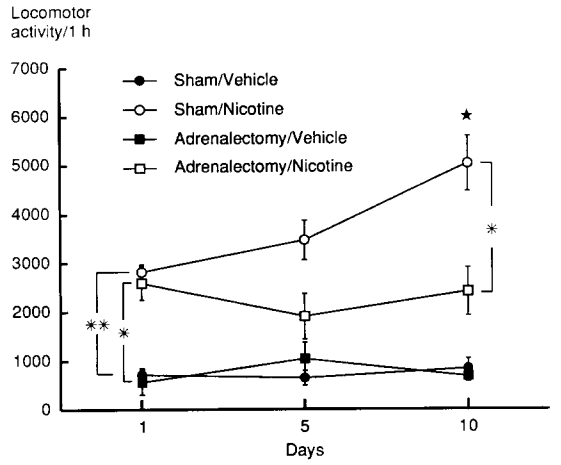

Fig. 1. Effects of adrenalectomy on locomotor activity in rats treated with nicotine (0.4 mg/kg s.c.) or vehicle daily for 10 days. Shown are means ± S.E.M., $n = 4–6$ all groups. Statistics: the Friedman test followed by Wilcoxon's signed ranks test for paired measures: $^{*}P < 0.05$, compared to day 1 value, the Kruskall-Wallis test followed by Mann-Whitney U-test for unpaired measures, $^{*}P < 0.05$, $^{**}P < 0.01$.

Experiment No. 4: Sham-operated rats receiving daily injections of dexamethasone and adrenalectomized rats receiving daily dexamethasone or vehicle were given vehicle once daily for 10 days, thus forming the groups: Sham + Dexamethasone/Vehicle, Adrenalectomy + Dexamethasone/Vehicle, Adrenalectomy + Vehicle/Vehicle ($n = 6$ or 9 all groups). Locomotor activity was recorded on day 1 and 10. On day 11, all animals were challenged with nicotine and locomotor activity was recorded.

Experiment No. 5: Adrenalectomized animals were given daily nicotine injections for 30 days. Locomotor activity was first tested on day 1. On day 10 and 30, one half of the animals (Adrenalectomy/Nicotine + Acute corticosterone) were given corticosterone (2.5 mg/kg p.o.), 1 and 6 h, respectively, before being tested for nicotine-induced locomotor activity. Control animals (Adrenalectomy/Nicotine + Acute vehicle) received the corresponding vehicle ($n = 6$ both groups).

Experiment No. 6: Animals ($n = 10$) received daily injections of nicotine for 15 days and were tested for locomotor activity on day 1 and 15. On day 16 the rats were subjected to adrenalectomy and were allowed to recover for 10 days (during this period no nicotine injections were given). On day 25, the animals were challenged with nicotine and locomotor activity was recorded.

2.6. Biochemical analysis of brain monoamines

Sixty-five minutes after drug administration the animals were decapitated and the brains were taken out and placed on a chilled Petri dish. The limbic forebrain (including the olfactory tubercles, the amygdala, the nucleus accumbens and septum), the striatum and the cortex were dissected out according to Carlsson and Lindqvist (1973) and were stored at −70°C until analyzed by high-performance liquid chromatography with electrochemical detection (HPLC-ED) according to standard principles (Svensson, 1986).

2.7. Statistics

The locomotor activity data were statistically analyzed by using the Friedman test, followed by Wilcoxon's signed ranks test for paired measures and the Kruskall-Wallis test, followed by the Mann-Whitney U-test, for unpaired measures. The monoamine data were evaluated by using a one-way analysis of variance (ANOVA), followed by Fisher's protected least-significant difference test (PLSD). 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. Locomotor activity studies

As shown in Fig. 1, the locomotor activity of adrenalectomized animals did not differ from that of sham-operated controls after an acute vehicle injection. Acute nicotine treatment (day 1) approximately doubled locomotor activity in both sham-operated and adrenalectomized rats, and this locomotor activity response to nicotine did not differ between the two groups. The nicotine-induced locomotor activity was significantly enhanced in sham-operared rats after 10 daily injections of nicotine, compared to that observed

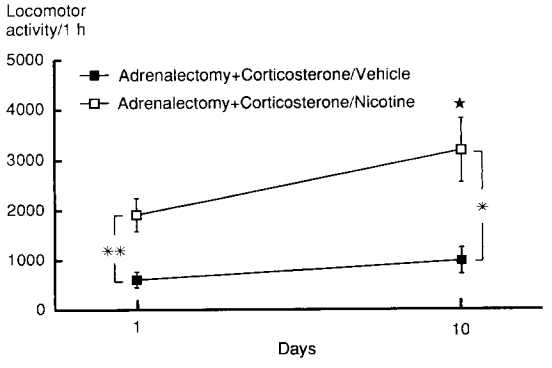

Fig. 2. Effects of daily injections of vehicle or nicotine (0.4 mg/kg s.c.) on locomotor activity in adrenalectomized animals receiving replacement treatment with corticosterone in their drinking fluid. Shown are means ± S.E.M., $n = 6$ both groups. Statistics: Wilcoxon's signed ranks test for paired measures: $^{*}P < 0.05$, Mann-Whitney U-test for unpaired measures, $^{*}P < 0.05$, $^{**}P < 0.01$.

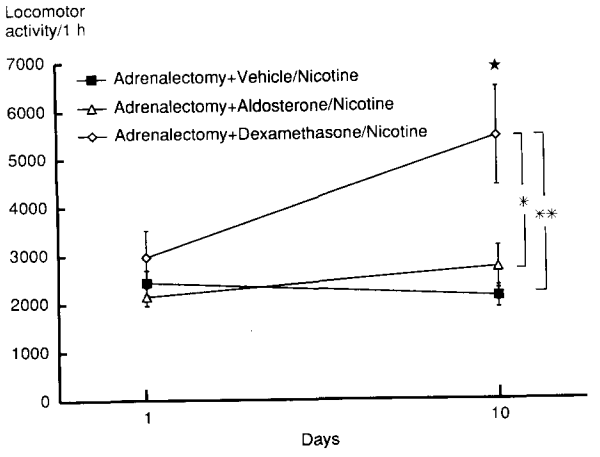

Fig. 3. Effects of adrenalectomy and replacement treatments with vehicle, dexamethasone (0.5 mg/kg s.c. once daily) or aldosterone (0.25 mg/kg s.c. once daily) on locomotor activity in rats treated with nicotine (0.4 mg/kg s.c.) daily for 10 days. Shown are means ± S.E.M., $n = 7$ or 8 all groups. Statistics: Wilcoxon's signed ranks test for paired measures: $^{*}P < 0.05$, the Kruskall-Wallis test followed by Mann-Whitney U-test for unpaired measures, $^{*}P < 0.05$, $^{**}P < 0.01$.

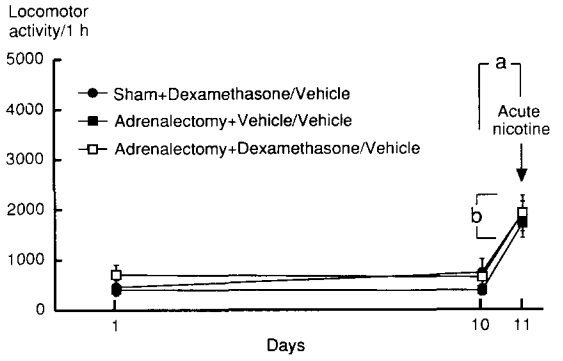

Fig. 4. Lack of effect of dexamethasone treatment (0.5 mg/kg s.c. once daily) on locomotor activity in sham-operated and adrenalectomized rats receiving daily injections of vehicle (day 1–10), and an acute nicotine challenge (0.4 mg/kg s.c.) (day 11). Shown are means ± S.E.M., $n = 6$ or 9 all groups. Statistics: Wilcoxon's signed ranks test for paired measures: [a] Sham + Dexamethasone/Vehicle and Adrenalectomy + Vehicle/Vehicle, $P < 0.05$; Adrenalectomy + Dexamethasone/Vehicle, $P < 0.01$, compared to day 10 value, Mann-Whitney U-test for unpaired measures; [b] no significant differences were seen between the groups.

on day 1, whereas in adrenalectomized animals no such enhancement was observed.

As shown in Fig. 2, repeated administration of nicotine to adrenalectomized animals receiving corticosterone replacement treatment in their drinking fluid significantly enhanced the locomotor activity response to nicotine on day 10, compared to that observed on day 1. Corticosterone replacement treatment by itself did not alter the locomotor activity of adrenalectomized animals.

As shown in Fig. 3, repeated nicotine administration to adrenalectomized animals subjected to replacement treatment with dexametasone, but not with aldosterone, resulted in a significantly increased locomotor activity response to nicotine on day 10, compared to that observed on day 1. No significant difference in lococomotor activity was observed between the groups on day 1.

As shown in Fig. 4, daily injections of dexamethasone to sham-operated or adrenalectomized animals did not affect locomotor activity after a vehicle challenge on day 1 or 10, compared to that observed in vehicle-treated adrenalectomized controls. Acute nicotine administration (day 11) to the dexamethasone- or vehicle-pretreated animals resulted in a significant increase in locomotor activity compared to day 10. The nicotine-induced locomotor activity did not differ between the groups.

As shown in Fig. 5, acute corticosterone treatment, 1 or 6 h before locomotor activity testing in adrenalectomized animals treated daily with nicotine (10 and 30 days), failed to alter nicotine-induced locomotor stimulation, compared to that observed on day 1.

As shown in Fig. 6, adrenalectomy in animals already sensitized to nicotine by 15 daily injections did not alter the sensitized locomotor activity response to an acute nicotine challenge 10 days postoperatively.

3.2. Brain monoamine studies

As shown in Figs. 7 and 8, there was a significant elevation of homovanillic acid (HVA) levels and a tendency for an increase in the dihydroxyphenylacetic acid (DOPAC)/dopamine quotient in the limbic fore-

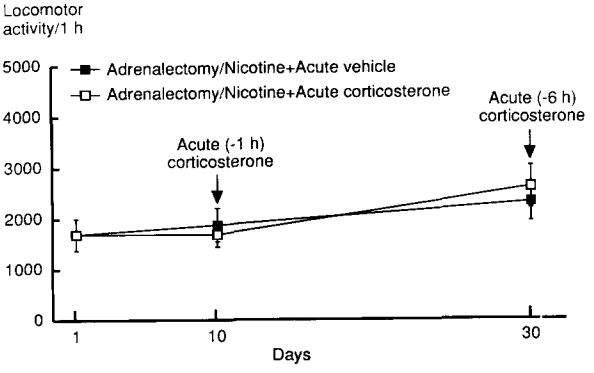

Fig. 5. Lack of effect of acute vehicle or corticosterone (2.5 mg/kg p.o.) on locomotor activity in adrenalectomized rats receiving daily injections of nicotine (0.4 mg/kg s.c.). Corticosterone or vehicle was given 1 or 6 h before locomotor activity recording on day 10 and 30, respectively. Shown are means ± S.E.M., $n = 6$ both groups. Statistics: Wilcoxon's signed ranks test for paired measures, Mann-Whitney U-test for unpaired measures. Locomotor activity did not differ significantly between groups or over time throughout the experiment.

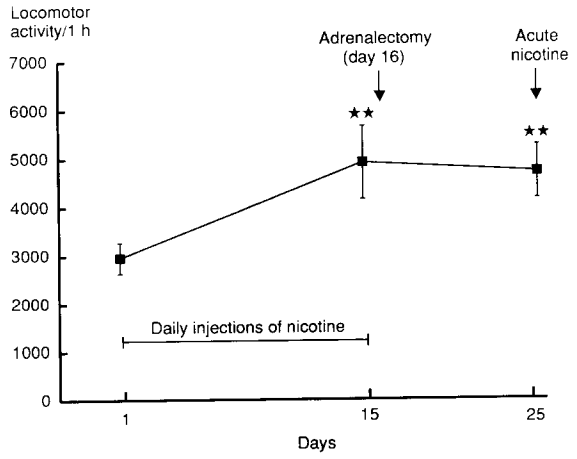

Fig. 6. Lack of effect of adrenalectomy on the expression of nicotine-induced locomotor activity in animals sensitized to nicotine (0.4 mg/kg s.c.). Shown are means ± S.E.M., $n = 10$. Statistics: Wilcoxon's signed ranks test: $^{**}P < 0.01$, compared to day 1 value. No significant difference was seen between day 15 and 25.

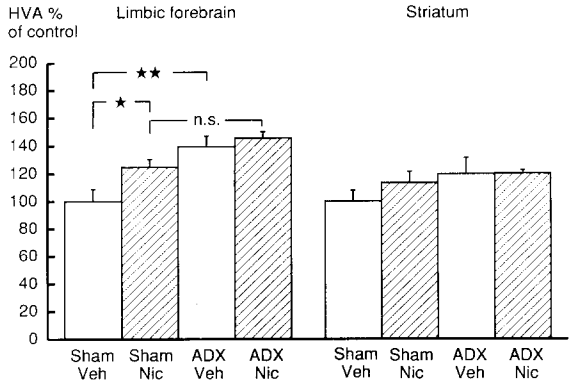

Fig. 8. Effects of 10 daily injections of vehicle or nicotine (0.4 mg/kg s.c.) in sham-operated and adrenalectomized (ADX) rats on homovanillic acid (HVA) levels in the limbic forebrain and the striatum. Results are expressed as percent of controls (limbic forebrain: 126 ng/g, striatum: 575 ng/g). Shown are means + S.E.M., $n = 4$–6 all groups. Statistics: ANOVA followed by Fisher's PLSD, $F(3,17) = 6.271$, $P = 0.0046$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{n.s.}P > 0.05$.

brain of adrenalectomized rats, compared to that in the limbic forebrain of sham-operated controls. Sham-operated rats receiving 10 days of nicotine treatment, compared to vehicle-treated animals, displayed an increased limbic forebrain HVA and DOPAC/dopamine quotient after acute nicotine. Nicotine-treated adrenalectomized rats, as compared to nicotine-treated sham-operated animals, showed a significantly higher DOPAC/dopamine quotient but no difference in HVA levels after acute challenge with nicotine.

In the striatum and cortex, no effects of nicotine treatment or adrenalectomy were observed on any of the measures. In the cortex HVA levels were not detectable. No significant alterations in dopamine, DOPAC, 5-HT, 5-HIAA or in the 5-HIAA/5-HT quotient were observed in any of the brain regions studied after nicotine treatment or adrenalectomy (data not shown).

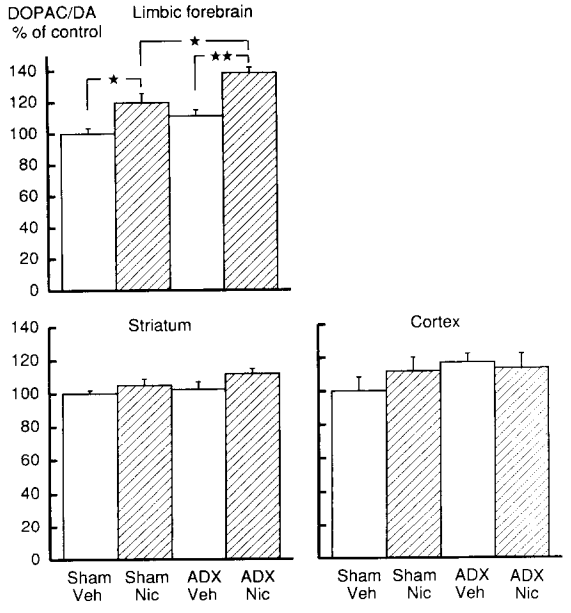

Fig. 7. Effects of 10 daily injections of vehicle or nicotine (0.4 mg/kg s.c.) in sham-operated and adrenalectomized (ADX) rats on the dihydroxyphenylacetic acid (DOPAC)/dopamine (DA) quotient in the limbic forebrain, striatum and cortex. Results are expressed as percent of controls. Shown are means + S.E.M., $n = 4$–6 all groups. Statistics: ANOVA followed by Fisher's PLSD, $F(3,17) = 9.451$, $P = 0.0007$, $^{*}P < 0.05$, $^{**}P < 0.01$, compared to controls.

4. Discussion

The present results demonstrate that sensitization to the locomotor stimulating effect of nicotine is prevented by adrenalectomy. This is consistent with the effect of adrenalectomy on amphetamine-induced sensitization (Rivet et al., 1989). Adrenalectomy, however, did not modify the acute locomotor activity enhancing effect of nicotine, which contrasts to recent findings showing that adrenalectomy reduces the acute locomotor activating effects of amphetamine (Cador et al., 1993a), cocaine and morphine (Marinelli et al., 1994).

The prevention of sensitization by adrenalectomy was abolished by replacement treatment with corticosterone or dexamethasone which both are agonists at the glucocorticoid (type II) receptor but not by the mineralocorticoid (type I) receptor agonist, aldosterone. The dose of aldosterone used was selected based on previous results showing that it reverses the increased salt intake observed in adrenalectomized animals and, hence, is physiologically active (Fahlke et al., 1994). This may argue against the possibility of the

present negative result being due to application of a too low dose of aldosterone. Furthermore, the present results are compatible with the findings of Rivet et al. (1989) and suggest that stimulation of brain glucocorticoid (type II), rather than mineralocorticoid (type I) receptors, is of importance for the initiation of sensitization. The importance of mineralocorticoid (type I) receptors in behavioural sensitization to nicotine can, however, not be ruled out based on the present experiments, since only one dose of aldosterone was used.

It should be noted that, although the results were obtained in different experiments, the nicotine sensitization obtained after dexamethasone replacement appeared more pronounced than after corticosterone. This could be explained by the fact that dexamethasone is a more potent agonist at the glucocorticoid receptor than corticosterone. These findings are consistent with those of Cador et al. (1993a), who demonstrated that high but not low levels of corticosterone sensitize animals to amphetamine. This together with the fact that corticosterone occupies mineralocorticoid receptors already at moderate levels, whereas glucocorticoid receptors are occupied only at high levels of the hormone, such as after stress or at the diurnal peak of corticosterone (Reul and De Kloet, 1985), further implicate the type II receptor in the sensitization phenomenon.

Acute injection of corticosterone to adrenalectomized animals treated daily with nicotine for 10 or 30 days did not change the locomotor activity observed after nicotine challenge. The dose of corticosterone used approximately equals the daily dose obtained by the above described corticosterone drinking substitution protocol and has previously been demonstrated to exert behavioural effects in adrenalectomized animals (Söderpalm and Engel, 1992). The results obtained weaken the possibility that acute receptor activation by corticosterone is required for expression of the sensitization phenomenon and suggests instead an inductive role for glucocorticoids in nicotine sensitization, possibly through genomic actions. This view is further substantiated by the finding that adrenalectomy had no effect on nicotine-induced locomotor activity in already sensitized animals. These results show that once sensitization to nicotine has been established the presence of glucocorticoids is not required for the expression of the phenomenon.

Chronic dexamethasone treatment of adrenalectomized or sham-operated rats did not influence spontaneous or nicotine-induced locomotor activity. These results differ from those of Deroche et al. (1992a), showing that repeated corticosterone treatment sensitizes animals to the locomotor stimulatory effect of amphetamine. One explanation to this discrepancy could be that amphetamine and nicotine sensitization do not involve identical neurochemical mechanisms (see Introduction). Another possibility is that the weight loss observed and possible associated malaise after dexamethasone (data not shown), counteract or conceal a sensitization or cross-sensitization phenomenon. However, the facts that also dexamethasone treated sham-operated animals, which displayed less of a weight loss, failed to show cross-sensitization, and that a similar dexamethasone treatment did allow the development of nicotine sensitization in adrenalectomized animals (experiment No. 4), weaken this hypothesis.

In the neurochemical part of the study it was found that the HVA levels in the limbic system but not in the striatum were significantly higher in vehicle-treated adrenalectomized animals compared to vehicle-treated sham-operated rats. The limbic and the cortical DOPAC/dopamine quotients also tended to be elevated in the former group. Rastogi and Singhal (1978) have previously obtained evidence that adrenalectomy increases dopamine turnover in the rat cortex, but also in the striatum, an effect that was reversed by chronic corticosterone administration.

In this study, acute administration of nicotine to animals that had been repeatedly treated with nicotine raised the DOPAC/dopamine quotient and the HVA levels in the limbic system, but not in the striatum or the cortex, compared to those observed after vehicle challenge of chronically vehicle-treated rats. These findings appear contrasting to those of Vezina et al. (1992) who observed a higher DOPAC/dopamine quotient in the prefrontal cortex than in the nucleus accumbens of rats repeatedly treated with nicotine. However, the results are not easily compared due to different dissection techniques; while Vezina et al. (1992) studied only the prefrontal cortex and the nucleus accumbens, the entire cortex and limbic system were included in the present analysis.

In adrenalectomized rats repeatedly treated with nicotine, dopamine turnover was unchanged or higher than in nicotine-sensitized sham-operated animals. Thus, adrenalectomy, if anything, enhanced rather than counteracted the neurochemical consequences of chronic nicotine treatment measured in the present study. Since the behavioral outcome of adrenalectomy was quite the opposite, these findings indicate that the steroid-dependent part of the sensitization phenomenon is not related to the measures of presynaptic dopamine activity studied here.

Although a presynaptic involvement cannot be totally ruled out until corresponding studies on nicotine-induced dopamine release have been performed, the above findings may indicate that the steroid-dependent component of the nicotine sensitization phenomenon is located distally to dopamine neurons. Indeed, adrenalectomy has previously been shown to reduce the number of dopamine D_1 and D_2 receptors in most brain regions, an effect that is reversed, and, in the

case of D_1 receptors, even potentiated by chronic dexamethasone treatment (Biron et al., 1992; see however Faunt and Crocker, 1989). Thus, one explanation to the prevention of nicotine sensitization caused by adrenalectomy could be that adrenal removal disrupts a tentative nicotine-induced up-regulation of post-synaptic dopamine receptor function. Increased dopamine D_1 as well as D_2 receptor responsivity has been demonstrated after sensitization to several other drugs of abuse, e.g. amphetamine and cocaine (Levy et al., 1988; Henry and White, 1991). Indeed, the neurochemical findings may lend some support for this hypothesis. A tentative reduction of postsynaptic dopamine receptor responsiveness in adrenalectomized animals would be expected to produce both a feedback-mediated compensatory increase in dopamine turnover and an enhanced dopamine turnover-stimulating effect after repeated nicotine. Both these alterations were observed in the present study.

As mentioned in the Introduction, repeated nicotine treatment is probably associated both with increased dopamine release in the nucleus accumbens and with increased postsynaptic dopamine receptor responsivity (Suemaro et al., 1993). The present findings may indicate that the latter effect is a prerequisite for the behavioral expression of nicotine sensitization. Interestingly, adrenalectomy has been shown to reduce the expression of G_{sa} and increase that of G_{ia}, changes that could reduce the nicotine-induced activation of the dopamine system (Saito et al., 1989). The mechanism(s) by which glucocorticoid receptor agonists modulate the signal transduction system possibly involved in initiating behavioural sensitization remain(s), however, to be elucidated.

In conclusion, the present study indicates that induction of sensitization to nicotine is dependent on glucocorticoid (type II) receptor stimulation, whereas the presence of corticosteroids is not required for the expression of nicotine-induced locomotor activity in non-sensitized or sensitized animals. Moreover, in our hands, chronic treatment with a selective glucocorticoid agonist alone does not sensitize rats to nicotine. Furthermore, the neurochemical results of the study suggest that the steroid-dependent part of the sensitization phenomenon to nicotine may be post-synaptically located in relation to mesolimbic dopamine neurons. Our data corroborate and extend earlier findings implicating corticosteroids in behavioural sensitization to psychostimulants.

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