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Subchronic Caffeine Exposure Induces Sensitization to Caffeine and Cross-Sensitization to Amphetamine Ipsilateral Turning Behavior Independent from Dopamine Release

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We have recently shown that repeated exposure to caffeine sensitizes rats to the motor activating effects of dopamine D_1 and D_2 receptor agonists. In order to study the role of dopamine in this effect, sensitization to caffeine and cross-sensitization between caffeine and amphetamine was evaluated by studying turning behavior and *in vivo* striatal dopamine release in unilaterally 6-hydroxydopamine-lesioned rats. Administration of caffeine (15 mg/kg) for 2 weeks, on alternate days, induced a significant increase in ipsilateral turning behavior during the course of treatment, indicating that sensitization to caffeine took place in the intact striatum. Caffeine modestly increased dopamine release in the intact dorsa-lateral striatum and no significant difference between the first (+38%) and the last (+51%) injection was observed. Amphetamine (2 mg/kg) induced a significantly higher ipsilateral turning behavior in caffeine-sensitized rats than in vehicle-pretreated rats, however, a similar increase in dopamine release (+900 and +800%) was observed in the two groups. The results are the first demonstration that caffeine pre-exposure sensitizes the motor-stimulant effects of caffeine itself and of amphetamine. Sensitized ipsilateral turning after caffeine and amphetamine are not correlated to modification in striatal dopamine release, rather, postsynaptic modifications in dopamine and adenosine receptor interaction might be involved in the sensitization phenomena observed.

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INTRODUCTION

Caffeine psychomotor effects are influenced by dopamine transmission and both acute and long-term interactions between caffeine and dopamine agonists have been described. The basis for this reciprocal influence are attributed to the negative interaction between dopamine D_1 and D_2 receptors and adenosine A_1 and A_{2A} receptors that are blocked by caffeine (Ferré *et al*, 1997; Fredholm *et al*, 1999). Caffeine and theophylline acutely potentiate the motor-activating effects induced by dopamine receptor agonists (Ferré *et al*, 1991; Fredholm *et al*, 1983; Garrett and Griffiths, 1997; Kuribara, 1994; Misra *et al*, 1986) and reverse catalepsy induced by dopamine receptor antagonists (Hauber *et al*, 2001; Malec, 1997; Mandhane *et al*, 1997), whereas sensitization or upregulation of dopamine receptors sensitize rats to the motor-activating effect induced by caffeine or theophylline (Fenu *et al*, 2000; Fenu and Morelli, 1998; Ferré *et al*, 1994).

We and others (Cauli and Morelli, 2002; Gasior *et al*, 2000) have recently demonstrated that low stimulant doses of caffeine administered either parenterally in spaced administration or continuously in drinking solution, in contrast to daily administration or continuous oral intake of high doses, which induce tolerance to caffeine motor stimulant effects (Garrett and Holtzman, 1995; Holtzman and Finn, 1988), sensitize rats to the motor-stimulant effects induced by dopamine agonists.

The rotational (turning) behavior of rodents lesioned unilaterally in the nigrostriatal pathway with 6-hydroxydopamine (6-OHDA) (Ungerstedt, 1971) allows the study of motor responses induced by drugs that modulate dopamine transmission in the striatum. Turning behavior provides a model that has been widely used to evaluate in a quantitative way the sensitizing effects of drugs that act as direct or indirect agonists at the dopamine receptor level (Badiani *et al*, 1997; Henry *et al*, 1998; Pinna *et al*, 2001).

In unilaterally 6-OHDA-lesioned rats, caffeine and related methylxanthines have been reported to induce contralateral

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turning behavior (Fredholm *et al*, 1983; Fuxe and Ungerstedt, 1974; Herrera-Marschitz *et al*, 1988). A previous study of our group, however, showed that caffeine elicited contralateral turning behavior only in rats repeatedly primed with a dopamine receptor agonist (Fenu and Morelli, 1998).

In order to evaluate the role of dopamine in the motor effects produced by caffeine, we administered caffeine in a subchronic protocol of spaced injections in unilaterally 6-OHDA-lesioned rats not repeatedly primed with dopamine receptor agonists and studied turning behavior and striatal dopamine release by *in vivo* microdialysis. Moreover, in order to investigate if spaced caffeine administrations modified the effects of drugs that increase dopamine release, amphetamine-induced turning behavior and dopamine release in the nonlesioned striatum of caffeine-sensitized rats was evaluated. Doses of caffeine used in this study correspond to human consumption of approximately four/five cups of coffee.

EXPERIMENTAL PROCEDURE

Animals

Male Sprague–Dawley rats (Charles River, Calco, Italy) were used in all experiments. Rats were housed in groups of five in polycarbonate cages (33 *w*, 56 *l*, 20 *h*, cm) and were allowed to settle under standard conditions (lights on 08.00–20.00; temperature 23°C) with free access to food and water. Behavioral tests were performed between 10.00 and 13.00.

All experiments were conducted in accordance with the guidelines for care and use of experimental animals of the European Communities Directive (86/609/EEC; D.L., 27.01.1992, No. 116).

6-OHDA Lesions

In order to lesion the dopaminergic nigrostriatal pathway, rats (275–300 g) were anesthetized with chloral hydrate (400 mg/kg i.p.), placed on a David Kopf stereotaxic apparatus and injected unilaterally into the left medial forebrain bundle at coordinates $A = -2.2$, $L = +1.5$, $V = -7.8$ according to the atlas of Pellegrino *et al* (1979). 6-OHDA-HCl (8 μg/4 μl of saline containing 0.05% ascorbic acid) was delivered through a stainless cannula, at a rate of 1 μl/min. The injection needle was kept in place for an additional 2 min, upon completion of the injection. All rats were pretreated with desipramine (10 mg/kg i.p.) in order to prevent damage to noradrenergic neurons.

Evaluation of Turning Behavior

Rotational behavior was measured by placing rats in hemispherical bowls with sawdust on the floor and connecting them to an automated rotameter system (Carnegie Medicine, Sweden). A direction-sensitive rotation sensor detected the number of full (360°) rotations in any direction. Rats were placed in each apparatus 30 min before drug administration in order to acclimatize and let them extinguish any spontaneous rotational behavior.

At 10 days after 6-OHDA infusion, rats were challenged with an injection of apomorphine (0.2 mg/kg s.c.). Rats not showing at least 100 contralateral rotations during the 1-h-testing period were eliminated from the study. Rats were divided into two experimental groups (caffeine or vehicle) on the basis of apomorphine-induced contralateral turning behavior .

Experiments consisted of two phases: induction of caffeine sensitization and expression of amphetamine cross-sensitization.

Induction of Caffeine Sensitization

At 2 days after apomorphine challenge, rats received caffeine (15 mg/kg i.p.) or vehicle (i.p.) in spaced injections, on every other day, for 2 weeks (seven total administrations). Turning behavior was measured in hemispherical bowls for 3 h after each injection as described above.

Expression of Amphetamine Cross-Sensitization

At 3 days after the last caffeine or vehicle administration, all rats received the challenge injection of D-amphetamine (0.25 or 2 mg/kg i.p.). Turning behavior was measured for 3 h after drug challenge as described above.

Microdialysis Studies

Surgery and treatments. A separate group of unilateral 6-OHDA-lesioned rats were anesthetized with chloral hydrate (400 mg/kg i.p.) placed in the David Kopf stereotaxic apparatus and implanted bilaterally in the dorsolateral striatum with vertical microdialysis probes. The microdialysis membranes (AN 69 Hospal membrane; 220 μm ID and 310 μm OD; molecular weight cutoff $>15\,000$ Da) were 3 mm long. The coordinates used for implantation of the microdialysis probe were 0.7 mm anterior and 3.2 mm lateral to the bregma and 6.5 mm ventral from dura madre (Paxinos and Watson, 1998). The external portion of the probe was fixed to the skull with dental cement. After surgery, rats were individually housed in hemispherical bowls that also served as the experimental environment. Microdialysis experiments were performed in the striatum 13 (effect of acute caffeine), 25 (effect of subchronic caffeine), and 28 (effect of amphetamine in caffeine- and vehicle-pretreated rats) days after unilateral infusion of 6-OHDA into the medial forebrain bundle. Rats were infused with 6-OHDA on day 1, screened with apomorphine on day 11, implanted with microdialysis probes in both striata on days 12, 24, 27, and used for the microdialysis experiments on days 13, 25, 28. Caffeine was injected at a dose of 15 mg/kg i.p., and amphetamine was injected at a dose of 0.25 or 2 mg/kg i.p.

Microdialysis procedure. Perfusion was started 24 h after implantation of the microdialysis probes, in freely moving rats, as previously described (Di Chiara *et al*, 1993). The inlet of the microdialysis probe was connected to a microperfusion pump (CMA/100 microinjection pump, Carnegie Medicine, Sweden), while the outlet was inserted into a 200-μl test tube. Microdialysis probes were perfused continuously with Ringer's solution (NaCl 147 mM, $CaCl_2$

2.2 mM, KCl 4.0 mM, pH 7.0) at a constant flow rate of 2 μl/min. After a 1.5-h stabilization period, 20-min samples were collected.

Dopamine assay. A total volume of 20 μl of dialysate samples were injected, without purification, into a high-performance liquid chromatograph equipped with a reverse-phase column (LC-18 DB, 15 cm, 5 μm particle size, Supelco) and a coulometric detector (ESA, Coulochem II, Bedford, MA, USA) in order to quantitate dopamine. The first electrode of the detector was set at +150 mV (oxidation) and the second was set at −250 mV (reduction).

The composition of the mobile phase was 50 mM NaH_2PO_4, 5 mM Na_2HPO_4, 0.1 mM Na_2-EDTA, 0.5 mM *n*-octyl sodium sulfate, and 15% methanol; pH was adjusted to 5.50. The mobile phase was pumped with an LKB 2150 pump at a flow rate of 1.0 ml/min. The sensitivity of the assay for dopamine was 2 fmol/sample.

Histological Control

At the end of experiments, rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and killed by decapitation. Coronal slices (50 μm) were cut using a microtome in order to verify the position of the dialysis probes. Samples obtained from rats in which the probes were not correctly positioned were discarded.

Drugs

6-OHDA hydrochloride, desipramine hydrochloride, *R*-(−) apomorphine hydrochloride, caffeine (free base), and D-amphetamine sulfate were purchased from Sigma-RBI Co. (Milano, Italy).

Caffeine, D-amphetamine, and desipramine were injected in a volume of 0.3 ml i.p./100 g body weight. All drugs were dissolved in saline.

Statistics

Means ± SE of the number of ipsilateral turns were calculated. Significance was evaluated by one-way or two-way ANOVA followed by Tukey's *post hoc* test. In microdialisys studies, the increase of dopamine release was expressed as percentage of basal values. Basal values were the means of five consecutive samples differing no more than 10%. The significance of differences between groups was evaluated by one-way or two-way ANOVA followed by Tukey's *post hoc* test.

RESULTS

Induction of Caffeine Sensitization

Caffeine (15 mg/kg i.p.) treatment on alternate days (seven total administrations) elicited an ipsilateral turning behavior in unilaterally 6-OHDA-lesioned rats, which significantly increased during the course of treatment (Figure 1) (one-way ANOVA, $F_{6,216} = 6.13$, $P < 0.0001$, $N = 32$–37). The ipsilateral turning induced by caffeine was significantly different on days 6 and 7 *vs* days 1 and 2. During the course of treatment, a few contralateral turns were observed. After the first caffeine administration, 1.5 ± 0.5 contralateral turns and after the seventh caffeine administration, 1 ± 0.4 contralateral turns were recorded in 3-h testing (data not shown).

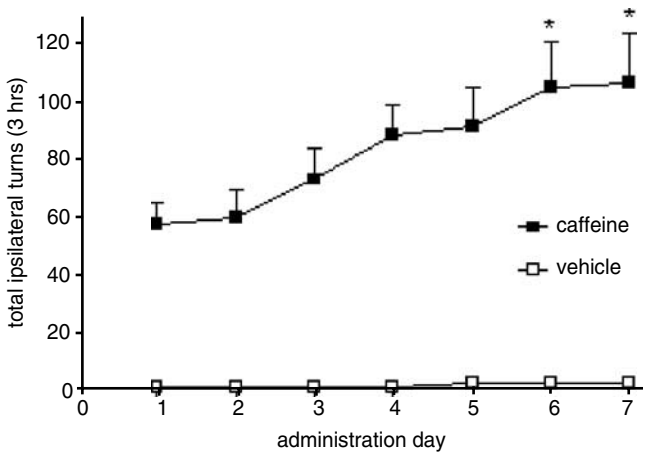

Figure 1 Mean ± SEM of ipsilateral turns, recorded in 3-h-testing period, induced by caffeine (15 mg/kg i.p.) or vehicle (i.p.) in unilaterally 6-OHDA-lesioned rats. Caffeine or vehicle was administered for 2 weeks, on alternate days (seven total administrations). The ipsilateral turning induced by caffeine was significantly different on day 6 *vs* days 1 and 2 and on day 7 *vs* days 1 and 2 (*$P < 0.001$; Tukey HSD *post hoc* test) ($N = 32$–37).

Expression of Amphetamine Cross-Sensitization

Administration of amphetamine (2 mg/kg i.p.) 3 days after the last caffeine or vehicle injection induced a significantly higher ipsilateral turning behavior in caffeine-pretreated as compared to vehicle-pretreated rats (Figure 2b). Two-way ANOVA analysis of the time-course of ipsilateral turning induced by amphetamine in caffeine- and vehicle-pretreated rats (Figure 2a) revealed a significant effect of group ($F_{1,17} = 5.36$; $P < 0.05$), a significant effect of time ($F_{11,187} = 15.94$; $P < 0.0001$), and a significant interaction ($F_{11,187} = 2.62$; $P < 0.01$) suggesting that sensitization in the control nonlesioned striatum took place. Administration of 0.25 mg/kg of amphetamine failed to induced turning behavior in both vehicle- and caffeine-pretreated rats (Figure 2a).

Effect of Acute and Subchronic Caffeine on Dopamine Release

Acute administration of caffeine (15 mg/kg i.p.) induced a significant increase of dopamine release as compared to basal dopamine release in the dorso-lateral nonlesioned striatum of unilaterally 6-OHDA-lesioned rats (Figure 3). The Tukey HSD *post hoc* test showed a statistically significant difference between basal and caffeine-induced dopamine release at 120 and 140 min. Similar to acute caffeine, administration of caffeine (15 mg/kg i.p.) to subchronically caffeine-treated rats induced a significant increase of dopamine release as compared to basal dopamine release in the dorso-lateral nonlesioned striatum (Figure 3). The Tukey HSD *post hoc* test showed a significant difference between basal and caffeine-induced

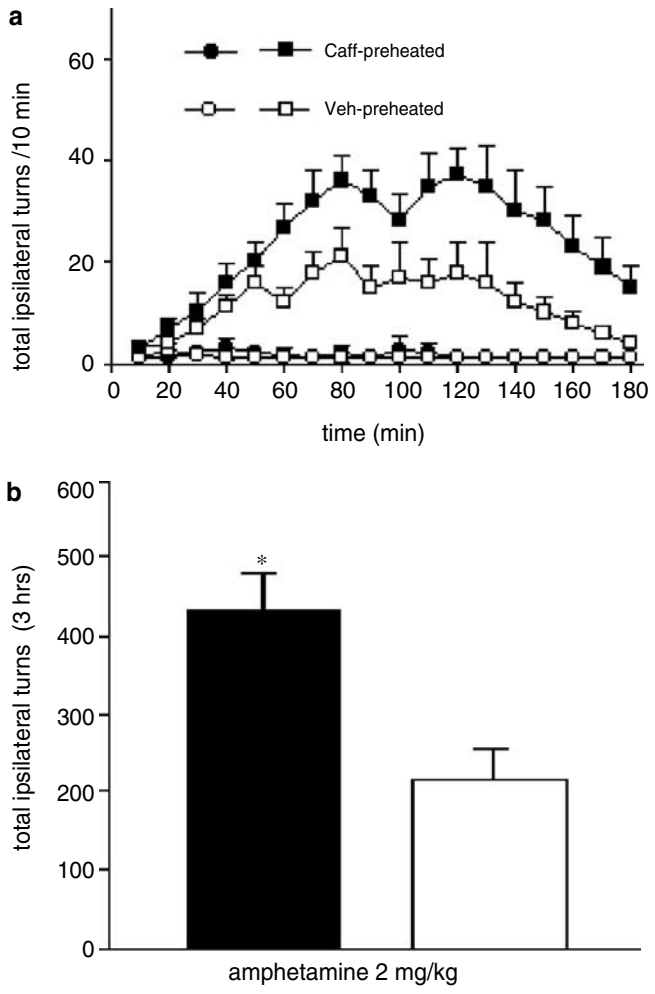

Figure 2 Mean ± SEM of ipsilateral turns, in unilaterally 6-OHDA-lesioned rats treated subchronically with caffeine (15 mg/kg i.p.) or vehicle (i.p.), induced by amphetamine 0.25 mg/kg i.p. (circles) and 2 mg/kg i.p. (squares), recorded every 10 min (a) or in total 2 h-testing period (2 mg/kg i.p.) (b). Significance was evaluated by two-way ANOVA followed by the Tukey HSD *post hoc* test ($F_{1,17} = 4.93$; $P < 0.05$, $N = 9$–10).

dopamine release at 140 and 180 min. Two-way ANOVA analysis of the time-course of the dopamine release between the first and the seventh administration of caffeine showed no significant effect of group ($F_{1,7} = 0.42$; $P = 0.54$), a significant effect of time ($F_{9,63} = 5.98$; $P < 0.00001$), and no significant interaction ($F_{9,63} = 0.60$; $P = 0.79$).

Basal release of dopamine was lower than the minimum value detectable (<2 fmol per sample) in the dorso-lateral striatum correspondent to the 6-OHDA-infused side.

Effect of Acute Amphetamine on Dopamine Release in Subchronically Caffeine- or Vehicle-Treated Rats

Administration of amphetamine (2 mg/kg i.p.) to vehicle-pretreated 6-OHDA-lesioned rats induced a significant increase of dopamine release as compared to basal dopamine release in the dorso-lateral nonlesioned striatum (Figure 4). In caffeine-pretreated rats, similar to vehicle-pretreated rats, administration of amphetamine (2 mg/kg i.p.) induced a significant increase of dopamine release as compared to basal dopamine release in the dorso-lateral

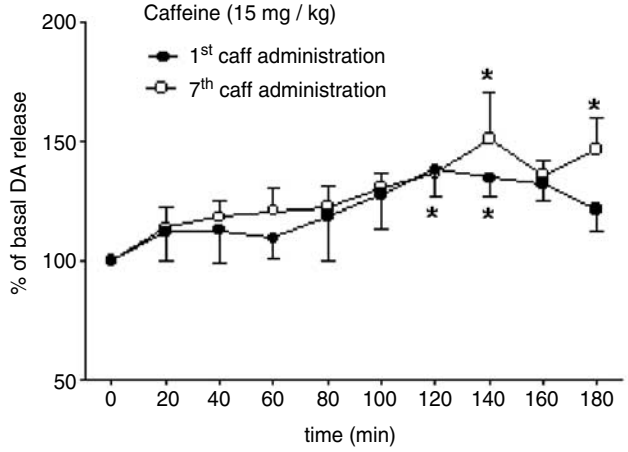

Figure 3 Effect of acute (first caffeine administration) and subchronic (seventh caffeine administration) caffeine (15 mg/kg i.p.) on dopamine (DA) release in the dorso-lateral nonlesioned striatum of unilaterally 6-OHDA-lesioned rats. The basal output of DA was 73 ± 13 and 85 ± 12 fmol/20 min sample, respectively ($F_{1,9} = 0.52$; $P = 0.49$). Microdialysis values are mean ± SEM; values are expressed as percentage of basal values. Significance was evaluated by one-way ANOVA ($F_{9,27} = 3.26$; $P < 0.0083$, $N = 4$ for the first caffeine administration; $F_{9,36} = 3.71$; $P < 0.0023$, $N = 5$ for the seventh caffeine administration) followed by the Tukey HSD *post hoc* test ($N = 4$–5). The Tukey HSD *post hoc* test showed a significant difference between basal and caffeine-induced dopamine release at 120 and 140 min (*$P < 0.05$) and at 140 and 180 min (*$P < 0.05$) for the first caffeine administration and the seventh caffeine administration, respectively. Two-way ANOVA did not reveal differences between the experimental groups.

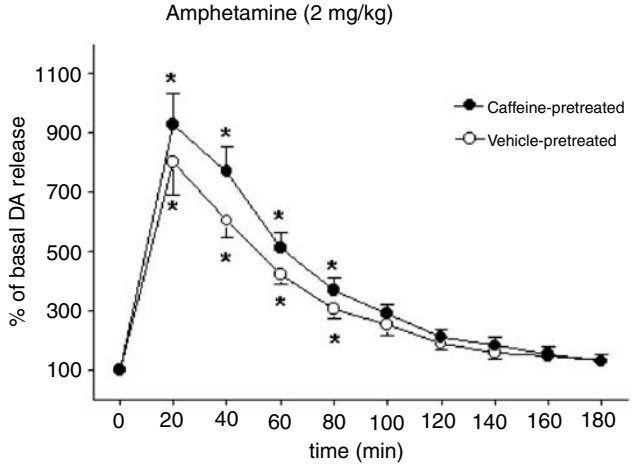

Figure 4 Effect of amphetamine (2 mg/kg i.p.) on DA release in the dorso-lateral nonlesioned striatum of unilaterally 6-OHDA-lesioned rats treated subchronically with caffeine (15 mg/kg i.p.) or vehicle (i.p.). The basal output of DA was 80 ± 9 and 81 ± 9 fmol/20 min sample, respectively. Microdialysis values are mean ± SEM; values are expressed as percentage of basal values. Significance was evaluated by one-way ANOVA ($F_{9,81} = 32.07$; $P < 0.0000$, $N = 10$ for vehicle-pretreated rats; $F_{9,81} = 45.49$; $P < 0.0000$, $N = 10$ for caffeine-pretreated rats) followed by the Tukey HSD *post hoc* test ($N = 10$). The Tukey HSD *post hoc* test showed a significant difference between basal and amphetamine-induced dopamine release at 20, 40, 60, 80 min (*$P < 0.05$) for both vehicle- and caffeine-pretreated rats. Two-way ANOVA did not reveal differences between the experimental groups.

nonlesioned striatum (Figure 4). Two-way ANOVA analysis of the time-course of the dopamine release after amphetamine administration in rats treated subchronically with vehicle or caffeine showed no significant effect of group

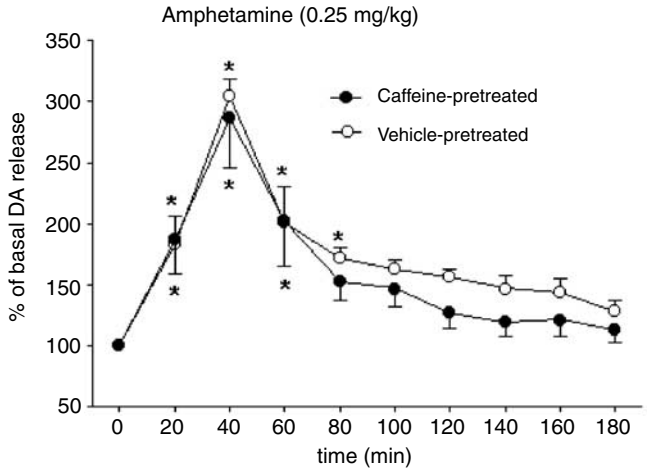

Figure 5 Effect of amphetamine (0.25 mg/kg i.p.) on DA release in the dorso-lateral nonlesioned striatum of unilaterally 6-OHDA-lesioned rats treated subchronically with caffeine (15 mg/kg i.p.) or vehicle (i.p.). The basal output of DA was 80 ± 9 and 81 ± 9 fmol/20 min sample, respectively. Microdialysis values are mean $\pm$ SEM; values are expressed as percentage of basal values. Significance was evaluated by one-way ANOVA ($F_{9,27} = 16.42$; $P < 0.0000$, $N = 4$ for vehicle-pretreated rats; $F_{9,36} = 10.81$; $P < 0.0000$, $N = 5$ for caffeine-pretreated rats) followed by the Tukey HSD *post hoc* test ($N = 4$–5). The Tukey HSD *post hoc* test showed a significant difference between basal and amphetamine-induced dopamine release at 20, 40, 60, 80 min and at 20, 40, 60 min (*$P < 0.05$) for vehicle- and caffeine-pretreated rats, respectively. Two-way ANOVA did not reveal differences between the experimental groups.

($F_{1,18} = 2.06$; $P = 0.17$), a significant effect of time ($F_{9,162} = 77.06$; $P < 0.0000$), and no significant interaction ($F_{9,162} = 0.93$; $P = 0.50$), showing no differences in dopamine release between the two groups.

Administration of amphetamine (0.25 mg/kg i.p.) induced a significant increase of dopamine release as compared to basal dopamine release in the dorso-lateral nonlesioned striatum of vehicle-pretreated rats (Figure 5). In caffeine-pretreated rats, similar to vehicle-pretreated rats, administration of amphetamine (0.25 mg/kg i.p.) induced a significant increase of dopamine release as compared to basal dopamine release in the dorso-lateral nonlesioned striatum (Figure 5). Two-way ANOVA analysis of the time-course of the dopamine release after amphetamine administration in rats treated subchronically with vehicle or caffeine showed no significant effect of group ($F_{1,7} = 0.66$; $P = 0.44$), a significant effect of time ($F_{9,63} = 23.57$; $P < 0.0000$), and no significant interaction ($F_{9,63} = 0.27$; $P = 0.98$), showing no differences in dopamine release between the two groups.

Basal release of dopamine was lower than the minimum value detectable (< 2 fmol per sample) in the dorso-lateral striatum corresponding to the 6-OHDA-infused side.

DISCUSSION

The present results, combining behavioral and microdialysis studies, show that: (I) subchronic caffeine administration sensitizes unilaterally 6-OHDA-lesioned rats to the ipsilateral turning behavior induced by caffeine itself; (II) rats sensitized to caffeine are cross-sensitized to amphetamine-induced ipsilateral turning behavior; (III) dopamine release induced by either caffeine or amphetamine in the intact striatum does not correlate to the sensitized turning behavior response.

Turning Behavior and Dopamine Release after Subchronic Caffeine

Adenosine A_{2A} receptor blockade has been shown to mediate the motor-activating effects induced by caffeine (Chen *et al*, 2001; Griebel *et al*, 1991; Ledent *et al*, 1997; Svenningsson *et al*, 1997b) and to potentiate the effect of stimulation of postsynaptic dopamine D_1 receptors (Pinna *et al*, 1996; Pollack and Fink, 1996) as well as dopamine D_2 receptors that are colocalized with adenosine A_{2A} receptors (Ferré *et al*, 1997; Fink *et al*, 1992; Schiffmann *et al*, 1991; Svenningsson *et al*, 1997a). A concurrent increase of dopamine release induced by caffeine in the intact striatum as shown by the present study might therefore synergize with postsynaptic adenosine A_{2A} receptor blockade inducing ipsilateral turning behavior. Therefore, in order for caffeine to express its motor-stimulant effects, it needs either endogenous dopamine as suggested by the present study or repeated priming with dopamine receptor agonists as suggested by previous studies (Fenu and Morelli, 1998), indicating that turning behavior by caffeine is dependent on dopamine transmission.

In contrast to previous studies showing that continuous oral intake or daily high dose of caffeine administration induces tolerance to caffeine-mediated contralateral turning behavior (Casas *et al*, 1999; Garrett and Holtzman, 1995), the present study evidences that intermittent caffeine administration (once a day on alternate days) induces a progressive increase of ipsilateral turning behavior during the course of treatment. These results argue that sensitization, and not only tolerance, to caffeine may take place. The induction of sensitization indicates that caffeine is able to induce neuronal long-term modifications that amplify its own motor-stimulant effects.

The mechanism at the basis of this increased motor behavioral response, however, does not appear to be related to a presynaptic effect on dopamine transmission. Evaluation of dopamine release by *in vivo* brain microdialysis, consistent with previous studies in which caffeine was directly infused through the microdialysis probe in the striatum (Okada *et al*, 1997), shows that, although modestly, systemic caffeine increased dopamine release in the dorso-lateral striatum. However, no significant difference on caffeine-induced dopamine release between the beginning and the end of the treatment was observed in the present study. Caffeine sensitization, therefore, does not appear to be due to an increased ability of caffeine to release dopamine. Release of dopamine by caffeine, however, might be important to trigger the induction of postsynaptic modifications at the dopamine receptor level which, together with modifications produced by repeated blockade of A_{2A} receptors by caffeine, produce caffeine ipsilateral turning behavior sensitization. A recent study by Lindskog *et al* (2002) reported that caffeine through its effect on A_{2A} receptors increases the state of phosphorylation of DARPP-32, indicating a new mechanism for the postsynaptic interaction between dopamine and adenosine A_{2A} receptors that might produce long-term postsynaptic changes.

Antagonistic interactions between dopamine D_1 and adenosine A_1 receptors have been demonstrated at both receptor and behavioral levels (Ferré *et al*, 1998; Gines *et al*, 2000; Popoli *et al*, 1996). These types of interactions, however, have been shown to play a pivotal role in limbic rather than in motor areas (Bonci and Williams, 1996; Kuzmin *et al*, 1999; Mayfield *et al*, 1999), whereas in turning behavior, A_1 receptor antagonists do not appear to play a central role (Pinna *et al*, 1996).

Turning Behavior and Dopamine Release after Amphetamine in Subchronically Caffeine-Treated Rats

Administration of amphetamine elicited a higher ipsilateral-turning behavior in caffeine-sensitized rats as compared to vehicle-pretreated rats, indicating that cross-sensitization between caffeine and amphetamine takes place.

Caffeine produces active metabolites that might stimulate, although to a low degree, locomotor activity. Our experimental protocol (alternate administrations plus 3 days washout) differs from continuous oral intake or daily administration, and renders the presence of active metabolites unlikely (Lau *et al*, 1995; Svenningsson *et al*, 1999), suggesting that a mechanism of sensitization rather than a potentiation is at the basis of the amplification of turning behavior induced by amphetamine. In previous studies, in fact, the lack of washout from the last caffeine injection or caffeine solution intake could have produced a potentiation, rather than a sensitization, to the amphetamine or cocaine motor-stimulant effects (Gasior *et al*, 2000; Schenk *et al*, 1990).

As shown by previous studies, amphetamine significantly increased dopamine release in the intact striatum (Badiani *et al*, 2000; Di Chiara *et al*, 1993), however, no significant difference on dopamine release between caffeine-sensitized rats and vehicle-pretreated rats was observed in the present study. In order to exclude that the very high increase in dopamine release after 2 mg/kg of amphetamine, +800% in vehicle, and +900% in caffeine-pretreated rats could prevent a further increase in dopamine release, we evaluated the effect of a lower dose of amphetamine (0.25 mg/kg) that produced a maximal increase of about 300%. Administration of a low dose of amphetamine that is ineffective in producing ipsilateral turning behavior, increased dopamine release to the same extent in caffeine- and vehicle-pretreated rats, confirming that subchronic caffeine did not modify the dopamine-releasing effect of amphetamine. This result suggests that cross-sensitization to amphetamine, as well as caffeine sensitization, are not correlated to presynaptic changes at the level of dopamine transmission, rather, postsynaptic mechanisms which involve the interaction between adenosine and dopamine receptors in the striatum might be responsible for these effects.

Manipulation of the dopamine system, such as dopamine neuron denervation or chronic haloperidol, have been shown to lead to an increase in intramembrane interaction between adenosine A_{2A} and dopamine D_2 receptors in the striatum (Ferré and Fuxe, 1992; Ferré *et al*, 1994). On this basis, we hypothesize that after repeated caffeine administration, long-term modifications at the level of the A_{2A} receptor or its second messenger might lead to an increase in A_{2A} receptor sensitivity and as a consequence, in the D_2 receptor leading to the sensitized ipsilateral turning observed after caffeine and amphetamine.

Similar to this report, a previous study by Badiani *et al* (2000) showed that amphetamine induces sensitization to turning behavior independent from dopamine release. The failure of modifications in dopamine release in response to amphetamine in caffeine-sensitized rats is in line with previous studies showing that modifications at the postsynaptic level such as increased affinity of dopamine for D_1 receptor stimulated adenylate cyclase, increased DARPP-32 phosphorylation as well as changes in specific G protein subunits, protein kinase A activity or expression of immediate-early genes are at the basis of sensitization phenomena (Barone *et al*, 1994; Nestler 1992; Pierce and Kalivas, 1997; Pinna *et al*, 1997; Striplin and Kalivas, 1993).

Conclusions

The present results show for the first time that caffeine, besides producing tolerance after continuous oral administration or repeated high dose administration, sensitizes rats to its own and amphetamine motor-stimulant effects, when administered in spaced injections. Long-term modifications of adenosine–dopamine receptor interaction at the postsynaptic level might be at the basis of the sensitization observed, since no correlation between dopamine release and sensitization to ipsilateral turning behavior was detected. Caffeine, although sharing no reinforcing properties or abuse potential with drugs of abuse, should be regarded as a drug potentially capable of priming responses induced by drugs that stimulate dopamine transmission such as amphetamine.

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