ELSEVIER

Pharmacology, Biochemistry and Behavior 69 (2001) 59–70

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

The role of dopamine in the locomotor stimulant effects and tolerance to these effects of caffeine

Kelly R. Powell*, P. Michael Iuvone, Stephen G. Holtzman

Department of Pharmacology, Emory University School of Medicine, 1510 Clifton Road Atlanta, GA 30322, USA

Received 28 August 2000; received in revised form 10 January 2001; accepted 22 January 2001

Abstract

Current evidence indicates that the acute locomotor stimulant effects of caffeine involve dopamine (DA) receptor activation; however, few studies have investigated the role of DA receptors in mediating the development of tolerance to caffeine. Therefore, the present study was designed to determine the degree to which DA receptors mediate the development of tolerance to the locomotor stimulant effects of caffeine. Caffeine was examined alone and in combination with haloperidol (HAL), GBR 12909, nisoxetine and fluoxetine. HAL dose-dependently and completely blocked the acute effects of caffeine on locomotor activity, and the highest dose of GBR 12909 enhanced the effects of caffeine. Neither nisoxetine nor fluoxetine altered the effects of caffeine. HAL was infused via osmotic pumps (0.1 mg/kg/day) during a 14-day regimen of chronic caffeine administered in a caffeinated drinking solution ($\approx$136 mg/kg/day). HAL did not block the development of tolerance to the locomotor stimulant effects of caffeine, but did impair the recovery from tolerance following withdrawal of caffeine. [^{3}H]SCH 23390 (DA D_1) binding sites were downregulated in the nucleus accumbens and striatum and were upregulated in the prefrontal cortex of caffeine-treated vs. control rats; however, the affinity of [^{3}H]SCH 23390 for these binding sites was unaltered. There were no differences between the caffeine-treated and control rats in number or affinity of [^{3}H]spiperone (DA D_2) binding sites. These results suggest that, although HAL did not alter the development of tolerance to caffeine, changes in DA D_1 receptors could be one component of the mechanism underlying caffeine-induced tolerance.

Keywords: Caffeine; Tolerance; Locomotor activity; Dopamine receptors; Rats

1. Introduction

Caffeine, a member of a class of compounds known as methylxanthines, is widely consumed by humans in a variety of foods, beverages and over-the-counter medicines (Daly, 1993; Nehlig et al., 1992). Subjective effects often reported following the consumption of caffeine include increased energy, alertness and concentration (Griffiths and Woodson, 1988; Griffiths et al., 1986), yet, little is known about the effects of chronic caffeine administration (see Griffiths and Mumford, 1995). In rodents, acute administration of caffeine produces reliable and concentration-dependent locomotor stimulation (Daly et al., 1981; Snyder et al., 1981), and chronic administration results in tolerance to this effect and some degree of physical dependence (Ahlijanian and Takemori, 1986; Holtzman, 1991; Holtzman and Finn, 1988). Thus, developing an understanding of the mechanisms underlying the effects of chronic caffeine administration is highly relevant to human health and to drug development.

Although there is a high correlation between the acute behavioral stimulant effects of caffeine and other methylxanthines and their ability to antagonize adenosine receptors (Daly et al., 1981; Snyder et al., 1981), little is known of the mechanisms contributing to the development of tolerance to the behavioral stimulant effects of caffeine. Current evidence suggests that the behavioral stimulant effects of caffeine may be the result of increased activity in dopaminergic neurotransmission, perhaps via dopamine (DA)–adenosine interactions. For example, DA depletion with reserpine or α-methyl-tyrosine (Finn and Holtzman, 1990) and antagonism of DA D_1 and D_2 receptors (Garrett and Holtzman, 1994a) attenuate the locomotor stimulant effects of caffeine in rats. Locomotor stimulation produced by various DA agonists is potentiated by caffeine and other

* Corresponding author. Department of Behavioral and Social Sciences, Lee University, 1120 North Ocoee Street, Cleveland, TN 37311, USA. Tel.: +1-423-614-8132; fax: +1-423-614-8129.

E-mail address: kpowell@leeuniversity.edu (K.R. Powell).

0091-3057/01/$ – see front matter
PII: S0091-3057(01)00497-X

adenosine receptor antagonists (Ferre et al., 1991a,b). Moreover, caffeine increases DA levels in the central nervous system (Kirch et al., 1990; Morgan and Vestal, 1989), although it shows no direct interactions with DA receptors (Watanabe and Uramoto, 1986). Finally, there is an abundance of evidence for both pre- and postsynaptic adenosine/DA receptor interactions where adenosine acts to inhibit dopaminergic activity. For example, adenosine A_1 receptor activation decreases binding of DA to DA D_1 receptors and blocks locomotor stimulation produced by DA D_1 receptor agonists (Ferre et al., 1994a, 1996). Adenosine A_{2A} receptor agonists inhibit DA D_2 receptor-mediated hyperactivity and inhibit DA D_2 receptor binding (Dasgupta et al., 1996; Ferre et al., 1991a,b, 1994b). Moreover, stimulation of adenosine A_{2A} receptors decreases affinity of DA D_2 receptors for DA (Ferre et al., 1991c; Salim et al., 2000). Both A_1 and A_{2A} adenosine receptors attenuate methamphetamine-induced overflow of DA in rat striatum (Golembiowska and Zylewska, 1998). In addition, adenosine A_1 and DA D_1 receptor activation and adenosine A_{2A} and DA D_2 receptor activation have opposing effects on GABA release in rat striatum (Corsi et al., 1999; Mayfield et al., 1999). Together, these reports suggest that caffeine very likely stimulates motor activity by blocking adenosine receptors and removing adenosine-mediated inhibition of DA neurotransmission. If this is indeed the mechanism underlying the behavioral stimulant effects of the acute administration of caffeine, it would be reasonable to propose that changes in the DA system underlie tolerance to these effects. More specifically, if repeated administration of caffeine results in chronic stimulation of DA receptors, compensatory changes such as DA receptor downregulation or decreased receptor–effector coupling might occur.

To date, few studies have investigated the role of DA in the development of tolerance to caffeine-induced behavioral stimulation. Rats made tolerant to the locomotor stimulant effects of caffeine are cross-tolerant to the locomotor stimulant effects of either DA D_1 or D_2 receptor agonists (Garrett and Holtzman, 1994b). Caffeine-tolerant rats are not, however, cross-tolerant to the locomotor stimulant effects produced by concurrent administration of DA D_1 and D_2 agonists (Garrett and Holtzman, 1994b) or to other non-xanthine behavioral stimulants such as D-amphetamine and cocaine, whose effects are mediated at least in part by concurrent activation of both DA D_1 and D_2 receptors (Finn and Holtzman, 1987; Holtzman, 1991). Based on these data, Garrett and Holtzman (1994b) proposed that tolerance to the locomotor stimulant effects of caffeine is mediated by both DA D_1 and D_2 receptors, even though cross-tolerance does not occur to drugs that activate both of these DA receptor subtypes simultaneously.

The present study was designed to investigate further the role of DA receptors in the locomotor stimulant effects of caffeine and tolerance to these effects in rats. Specifically, we examined the effects of DA receptor activation with a DA uptake inhibitor GBR 12909 and DA receptor blockade with a DA receptor antagonist HAL on caffeine-induced locomotor stimulation. Based on evidence that tolerance to caffeine is mediated by both DA D_1 and D_2 receptors, haloperidol (HAL) was coadministered with caffeine using a chronic regimen to determine whether DA receptor blockade would block the development of tolerance to caffeine-induced locomotor stimulation. Although HAL may show some selectivity for DA D_2 receptors over DA D_1 receptors, its behavioral profile suggests that it is still fairly non-selective and serves our purposes in the present study. Finally, the effects of chronic caffeine administration on the binding parameters of both DA D_1 and D_2 receptors were examined to determine whether compensatory changes occur in the DA system that might underlie tolerance to the locomotor stimulant effects of caffeine.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (Charles River, Raleigh, NC) weighing 300–325 g at the start of the experiments were used. Rats used in the acute testing experiment ($n = 8$) were housed in pairs in an environmentally controlled colony room and were allowed continuous access to water in their home cages. Rats used in the caffeine tolerance experiments ($n = 32$; 8 per group) were housed individually in a climate-controlled cabinet where access to their drinking solution was controlled via electronic timer connected with electronic bottle holders above each cage. All rats were maintained on a 12-h light/dark cycle and were allowed continuous access to food in their home cages.

2.2. Apparatus

Locomotor activity was measured using eight Omnitech Digiscan Activity Monitors (Omnitech, Columbus, OH). Each rat was placed in an acrylic box (40×40 cm) surrounded by a framework of photobeams. The photobeams were designed in a 16×16-cm beam array around the bottom of the box and 2.5 cm from the floor. Also, 16 photobeams were mounted 10.5 cm above the bottom photobeams on the left and right sides of the box in order to measure vertical locomotor activity. Locomotor movements were determined by breaks in photobeams and were converted into locomotor activity counts by a Digiscan Analyser (Omnitech) that was interfaced with a microcomputer (IBM PC clone).

2.3. Experiment 1

2.3.1. Acute locomotor activity tests

Rats were first habituated to the activity chambers for 30 min each day for 5 days prior to testing. Locomotor activity tests were then conducted two times per week and were

separated by at least 5 days to allow for recovery from residual drug effects and to prevent the development of tolerance to caffeine's effects. Initially, on two separate days, rats were given five consecutive injections of saline 40 min apart, and locomotor activity was measured during the final 20 min of each interinjection interval in order to obtain baseline data under a five-component, cumulative dosing timeline. Following this, locomotor activity was measured on two separate days following administration of cumulative doses of caffeine (3–100 mg/kg ip) over five components. The first component followed saline administration and served as a baseline measure of locomotor activity for that day. As described above, injections were given every 40 min, and locomotor activity was measured during the final 20 min of each component. Immediately following each 20-min measuring period, rats were injected with the next dose of caffeine that, when added to the previous dose(s), equaled a dose one-half log unit greater than the previous cumulated dose. For example, 3 mg/kg followed by 7 mg/kg equals a cumulative dose of 10 mg/kg. Rats remained in the locomotor test chambers at all times except briefly when removed for injections.

The effects of caffeine (3.0–56 mg/kg), HAL (0.1–3.0 mg/kg), GBR 12909 (1.0–17.5 mg/kg), nisoxetine (0.1–3.0 mg/kg) and fluoxetine (0.1–3.0 mg/kg) were examined on locomotor activity using the cumulative dosing procedure. When testing drug combinations, a single dose of HAL, GBR 12909, nisoxetine or fluoxetine instead of saline was administered prior to redetermining the cumulative dose–response curve for caffeine. These doses of HAL, GBR 12909, nisoxetine and fluoxetine combined with caffeine were doses that did not produce any effects on locomotor behavior when tested alone (lower doses of HAL and GBR 12909 alone not shown).

2.3.2. Data analysis

Locomotor activity was recorded and analyzed as total horizontal activity counts. For the acute experiment, area under the curve was calculated from the mean dose–response curves for caffeine alone and in combination with HAL, GBR 12909, nisoxetine and fluoxetine and were compared using a one-factor ANOVA with a P value of .05.

2.4. Experiment 2

2.4.1. Caffeine tolerance locomotor activity tests

Rats were initially habituated to the locomotor activity chambers, and dose–response curves were obtained for caffeine as described for the acute testing. In addition, rats were tested with a single dose of caffeine (30 mg/kg) on a separate day for comparison with the effects of this dose of caffeine during and after the tolerance regimen. Rats were then water-deprived for 24 h in order to motivate them to drink a caffeinated solution. Approximately 4 h prior to reinstatement of access to drinking solutions, HAL-filled or sham osmotic minipumps were surgically implanted in each rat. The HAL pumps infused a dose of 0.1 mg/kg/day, the highest dose that could be infused without general behavioral disruption (personal observation). Access to drinking solutions was reinstated on a schedule of 10 min access every 6 h. Half the rats were given a caffeinated drinking solution (1 mg/ml) and the other half were given tap water. During the tolerance regimen, drinking bottles were weighed daily to determine the amount of caffeine or water consumed (1 g bottle weight = 1 ml solution). Locomotor activity was measured over two components every second day for 12 days, the first component following saline administration and the second following a single challenge dose of caffeine (30 mg/kg). This testing schedule (Table 1) allowed assessment of the development of tolerance to caffeine in rats drinking the caffeinated solution without producing tolerance in the control rats (drinking water). On Day 20, osmotic pumps were removed and all rats were given continuous access to water for the remainder of the experiment. Caffeine (30 mg/kg) was tested on Day 24 or approximately 4 days after removal of the pumps and caffeinated drinking solution.

2.4.2. Surgical procedure

In the caffeine tolerance experiment, approximately 4 h prior to the first access to caffeine or water drinking solutions, rats were lightly anesthetized with Halothane and osmotic minipumps (Model 2ML2, Alzet,, Palo Alto, CA) were implanted subcutaneously through a small incision in the upper midregion of the back. Two groups of rats ($n = 8$) were implanted with empty pumps (sham pumps), and two groups of rats were implanted with pumps that delivered 0.1 mg/kg/day of HAL for 14 days. On Day 14, rats were lightly anesthetized with Halothane, and the pumps were removed through the original incision.

2.4.3. Data analysis

The effects of the challenge dose of caffeine (30 mg/kg) were compared between groups prior to the start of the tolerance regimen, on each test day during the tolerance

Table 1
Outline of events in caffeine tolerance experiment

Days 1–5	Rats habituated to locomotor test chambers for 30 min per day
Day 6	Rats tested following saline then 30 mg/kg of caffeine (premeasure)
Day 7	Rats water-deprived for 24 h
Day 8	4 h prior to end of 24-h water-deprivation, rats implanted with pumps 24 h after start of water-deprivation, rats given access to drinking solution for 10 min every 6 h
Days 10, 12, 14, 16, 18, 20	Rats tested following saline then 30 mg/kg of caffeine
Day 20	Following test session, pumps were surgically removed and continuous access to tap water was reinstated (caffeine removed from drinking solution)
Day 24	Rats were tested following saline then 30 mg/kg of caffeine (postmeasure)

regimen, and on Day 24, 4 days after the end of the tolerance regimen. Comparisons were made within groups between the premeasures and measures taken on each test day after using a two-factor ANOVA with repeated measures on one factor (test day), and post hoc analyses were made using Tukey's Multiple Comparison Test.

2.5. Experiment 3

2.5.1. Subjects

Subjects were male Sprague–Dawley rats of the same approximate age and weight as rats used in the behavioral experiments ($n = 80–100$). Rats were housed in the same manner as the rats used in the caffeine tolerance experiment. After 24 h of water deprivation, half the rats were given access to the same caffeinated drinking solution used in the caffeine tolerance experiment and half were given access to tap water for 10 min every 6 h for 14 days.

2.5.2. DA receptor binding

On Day 14 of scheduled access to caffeine or water, approximately 2–3 h after the last access to caffeine, rats were sacrificed by rapid decapitation. Brains were rapidly removed and the prefrontal cortex, nucleus accumbens and striatum were isolated by freehand dissection. Each tissue was immediately placed in 40 vol. (milligram per gram tissue weight) of ice cold Tris–HCl buffer (50 mM, pH 7.4) and homogenized while on ice. The tissue was centrifuged at 49,000 × *g* for 15 min (4°C).

[^{3}H]SCH 23390 binding was determined using slight modifications of the methods of Ferre et al. (1990). Protein content for each sample was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard. Incubations were carried out for 30 min at 37°C in the presence of 0.039–10 nM [^{3}H]SCH 23390 to determine equilibrium binding constants (B_{max} and K_D). During competition binding assays, samples were incubated for 30 min in the presence of 1 nM [^{3}H]SCH 23390 and eight to nine concentrations of DA ranging from 0.001 to 10 μM in 0.1% ascorbic acid. IC_{50} values were determined in the presence and absence of 1 μM guanesine trisphosphate (GTP). Incubations were initiated by the addition of 100 (prefrontal cortex) or 60 μg (nucleus accumbens and striatum) of protein in a final assay volume of 1 ml. The incubation was terminated by rapid filtration over GF/C filters presoaked in 50 mM Tris–HCl and washed three times with 4 ml of ice cold Tris–HCl (50 mM). Nonspecific binding, defined by 1 μM *cis*-flupenthixol, was determined for every concentration of [^{3}H]SCH 23390. At a radioligand concentration of 1 nM, nonspecific binding was approximately 36% of the total for prefrontal cortex, 20% of the total for nucleus accumbens and 14% of the total for striatum. Radioactivity was determined by liquid scintillation counting (Beckman LS 6500) at a counting efficiency of 55%.

[^{3}H]Spiperone binding was determined using modifications of the methods of Hess et al. (1988) and Kirch et al. (1992). Protein content for each sample was determined and incubations were carried out for 60 min at 30°C in the presence of 4.7–600 pM [^{3}H]spiperone to determine B_{max} and K_D values. During competition assays, samples were incubated with 0.1 nM [^{3}H]spiperone and seven to nine concentrations of DA ranging from 0.001 to 10 μM in 0.01% ascorbic acid. IC_{50} values were determined in the presence and absence of 10 μM GTP. The incubations were initiated by addition of 150 μg of protein for nucleus accumbens and striatum tissue in a final assay volume of 3 ml. Incubations were terminated by rapid filtration over GF/B filters presoaked in 50 mM Tris–HCl and washed three times with 4 ml of Tris–HCl (50 mM). Nonspecific binding, defined by 1 μM butaclamol, was determined for every concentration of [^{3}H]spiperone. At a radioligand concentration of 0.1 nM, nonspecific binding was approximately 34% of the total for nucleus accumbens and 17% of the total for striatum.

2.5.3. Data analysis

Radioligand binding saturation and competition curves were analyzed by nonlinear regression analysis program (PRISM, GraphPAD, San Diego, CA). B_{max} and K_D values were determined for individual saturation curves and the mean values ± 1 S.E.M. were calculated. Mean B_{max} and K_D values for caffeine- and control-treated tissues were compared within each brain region using a two-tailed Student's *t*-test. Significant differences were determined by a P value $< .05$. IC_{50} values for one- or two-site best-fit analysis were calculated and selected based on the best fitting model. These values for caffeine- and control-treated tissues were compared in the same manner as B_{max} and K_D values.

2.6. Drugs

The caffeine drinking solution was made with anhydrous caffeine (Sigma, St. Louis, MO) dissolved in tap water in a concentration of 1 mg/ml. Caffeine sodium benzoate and HAL (Sigma) and nisoxetine and fluoxetine (Research Biochemicals, Natick, MA) were dissolved in 0.9% saline. GBR 12909 (Research Biochemicals) was dissolved in one part desmethyl sulfoxide and two parts distilled water. All drugs were administered intraperitoneally in a volume of 1 ml/kg body weight. Drug doses are expressed as the free base. For binding experiments, [^{3}H]SCH 23390 and [^{3}H]spiperone were obtained from Amersham Pharmacia Biotech (Piscataway, NJ).

3. Results

3.1. Acute drug administration tests

Caffeine (3.0–100 mg/kg) produced a biphasic effect on locomotor activity, with peak stimulant effects

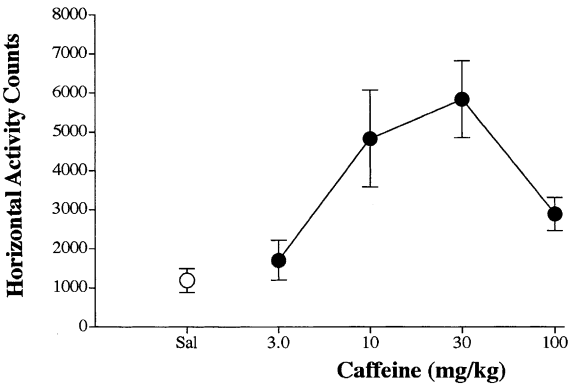

Fig. 1. The dose–effect curve for caffeine (3.0–100.0 mg/kg) on locomotor activity expressed as the mean (±1 S.E.M.) total horizontal activity counts ($n=8$). The point above SAL represents the mean effect of saline administration.

observed following 30 mg/kg (Fig. 1). The DA uptake inhibitor GBR 12909 increased significantly locomotor activity, whereas the noradrenergic uptake inhibitor nisoxetine and the serotonergic uptake inhibitor fluoxetine did not alter locomotor activity compared to saline (Fig. 2A). When combined with caffeine, the highest dose of GBR 12909 (1.0 mg/kg) enhanced the effects of caffeine on locomotor activity, but neither nisoxetine nor fluoxetine altered the effects of caffeine (Fig. 2B). The locomotor stimulant effects of caffeine were dose-dependently attenuated when caffeine was combined with HAL, with complete antagonism at the highest dose of HAL (0.3 mg/kg; Fig. 3).

3.2. Caffeine tolerance and locomotor activity

Both control and caffeine-drinking rats consumed an average of 41 ml of liquid daily. That equals approximately 136 mg/kg of caffeine consumed by each caffeine-drinking rat daily over the 14-day period. Caffeine-drinking rats implanted with sham pumps showed tolerance to the

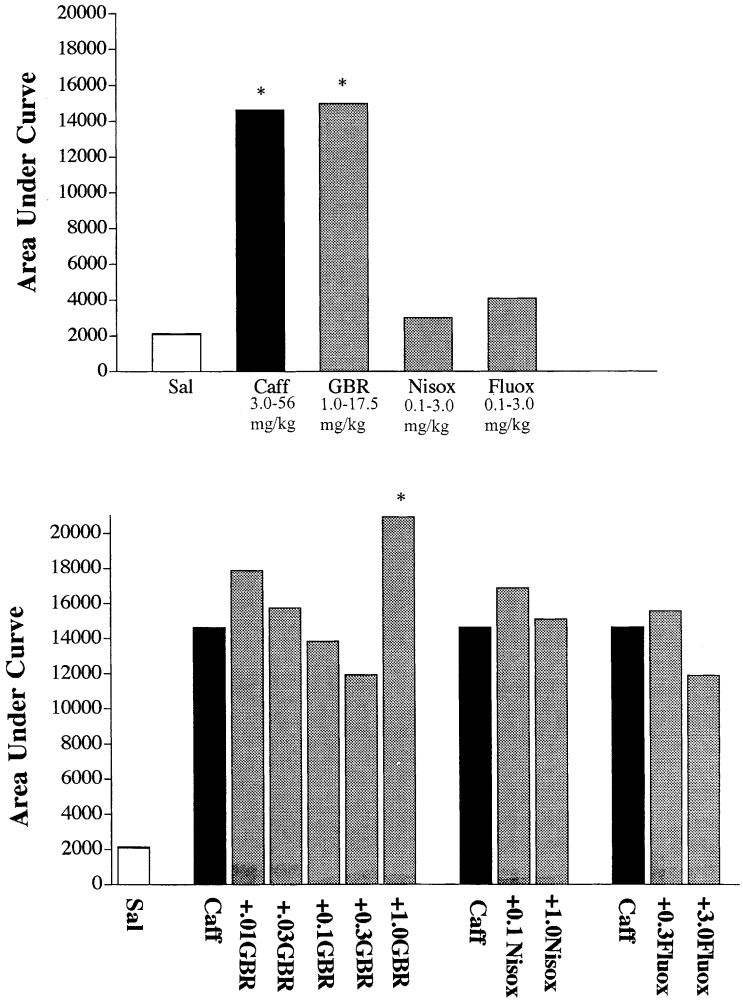

Fig. 2. Panel A: The effects of caffeine (3.0–56.0 mg/kg), GBR 12909 (1.0–17.5 mg/kg), nisoxetine (0.1–3.0 mg/kg) and fluoxetine (0.1–3.0 mg/kg) on locomotor activity expressed as area under the mean dose–effect curve ($n=8$). The white bar above SAL represents the effect of saline administered in the same cumulative manner as each of the drugs (* $P<.05$ compared to saline). Panel B: The effect of caffeine alone and in combination with various doses of GBR 12909, nisoxetine and fluoxetine on locomotor activity expressed as area under the mean dose–effect curve ($n=8$; * $P<.05$ compared to caffeine alone).

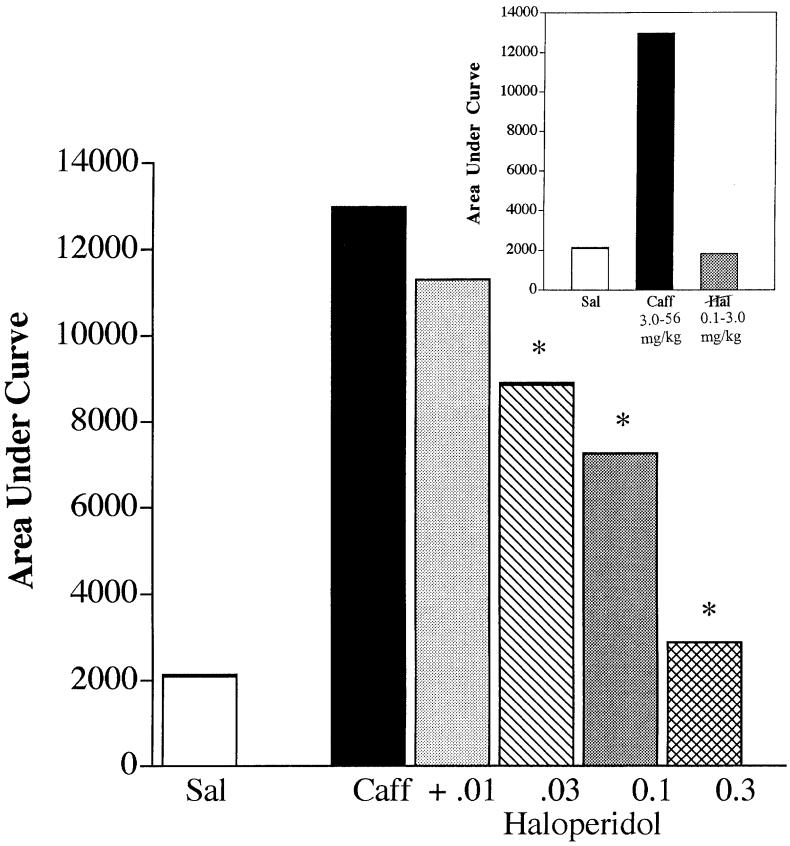

Fig. 3. Effects of HAL alone (inserted graph) and combined with caffeine on locomotor activity expressed as area under the mean dose–effect curve ($n = 8$; * $P < .05$ compared to caffeine alone).

locomotor stimulant effects of the challenge dose of 30 mg/kg of caffeine by Day 2 of exposure to caffeine (Fig. 4). Water-drinking rats implanted with sham pumps did not show tolerance to caffeine. Rats implanted with pumps infusing 0.1 mg/kg HAL over 24 h for 14 days were exposed to the same conditions and tests as rats implanted with sham pumps. Caffeine-drinking rats with HAL pumps showed tolerance to caffeine's effects by Day 2 of exposure to caffeine, whereas water-drinking rats with HAL pumps did not show tolerance to caffeine.

On Day 14 of chronic caffeine exposure, osmotic pumps were removed and all rats were switched to tap water for drinking and were retested with 30 mg/kg of caffeine 4 days later. The rats with sham pumps that had received caffeine for 14 days showed a loss of tolerance to caffeine by Day 4 following removal of caffeine from the drinking water (Fig.

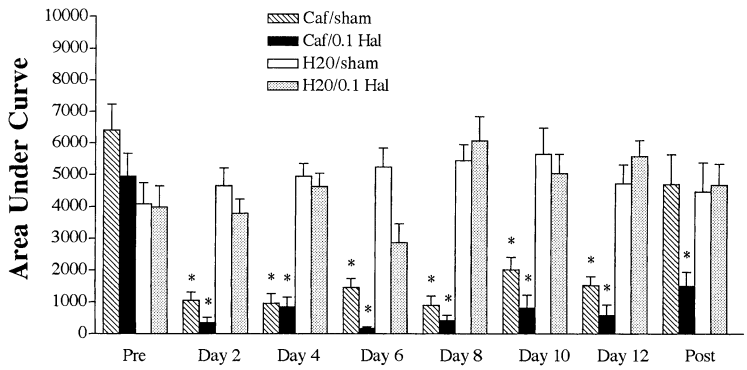

Fig. 4. Effects of a 30-mg/kg challenge dose of caffeine before, during and after the chronic treatment regimen in rats with sham pumps drinking caffeine (hatched bars), rats with HAL pumps drinking caffeine (dark bars), rats with sham pumps drinking water (white bars) and rats with HAL pumps drinking water (shaded bars; $n = 8$ per group). The bars above PRE represent the effects of 30 mg/kg of caffeine before pumps were implanted and caffeine was added to the drinking solution. The bars above POST represent the effects of 30 mg/kg of caffeine 4 days after pumps were removed and caffeine was removed from the drinking solution. * $P < .05$ compared to PRE activity levels.

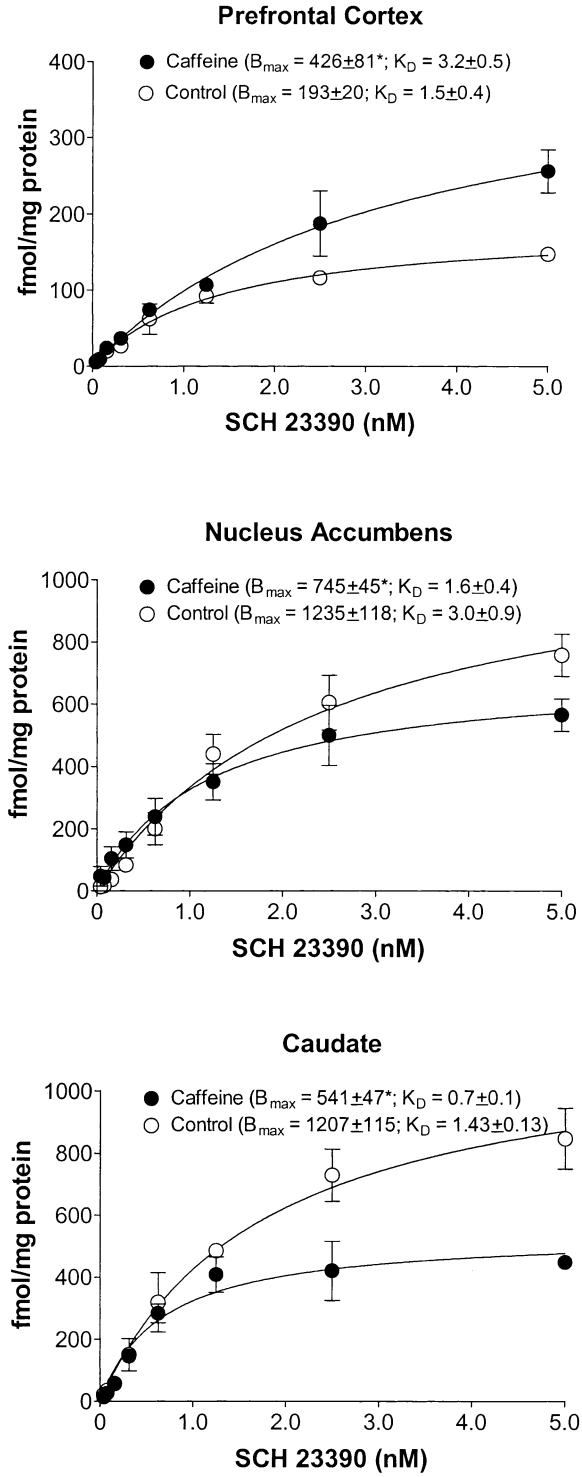

Fig. 5. Effects of chronic caffeine treatment on [^{3}H]SCH 23390 binding sites in tissue from rat prefrontal cortex (top), nucleus accumbens (middle) and striatum (bottom). Rats were given scheduled access to a caffeinated solution (caffeine) or drug-free tap water (control) for 14 days. Each point is the mean of triplicate determinations from three (prefrontal cortex, striatum) or four (nucleus accumbens) experiments. Mean B_{max} and K_D values ± 1 S.E.M. are listed in the key for each graph. * $P < .05$ caffeine vs. control.

4). Rats with HAL pumps that had received caffeine for 14 days showed a slight loss of tolerance, although the locomotor stimulant effects of caffeine did not return to the level observed in the prechronic test. Rats with sham and HAL pumps that received only tap water to drink for 14 days continued to show the same response to caffeine observed prior to pump implantation.

3.3. Caffeine tolerance and DA receptor binding

Rats used for the binding experiments consumed an average of 44 ml of tap water daily (control rats) or an average of 35 ml of caffeinated solution daily (caffeine-treated rats), which equals approximately 116 mg/kg of caffeine consumed by each rat daily over the 14-day period. Saturation analysis of binding of the DA D_1 receptor-selective antagonist [^{3}H]SCH 23390 and the relatively

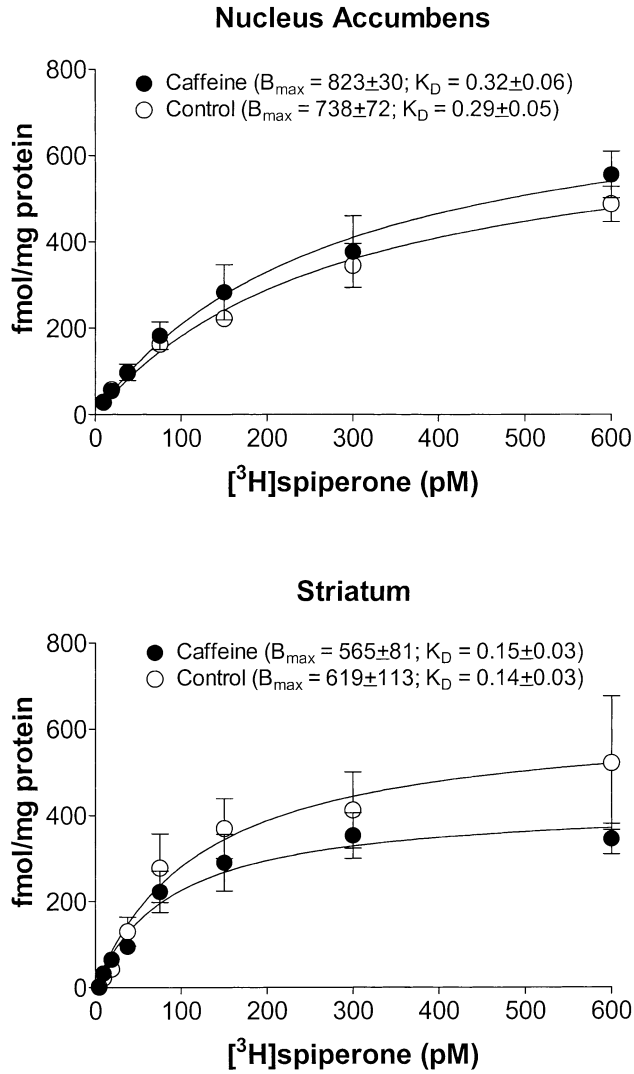

Fig. 6. Effects of chronic caffeine treatment on [^{3}H]spiperone binding sites in tissue from rat nucleus accumbens (top) and striatum (bottom). Rats were given scheduled access to a caffeinated solution (caffeine) or drug-free tap water (control) for 14 days. Each point is the mean of triplicate determinations from three experiments. Mean B_{max} and K_D values ± 1 S.E.M. are listed in the key for each graph.

Table 2
DA displacement of [^{3}H]SCH 23390 and [^{3}H]spiperone binding in prefrontal cortex, nucleus accumbens and striatum

	No GTP			With GTP		
	IC_{50_H}	IC_{50_L}	% high affinity	IC_{50_H}	IC_{50_L}	% high affinity
[^{3}H]SCH 23390 (μM)						
nucleus accumbens						
caffeine-treated	0.18 ± 0.01	0.88 ± 0.03	53 ± 3[a]	0.27 ± 0.03	0.79 ± 0.12	35 ± 1[a]
control	0.26 ± 0.06	0.71 ± 0.10	59 ± 7[a]	0.19 ± 0.02	0.87 ± 0.02	33 ± 12[a]
striatum						
caffeine-treated	0.20 ± 0.06	0.89 ± 0.02	67 ± 4[b]	0.18 ± 0.01	0.89 ± 0.002	57 ± 6[b]
control	0.22 ± 0.03	0.92 ± 0.01	23 ± 6[b]	0.45 ± 0.21	0.73 ± 0.19	23 ± 6[b]
prefrontal cortex						
caffeine-treated	0.82 ± 0.28			1.05 ± 0.38		
control	0.79 ± 0.08			1.05 ± 0.17		
[^{3}H]spiperone (μM)						
nucleus accumbens						
caffeine-treated	0.22 ± 0.03			0.36 ± 0.08		
control	0.12 ± 0.02			0.21 ± 0.05		
striatum						
caffeine-treated	0.23 ± 0.10			0.67 ± 0.40		
control	0.58 ± 0.21			2.2 ± 1.1		

Mean (± 1 S.E.M.) IC_{50} values for DA displacement of [^{3}H]SCH 23390 (μM) and [^{3}H]spiperone (μM) binding in prefrontal cortex, nucleus accumbens and striatum from rats drinking (14 days) a caffeinated solution or tap water. Each value represents the mean of three or four assays. Correlation coefficient values for [^{3}H]SCH 23390 for two-site binding analysis (.99 ± .01) reflect significantly better fit than for one-site binding analysis (.72 ± .13) in nucleus accumbens and striatum, but not in prefrontal cortex (one-site: .98 ± .01; two-site: .99 ± .01). Correlation coefficient values for [^{3}H]spiperone for one-site (.95 ± .05) and two-site (.93 ± .08) binding analyses were not significantly different for either nucleus accumbens or striatum, so IC_{50} values for these binding sites are listed under IC_{50_H}.

[a] No GTP vs. with GTP; $P < .05$.

[b] Caffeine-treated vs. control; $P < .05$.

selective DA D_2 receptor antagonist [^{3}H]spiperone was determined in crude membrane fractions of prefrontal cortex, striatum and nucleus accumbens of control and caffeine-treated rats.

The radioligand binding data indicate that chronic caffeine altered DA D_1 receptor expression, but not affinity, and had no apparent effect on DA D_2 receptors. Specifically, B_{max} values for specific [^{3}H]SCH 23390 binding were significantly decreased in the nucleus accumbens and striatum and significantly increased in the prefrontal cortex of caffeine-treated compared to control rats (Fig. 5). On the other hand, K_D values were not significantly changed in any of these brain regions in caffeine-treated vs. control rats. No significant differences between caffeine-treated and control rats were observed for either B_{max} or K_D values for [^{3}H]spiperone binding to striatal or nucleus accumbens membrane preparations (Fig. 6). [^{3}H]Spiperone binding in prefrontal cortex was not measured.

Analysis of IC_{50} values for displacement of [^{3}H]SCH 23390 binding by DA revealed that there are probably two binding sites or binding states (low and high affinity) in nucleus accumbens and striatum, but not in prefrontal cortex (Table 2). In striatum, the ratio of high- to low-affinity binding states increased significantly in caffeine-treated compared to control tissue both in the presence and absence of GTP. No difference in this ratio was observed in the nucleus accumbens of caffeine-treated vs. control rats. IC_{50} values for DA were not significantly different between caffeine-treated and control tissue in any of the three brain regions. Only a single affinity state was detected for DA binding to sites labeled with [^{3}H]spiperone. IC_{50} values for DA displacement of [^{3}H]spiperone binding were not different between caffeine-treated and control tissue in either nucleus accumbens or striatum (Table 2).

4. Discussion

Consistent with previous studies (Finn and Holtzman, 1986; Garrett and Holtzman, 1994a; Holtzman, 1983), these data show that caffeine produces biphasic effects on locomotor activity, increasing locomotor activity at lower doses and decreasing this effect at higher doses. Acute administration of the nonselective DA receptor antagonist HAL produced a dose-dependent and complete antagonism of the locomotor stimulant effects of caffeine. These data are also consistent with previous reports that DA depletion (Finn and Holtzman, 1990) and DA receptor blockade (Garrett and Holtzman, 1994a) can block the locomotor stimulant effects of acutely administered caffeine. Despite the fact that acute caffeine administration alters extracellular concentrations of DA, norepinephrine and serotonin (Daly, 1993), only the highest dose of the DA uptake inhibitor GBR 12909 enhanced the effects of caffeine, whereas the noradrenergic uptake inhibitor nisoxetine and the serotonergic uptake inhibitor fluoxetine at comparable

or higher doses did not. Although it is not clear why only the highest dose of GBR 12909 enhanced the effects of caffeine, together, these results strongly suggest that caffeine's acute locomotor stimulant effects are, at least in part, DA receptor-mediated.

In the present study, scheduled access to a caffeinated drinking solution produced tolerance to the locomotor stimulant effects of caffeine in a matter of a few days. These data are consistent with earlier reports of rapid and complete tolerance to the locomotor stimulant effects of caffeine using a similar method of scheduled access to a caffeinated drinking solution (Finn and Holtzman, 1986; Garrett and Holtzman, 1994b; Holtzman, 1983). Concurrent chronic administration of HAL and caffeine did not alter the development of tolerance to caffeine's locomotor stimulant effects in the present study. Other investigators have reported that rats made tolerant to caffeine are not cross-tolerant to other nonxanthine psychomotor stimulants, most notably D-amphetamine and cocaine, that produce locomotor stimulation by indirect activation of DA receptors (Garrett and Holtzman, 1994b; Finn and Holtzman, 1987; Holtzman, 1991). Moreover, Garrett and Holtzman (1994b) showed that rats made tolerant to caffeine are cross-tolerant to the locomotor stimulant effects of either D_1 or D_2 DA receptor agonists when administered separately, but not when administered concurrently. If concurrent activation of DA D_1 and D_2 DA receptors precludes cross-tolerance to caffeine, it is possible that concurrent blockade of DA D_1 and D_2 receptor subtypes does something to prevent blockade of the development of tolerance to caffeine. On the other hand, since HAL does show more selectivity for DA D_2 receptors over DA D_1 receptors, one must consider the possibility that HAL is simply not effective enough at antagonizing DA D_1 receptors, which seem to play a more important role in mediating caffeine-induced tolerance as suggested by the binding results.

Another possibility is that the dose of HAL infused during chronic caffeine exposure was not high enough to prevent the development of tolerance to caffeine. In the present study, HAL was infused over 24 h to achieve a dose of 0.1 mg/kg/day. Higher doses of HAL were not used in the chronic experiment because a pilot experiment from our laboratory showed that infusion of 0.3-mg/kg/day HAL produced a general disruption in behavior in control rats. It is noteworthy that studies reporting changes in extracellular DA concentrations following chronic HAL treatment (Ichikawa and Meltzer, 1990, 1991; Zhang et al., 1989) have used higher doses than the present study and studies finding no changes (Hernandez and Hoebel, 1989; Moghaddam and Bunney, 1993). However, in the present study, higher doses of HAL could not be infused without producing a general disruption in behavior. Failure to adequately block DA receptors in the present study might explain why chronic HAL did not block caffeine-induced tolerance. Furthermore, data from other pilot studies in our laboratory indicate that more selective DA receptor antagonists such as SCH 23390 (D_1) and eticlopride (D_2) are not stable enough to be continuously infused. Thus, HAL-induced DA receptor blockade may not have been adequate to reveal the role of DA receptors in mediating tolerance to caffeine's locomotor stimulant effects.

Interestingly, rats infused with HAL and drinking caffeine did not show a complete recovery from tolerance by the fourth day after removal of the HAL pumps and the caffeine. This indicates that chronic infusion of HAL produces long-term effects on the mechanisms mediating the locomotor response to acute caffeine. However, if this were the case, one might expect to observe a decreased response to the caffeine challenge tests in rats infused with HAL and drinking water. Another possibility is that the combination of HAL and caffeine produced changes in these mechanisms that did not completely recover by the postchronic test day. If recovery from tolerance to caffeine involves DA D_2 receptors, any long-term changes in these receptors due to chronic HAL administration could have altered the recovery process. Evaluation of changes in DA receptors in rats infused with HAL and drinking caffeine should be able to address this possibility.

Other reports have indicated a pharmacologic specificity of cross-tolerance to caffeine-induced locomotor stimulation that is limited to methylxanthines and a few nonxanthine stimulants, including theophylline and CGS 15943, respectively (Finn and Holtzman, 1987, 1988; Holtzman, 1991). Similarities in the mechanisms between these drugs and caffeine include blockade of adenosine A_1 and A_2 receptors. Although these reports suggest that the main mechanism(s) underlying caffeine-induced tolerance are likely to be found in the adenosine receptor system, there is little consensus on how adenosine receptors mediate caffeine-induced tolerance. Ferre et al. have gathered an impressive amount of evidence indicating an antagonistic interaction between adenosine and DA receptors. Specifically, adenosine receptor antagonists such as caffeine release a GABA- or acetylcholine-mediated inhibition on DA D_1 and D_2 receptors in striatopallidal and striatonigral neurons (see Ferre, 1997 for review). The results of one study showed changes in DA D_2, but not DA D_1 receptor binding in rat striatum by in vitro application of an adenosine agonist CGS 21680 (Ferre et al., 1991a). In contrast, the present data indicate that DA D_1 receptors are significantly upregulated in prefrontal cortex and downregulated in nucleus accumbens and striatum, and that D_2 DA receptors are not significantly changed in response to chronic in vivo caffeine exposure. Of course, the method of exposure to CGS 21680 and caffeine was considerably different between these two studies and could easily explain the inconsistent results.

Differential changes in DA neurotransmission between prefrontal cortex and the mesolimbic regions of the nucleus accumbens and striatum have been reported pre-

viously in response to various dopaminergic drugs (Hernandez and Hoebel, 1989; Ichikawa and Meltzer, 1991; Kuroki et al., 1999). Also, acute caffeine administration inhibits mesolimbic–mesocortical projecting DA neurons, but has no effect on DA neurons that project to the striatum (Stoner et al., 1988). Furthermore, the motoric effects of caffeine are believed to be mediated via adenosine–DA interactions in terminal regions of nigrostriatal neurons, whereas the cognitive effects of caffeine are believed to be mediated via adenosine–DA interactions in brain areas receiving input from mesolimbic–mesocortical DA projecting neurons (see Ferre, 1997 for review). Therefore, it is possible that the different regional changes in DA D_1 receptors following chronic caffeine administration in the present study are the result of different effects of caffeine on DA neurons that project to these regions. The fact that chronic caffeine administration significantly alters DA D_1 receptors in the present study seems consistent with the clear role of DA in mediating the acute locomotor stimulant effects of caffeine.

It is conceivable that caffeine-induced DA D_1 receptor downregulation in the striatum and nucleus accumbens is the result of the removal of adenosine's inhibitory effects on DA D_1 receptors, thereby producing overstimulation of these receptors. These data are consistent with some behavioral reports that tolerance to caffeine confers cross-tolerance to DA D_1 or D_2 agonists alone, but not to DA D_1 and D_2 receptor agonists administered together or to D-amphetamine and cocaine (Garrett and Holtzman, 1994b; Finn and Holtzman, 1987; Holtzman, 1991). However, it remains unclear why DA D_2 receptor agonists would show cross-tolerance to caffeine if DA D_2 receptors are not altered in response to chronic caffeine administration. Evidence for a functional interaction between DA D_1 and D_2 receptors may shed some light on this. For example, some studies have shown that selective DA D_1 receptor antagonists can block the locomotor stimulant effects of DA D_2 receptor agonists, and, conversely, selective DA D_2 receptor antagonists can block the locomotor stimulant effects of DA D_1 receptor agonists (Barone et al., 1988; Garrett and Holtzman, 1994a; Mailman et al., 1984; Martin-Inverson et al., 1988). A more recent report failed to show cross-tolerance between caffeine and a DA D_2 agonist, whereas cross-tolerance was confirmed between caffeine and a DA D_1 agonist (Jaszyna et al., 1998). The more recent study is most consistent with the present findings that DA D_2 receptors are not altered in response to chronic caffeine administration and may explain the lack of cross-tolerance between caffeine and other psychomotor stimulants such as cocaine and D-amphetamine. In addition, two recent reports indicate that the nonselective DA agonists, bromocriptine and pergolide, both block development of caffeine-induced tolerance on rotational behavior (Casas et al., 1999; Prat et al., 2000). These reports indicate that DA D_1 receptors may, indeed, be the key to changes in the dopaminergic system in response to chronic caffeine, and that tolerance to caffeine may be prevented by blocking caffeine's interactions with these receptors.

Despite the present evidence that changes in DA D_1 receptors underlie the development of tolerance to caffeine, at least one other possible explanation remains unchallenged. Jacobson et al. (1996) proposed an explanation for caffeine-induced tolerance based on the biphasic acute dose–response curve for caffeine. The low doses of caffeine are presumed to increase locomotor activity by antagonizing adenosine receptors, whereas high doses of caffeine decrease locomotor activity by some other mechanism (possibly inhibition of phosphodiesterases). Chronic caffeine administration may increase the threshold for the stimulatory action of caffeine by increasing adenosine receptor function, thereby allowing the depressant effects of caffeine to override its stimulant effects. This could also explain why tolerance to the locomotor stimulant effects of caffeine is insurmountable.

In conclusion, these results provide further evidence that DA receptor activation is probably the chief mechanism underlying the acute stimulatory effects of caffeine on locomotor activity. Even though HAL infusion concurrent with caffeine administration did not block the development of tolerance to caffeine-induced behavioral stimulation, there were clear changes in DA D_1 receptors in the striatum, nucleus accumbens and prefrontal cortex in rats treated chronically with caffeine. Thus, specific changes in DA D_1 receptor numbers are possible candidates for the mechanism underlying tolerance to the locomotor stimulant effects of caffeine.

Acknowledgments

The authors wish to thank Leon Goe for his technical assistance. This research was supported by the Individual Postdoctoral Fellowship Award F32 DA05709 (KRP), Research Scientist Award K05 DA00008 (SGH) and Grant DA03413 from the National Institute on Drug Abuse, NIH.

References

Ahlijanian MK, Takemori AE. Cross-tolerance studies between caffeine and (−)-N^6 (phenylisopropyl)adenosine (PIA) in mice. Life Sci 1986; 38:577–88.

Barone P, Tucci I, Parashos SA, Chase TN. Supersensitivity to a D-1 dopamine receptor agonist and subsensitivity to a D-2 receptor agonist following chronic D-1 receptor blockade. Eur J Pharmacol 1988;149: 225–32.

Casas M, Prat G' Robledo P, Barbanoj M, Kulisevsky J, Jane F. Repeated co-administration of caffeine and bromocriptine prevents tolerance to the effects of caffeine in the turning behavior animal model. Eur Neuropsychopharmacol 1999;9:515–21.

Corsi C, Melani A, Bianchi L, Pepeu G, Pedata F. Effect of adenosine A_{2A} receptor stimulation on GABA release from the striatum of young and aged rats in vivo. NeuroReport 1999;10:3933–7.

Daly JW. Mechanism of action of caffeine. In: Garratini S, editor. Caffeine, coffee and health. New York: Raven Press, 1993. pp. 7–150.

Daly JW, Bruns RE, Snyder SH. Adenosine receptors in the central nervous system: relationship to the central actions of methylxanthines. Life Sci 1981;28:2083–97.

Dasgupta S, Ferre S, Kull B, Hedlund PB, Finnman U-B, Ahlberg S, Arenas E, Fredholm BB, Fuxe K. Adenosine A_{2A} receptors modulate the binding characteristics of dopamine D_2 receptors in stably cotransfected fibroblast cells. Eur J Pharmacol 1996;316:325–31.

Ferre S. Adenosine–dopamine interactions in the ventral striatum. Psychopharmacology 1997;133:107–20.

Ferre S, Guix T, Salles J, Badia A, Parra P, Jane' F, Herrera-Marschitz M, Ungerstedt U, Casas M. Paraxanthine displaces binding of [^{3}H]SCH 23390 from rat striatal membranes. Eur J Pharmacol 1990;179:295–9.

Ferre S, Herrera-Marschitz M, Grabowski-Anden M, Ungerstedt U, Casas M, Anden N-E. Postsynaptic dopamine/adenosine interaction: I. Adenosine analogues inhibit D-2 mediated behaviour in short-term reserpinized mice. Eur J Pharmacol 1991a;192:30–5.

Ferre S, Herrera-Marschitz M, Grabowski-Anden M, Casas M, Ungerstedt U, Anden NE. Postsynaptic dopamine/adenosine interaction: II. Postsynaptic dopamine agonism and adenosine antagonism of methylxanthines in short-term reserpinized mice. Eur J Pharmacol 1991b; 192:31–7.

Ferre S, von Euler G, Johansson B, Fredholm BB, Fuxe K. Stimulation of high-affinity adenosine A_2 receptors decreases the affinity of dopamine D_2 receptors in rat striatal membranes. Proc Natl Acad Sci USA 1991c; 88:7238–41.

Ferre S, Popoli P, Giminez-Llort L, Finneman UB, Martinez E, de Carolis AS, Fuxe K. Postsynaptic antagonistic interaction between adenosine A_1 and dopamine D_1 receptors. NeuroReport 1994a;6:73–6.

Ferre S, O'Connor WT, Snaprud P, Ungerstedt U, Fuxe K. Antagonistic interaction between adenosine A_{2A} receptors and dopamine D_2 receptors in the ventral striopallidal system. Implications for treatment of schizophrenia. Neuroscience 1994b;63(3):765–73.

Ferre S, Popoli P, Tinner-Staines B, Fuxe K. Adenosine A_1 receptor–dopamine D_1 receptor interaction in the rat limbic system: modulation of dopamine D_1 receptor antagonist binding sites. Neurosci Lett 1996;208: 109–12.

Finn IB, Holtzman SG. Tolerance to caffeine-induced stimulation of locomotor activity in rats. J Pharmacol Exp Ther 1986;238:542–6.

Finn IB, Holtzman SG. Pharmacologic specificity of tolerance to caffeine-induced stimulation of locomotor activity. Psychopharmacology 1987;93:428–34.

Finn IB, Holtzman SG. Tolerance and cross-tolerance to theophylline-induced stimulation of locomotor activity in rats. Life Sci 1988;42: 2475–82.

Finn IB, Holtzman SG. Depletion of catecholamines in the brain of rats differentially affects stimulation of locomotor activity by caffeine, D-amphetamine and methylphenidate. Neuropharmacology 1990;29: 625–31.

Garrett BE, Holtzman SG. D_1 and D_2 dopamine receptor antagonists block caffeine-induced stimulation of locomotor activity in rats. Pharmacol Biochem Behav 1994a;47:89–94.

Garrett BE, Holtzman SG. Caffeine cross-tolerance to selective dopamine D_1 and D_2 receptor agonists but not to their synergistic interaction. Eur J Pharmacol 1994b;262:65–75.

Golembiowska K, Zylewska A. Agonists of A_1 and A_{2A} adenosine receptors attenuate methamphetamine-induced overflow of dopamine in rat striatum. Brain Res 1998;806:202–9.

Griffiths RR, Mumford GK. Caffeine — a drug of abuse? In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: the fourth generation of progress. New York: Raven Press, 1995. pp. 1699–713.

Griffiths RR, Woodson PP. Reinforcing effects of caffeine in humans. J Pharmacol Exp Ther 1988;246:21–9.

Griffiths RR, Bigelow GE, Liebson IA, O'Keefe M, O'Leary D, Russ N. Human coffee drinking: reinforcing and physical dependence producing effects of caffeine. J Pharmacol Exp Ther 1986;239:416–25.

Hernandez L, Hoebel BG. Haloperidol given chronically decreases basal dopamine in prefrontal cortex more than in striatum or nucleus accumbens as simultaneously measured by microdialysis. Brain Res Bull 1989;22:763–9.

Hess EJ, Norman AB, Creese I. Chronic treatment with dopamine receptor antagonists: behavioral and pharmacologic effects of D_1 and D_2 dopamine receptors. J Neurosci 1988;8:2361–70.

Holtzman SG. Complete, reversible, drug-specific tolerance to stimulation of locomotor activity by caffeine. Life Sci 1983;33:779–87.

Holtzman SG. CGS 15943, a nonxanthine adenosine receptor antagonist: effects on locomotor activity of nontolerant and caffeine-tolerant rats. Life Sci 1991;49:1563–70.

Holtzman SG, Finn IB. Tolerance to behavioral effects of caffeine in rats. Pharmacol Biochem Behav 1988;29:411–8.

Ichikawa J, Meltzer HY. The effects of chronic clozapine and haloperidol on basal dopamine release and metabolism in rat striatum and nucleus accumbens studies by in vivo microdialysis. Eur J Pharmacol 1990; 176:371–4.

Ichikawa J, Meltzer HY. Differential effects of repeated treatment with haloperidol and clozapine on dopamine release and metabolism in the striatum and nucleus accumbens. J Pharmacol Exp Ther 1991;256:348–57.

Jacobson KA, von Lubitz DKJE, Daly JW, Fredholm BB. Adenosine receptor ligands: differences with acute versus chronic treatment. TIPS 1996;17:108–13.

Jaszyna M, Gasior M, Shoaib M, Yasar S, Goldberg SR. Behavioral effects of nicotine, amphetamine and cocaine under a fixed-interval schedule of food reinforcement in rats chronically exposed to caffeine. Psychopharmacology 1998;140:257–71.

Kirch DG, Taylor TR, Gerhardt GA, Benoqitz NL, Stephen C, Wyatt RJ. Effect of chronic caffeine administration on monoamine and monoamine metabolite concentrations in rat brain. Neuropharmacology 1990;29(6):599–602.

Kirch DG, Taylor TR, Creese I, Xu S-X, Wyatt RJ. Effect of chronic nicotine treatment and withdrawal on rat striatal D_1 and D_2 dopamine receptors. J Pharm Pharmacol 1992;42:89–92.

Kuroki T, Meltzer HY, Ichikawa J. Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. J Pharmacol Exp Ther 1999;288:774–81.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265–75.

Mailman RB, Schultz DW, Lewis MH, Staples L, Rollema J, Dehaven DL. SCH-23390: a selective D_1 dopamine antagonist with potent D_2 behavioral actions. Eur J Pharmacol 1984;101:159–65.

Martin-Iverson MT, Iverson SD, Stahl SM. Long-term motor stimulant effects of (+)-4-propyl-9-hydroxynaphthoxazine (PHNO), a dopamine D-2 receptor agonist: interactions with a dopamine D-1 receptor antagonist and agonist. Eur J Pharmacol 1988;149:25–31.

Mayfield RD, Jones BA, Miller HA, Simonsky JK, Larson GA, Zahniser NR. Modulation of endogenous GABA release by an antagonistic adenosine A_1/dopamine D_1 receptor interaction in rat brain limbic regions but not basal ganglia. Synapse 1999;33:274–81.

Moghaddam G, Bunney BS. Depolarization inactivation of dopamine neurons: terminal release characteristics. Synapse 1993;14:195–200.

Morgan ME, Vestal RE. Methylxanthine effects on caudate dopamine release as measured by in vivo electrochemistry. Life Sci 1989;45: 2025–39.

Nehlig A, Daval JL, Debry G. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res Rev 1992;17:139–70.

Prat G, Robledo P, Rubio A, Barbanoj M, Jane' F, Casas M. Effects of subchronic combined treatment with pergolide and caffeine on contralateral rotational behavior in unilateral 6-hydroxydopamine-denervated rats. Brain Res 2000;868:376–9.

Salim H, Ferre S, Dalal A, Peterfreund RA, Fuxe K, Vincent JD, Lledo PM. Activation of adenosine A_1 and A_{2A} receptors modulates dopamine D_2 receptor-induced responses in stably transfected human neuroblastoma cells. J Neurochem 2000;74:432–9.

Snyder SH, Katims JJ, Annau A, Bruns RF, Daly JW. Adenosine receptors and the behavioral actions of methylxanthines. Proc Natl Acad Sci USA 1981;78:3260–4.

Stoner GR, Skirboll LR, Werlman S, Hommer DW. Preferential effects of caffeine on limbic and cortical dopamine systems. Biol Psychiatry 1988;23:761–8.

Watanabe H, Uramoto H. Caffeine mimics dopamine receptor agonists without stimulation of dopamine receptors. Neuropharmacology 1986; 25:577–82.

Zhang W, Tilson H, Stachowiak MK, Hong JS. Repeated haloperidol administration changes basal release of striatal dopamine and subsequent response to haloperidol challenge. Brain Res 1989;484:389–92.