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D_1 and D_2 Dopamine Receptor Antagonists Block Caffeine-Induced Stimulation of Locomotor Activity in Rats

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GARRETT, B. E. AND S. G. HOLTZMAN. *D_1 and D_2 dopamine receptor antagonists block caffeine-induced stimulation of locomotor activity in rats.* PHARMACOL BIOCHEM BEHAV 47(1) 89–94, 1994.—The mechanism of action for the behavioral stimulant effects of caffeine has been extensively studied, but results have been ambiguous and inconsistent. The present study examined the role of dopamine in caffeine-induced stimulation of locomotor activity in rats. *d*-Amphetamine was also tested for comparison. Locomotor activity of male Sprague–Dawley rats (300–350 g) was measured using two-channel electronic activity monitors. Activity counts were recorded for 30 min following a 30-min pretreatment with either caffeine (3.0–100 mg/kg, IP) or *d*-amphetamine (0.1–3.0 mg/kg, IP) alone and in combination with the D_1 dopamine antagonist SCH23390 (0.01 and 0.003 mg/kg, SC) or the D_2 dopamine antagonists sulpiride (30 mg/kg, SC) or eticlopride (0.03 mg/kg, SC). Caffeine and *d*-amphetamine dose dependently increased locomotor activity. This effect of both caffeine and *d*-amphetamine was blocked by SCH23390 as well as by eticlopride. Sulpiride blocked the stimulatory effects of caffeine but not *d*-amphetamine. These results suggest that the locomotor stimulant effect of caffeine, like that of *d*-amphetamine, is mediated through dopaminergic systems; both D_1 and D_2 receptors appear to be involved.

Caffeine *d*-Amphetamine SCH23390 Eticlopride Sulpiride Quinpirole SK&F38393

THE behavioral stimulant effects of caffeine have been well documented. Caffeine appears to enhance alertness, feelings of well-being, motivation, energy, and concentration in humans (13), and produces a dose-dependent increase in locomotor activity in animal species (8,21). However, the mechanisms of action that underlie these effects remain unclear. Caffeine is a competitive antagonist at adenosine receptors. Snyder et al. (21) have demonstrated that the behavioral stimulant effects of caffeine and other methylxanthines correlate with their in vitro binding affinities at adenosine receptors, suggesting that the stimulant effects of caffeine are mediated by blockade of the adenosine receptor. In addition to antagonism at adenosine receptors, stimulatory effects may be mediated by the ability of caffeine to inhibit cyclic nucleotide phosphodiesterase or alter intracellular calcium concentration (6).

Recently, the existence of a central dopamine/adenosine interaction has been suggested, adenosine causing an inhibition of dopamine receptor stimulation (7,12). Ferré et al. (9) have also demonstrated behavioral evidence of the existence of a postsynaptic D_2/adenosine interaction. This interaction appears to involve a high-affinity A_2 adenosine receptor [A_{2a} receptor, following the nomenclature suggested by (4)]. Caffeine, by adenosine antagonism, may act to remove adenosine inhibition on dopaminergic activity—thus producing its behavioral stimulant effect.

Numerous studies have demonstrated the role of dopamine in the behavioral effects of other stimulant drugs, such as *d*-amphetamine. The enhancement of locomotor activity that is seen after low doses of *d*-amphetamine and the stereotyped behavior that is seen after higher doses can be blocked by antagonists selective for the D_1 and D_2 dopamine receptors (18). The discriminative stimulus properties of *d*-amphetamine can also be blocked by D_1 and D_2 dopamine receptor antagonists (5). Fewer studies have investigated the involvement of dopamine in the behavioral stimulant effects of caffeine. Therefore, the objective of the present study was to assess the role of dopamine in caffeine-induced stimulation of locomotor activity in the rat. This was accomplished by examining the effects of selective D_1 and D_2 dopamine receptor antagonists on caffeine-induced increases in locomotor activity. *d*-Amphetamine, which acts indirectly at dopamine receptors, was used as a comparison.

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METHOD

Subjects

Male rats of Sprague–Dawley descent (Sasco, Inc., Omaha, NE) weighing 300–350 g at the start of the experiment were used. All rats were grouped housed in polycarbonate cages and maintained in a temperature-controlled colony room with a 12 L : 12 D cycle. Food (Purina Rodent Chow, Purina Mills, St. Louis, MO) and water were available ad lib.

Drug Administration

Rats were administered, in a random sequence, doses of caffeine (3.0, 10, 30, and 100 mg/kg, IP) alone and in combination with the D_1 dopamine receptor antagonist SCH23390 (0.01 and 0.003 mg/kg, SC) or the D_2 dopamine receptor antagonists sulpiride (30 mg/kg, SC) or eticlopride (0.03 mg/kg, SC). *d*-Amphetamine (0.1, 0.3, 1.0, and 3.0 mg/kg, IP) was tested under similar conditions. To assess the specificity of the drug effects, stimulation of locomotor activity by SK&F38393 (1.0, 3.0, 10, and 30 mg/kg, SC), a partial agonist at D_1 dopamine receptors, and the D_2 dopamine receptor agonist quinpirole (0.1, 0.3, 1.0, and 3.0 mg/kg, SC) were examined in the presence of either SCH23390 (0.003 mg/kg) or eticlopride (0.03 mg/kg). Doses of antagonists were selected for testing that had little effect on locomotor activity, based upon results from pilot experiments in experimentally naive rats. All drugs were administered 30 min before each experimental session except for sulpiride, which was administered 1 h before each test session. The drug pretreatment times were selected from pilot experiments and from previous literature that demonstrated the time of onset of action of these drugs.

Locomotor Activity

Locomotor activity was measured with six two-channel Electronic Activity Monitors (31404, Stoelting Co., Chicago, IL). Each rat was placed in a polycarbonate rat cage (51 × 41 × 22 cm), which was centered on a sensor platform (SA1566, Stoelting). Both the cage and sensor platform were placed in a ventilated, sound-attenuating chamber that was illuminated by a fluorescent lightbulb. The counting threshold of each sensor was calibrated with a swinging pendulum so that one channel measured gross movements in the horizontal plane corresponding to locomotion and the other channel measured total movements; the difference between the two channels represented the fine movements, such as grooming and sniffing. Rats were allowed to habituate to the activity chambers for at least 5 days prior to the start of activity testing. Activity testing was conducted twice weekly (Monday and Friday) to avoid the development of tolerance to caffeine and minimize any sensitization to the effects of amphetamine. Each rat received the appropriate drug pretreatment before being placed in the activity chamber. The last 15 min of the pretreatment interval consisted of an acclimation period during which activity was not recorded. Locomotor activity was then measured for 30 min.

Data Analysis

Dose-effect curves for caffeine and *d*-amphetamine were compared for agonist alone and agonist/antagonist combination treatment by a two-factor analysis of variance (ANOVA) with repeated measures on both factors. This was followed by a posthoc comparison using Student's *t*-test protected for multiple pairwise comparisons.

Drugs

Caffeine sodium benzoate and *d*-amphetamine sulfate were obtained from Sigma Chemical Co. (St. Louis, MO.) Other drugs used in this study were sulpiride, SCH23390 HCl, eticlopride HCl, SK&F38393 HCl, and quinpirole HCl (Research Biochemicals, Inc., Natick, MA). All drugs were dissolved in 0.9% saline with the exception of sulpiride and SK&F38393 HCl. Sulpiride was dissolved in three parts 8.5% lactic acid and the pH of the solution was adjusted to 5.0–6.0 with two parts 1.0 N NaOH. The solution was brought to final volume with 0.9% saline. SK&F38393 HCl was dissolved in distilled water. All drugs were administered in a volume of 1 ml/kg body weight with doses expressed as the free base.

RESULTS

Caffeine (3.0–100 mg/kg) dose dependently increased all three measures of activity (fine, gross, and total), with the peak increase occurring at 30 mg/kg (Figs. 1A–1C). The highest dose of caffeine, 100 mg/kg, appeared on the descending limb of the dose-effect curve, increasing activity over vehicle, but to a lesser degree than did 30 mg/kg. This type of biphasic dose–response curve for caffeine has been reported previously (10). The antagonists alone did not affect locomotor activity significantly compared to vehicle. However, both doses of SCH23390 (0.01 and 0.003 mg/kg) attenuated the locomotor response to the 10- and 30-mg/kg doses of caffeine, $F(4, 32) = 19.6$, $p < 0.0001$, and $F(4, 32) = 7.35$, $p = 0.0004$, respectively. Because the intermediate doses of caffeine were blocked by the 0.01-mg/kg dose of SCH23390 and the highest dose was unaffected, there appeared to be a complete flattening of the dose–response curve. Both eticlopride (0.03 mg/kg) and sulpiride (30 mg/kg) attenuated the locomotor response to the 30-mg/kg dose of caffeine, $F(4, 32) = 5.93$, $p = 0.0011$, and $F(4, 32) = 3.8$, $p = 0.0056$, respectively, and, in the case of eticlopride, to the 10-mg/kg dose as well, $F(4, 32) = 5.93$, $p = 0.0011$.

Rats administered *d*-amphetamine (0.1–3.0 mg/kg) also showed a dose-dependent increase in locomotor activity, which was approximately twice that produced by caffeine (Figs. 2A–2C). SCH23390 (0.003 mg/kg) and eticlopride (0.03 mg/kg) were equieffective in attenuating *d*-amphetamine-induced stimulation of locomotor activity at doses that had no significant effect on locomotor activity alone. As it did with caffeine, the highest dose of SCH23390 resulted in a complete flattening of the *d*-amphetamine dose–response curve. The results of the ANOVA indicated that the *d*-amphetamine curves that followed pretreatment with 0.03 mg/kg eticlopride and 0.01 and 0.003 mg/kg SCH23390 differed significantly from the curve for *d*-amphetamine alone, $F(4, 32) = 20.33$, $p < 0.0001$; $F(4, 32) = 38$, $p < 0.0001$; and $F(4, 32) = 11.32$, $p < 0.0001$, respectively. In contrast, sulpiride had no significant effect on *d*-amphetamine-induced stimulation of locomotor activity, $F(4, 32) = 0.642$, $p = 0.7143$.

Administration of SK&F38393 (1.0–30 mg/kg) resulted in a dose-dependent but modest increase in locomotor activity (Fig. 3A). Intense grooming behavior was observed after administration of the 30-mg/kg dose of SK&F38393, which correlates with the reduction in locomotor activity that was seen at this dose. SCH23390 and eticlopride were equally effective in attenuating the increases in locomotor activity and grooming behavior induced by 30 mg/kg SK&F38393 ($p < 0.05$). The antagonists alone did not significantly affect activity.

Like SK&F38393, quinpirole (0.1–3.0 mg/kg) dose depen-

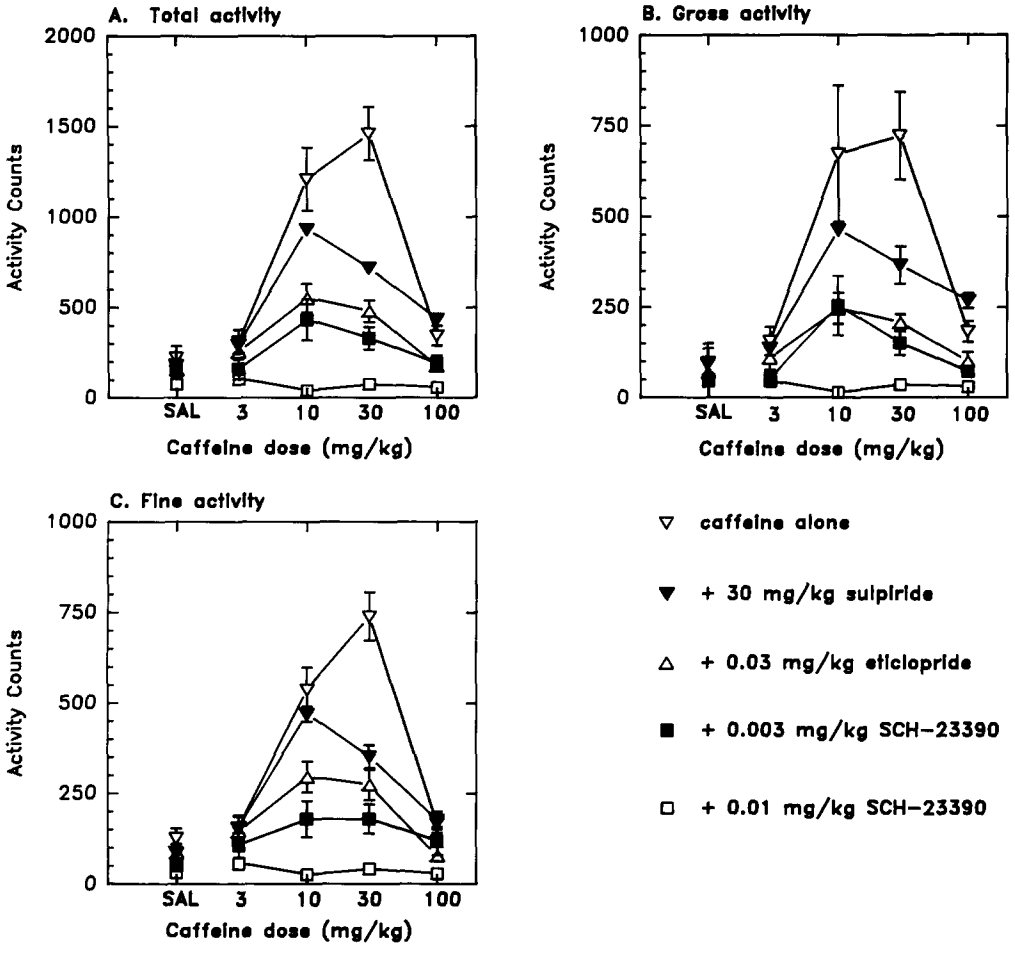

FIG. 1. Caffeine dose dependently increased total (A), gross (B), and fine (C) activity counts in rats (n = 9). This increase in activity was antagonized by administration of sulpiride (30 mg/kg), eticlopride (0.03 mg/kg), and SCH23390 (0.003 mg/kg). Doses of caffeine (3.0–100 mg/kg, IP) or saline (IP, points above SAL) were administered in random order alone or in combination with SC injections of antagonists 30 min prior to a 30-min test session. Pretreatment times before each test session were 30 min for eticlopride and SCH23390 and 60 min for sulpiride. Each point represents the mean activity counts ± SEM.

dently increased locomotor activity (Fig. 3B). However, the effect of quinpirole was more robust than the effect of SK&F38393. The effects of SCH23390 and eticlopride on quinpirole-induced stimulation of locomotor activity were comparable to their effects on activity induced by SK&F38393. Both drugs significantly reduced the locomotor response to the 3.0-mg/kg dose of quinpirole ($p < 0.01$). The antagonists alone had no significant effect on locomotor activity.

DISCUSSION

The results of the present study show that selective dopamine antagonists are effective in attenuating the locomotor response to caffeine as well as to *d*-amphetamine. The D_1 dopamine receptor antagonist SCH23390 and the D_2 dopamine antagonist eticlopride significantly reduced the locomotor stimulant effects produced by both caffeine and *d*-amphetamine. The less potent D_2 receptor antagonist sulpiride was only capable of antagonizing the 30-mg/kg dose of caffeine and had no effect on any *d*-amphetamine dose tested. These results are in contrast with those of Swerdlow et al. (22), which suggested that the behavioral stimulant effects of caffeine are independent of dopamine. These findings were based on the ability of flupenthixol or lesions of the nucleus accumbens with 6-hydroxydopamine to inhibit significantly the behavioral stimulant effect of one dose of *d*-amphetamine but not that of one dose of caffeine. The seeming discrepancies in the results between studies are unexplainable, but might be resolved by testing flupenthixol and neurochemically induced lesions of the nucleus accumbens with a full range of doses of *d*-amphetamine and caffeine.

Eticlopride and sulpiride are both selective for the D_2 dopamine receptor (14,15); eticlopride was the more potent of the two in antagonizing caffeine and *d*-amphetamine-induced stimulation of locomotor activity. Although sulpiride is selective for the D_2 receptor, it has been reported to be limited in its ability to cross the blood-brain barrier (19,22). This could explain why sulpiride was ineffective in attenuating the locomotor response to *d*-amphetamine. It is more difficult to ex-

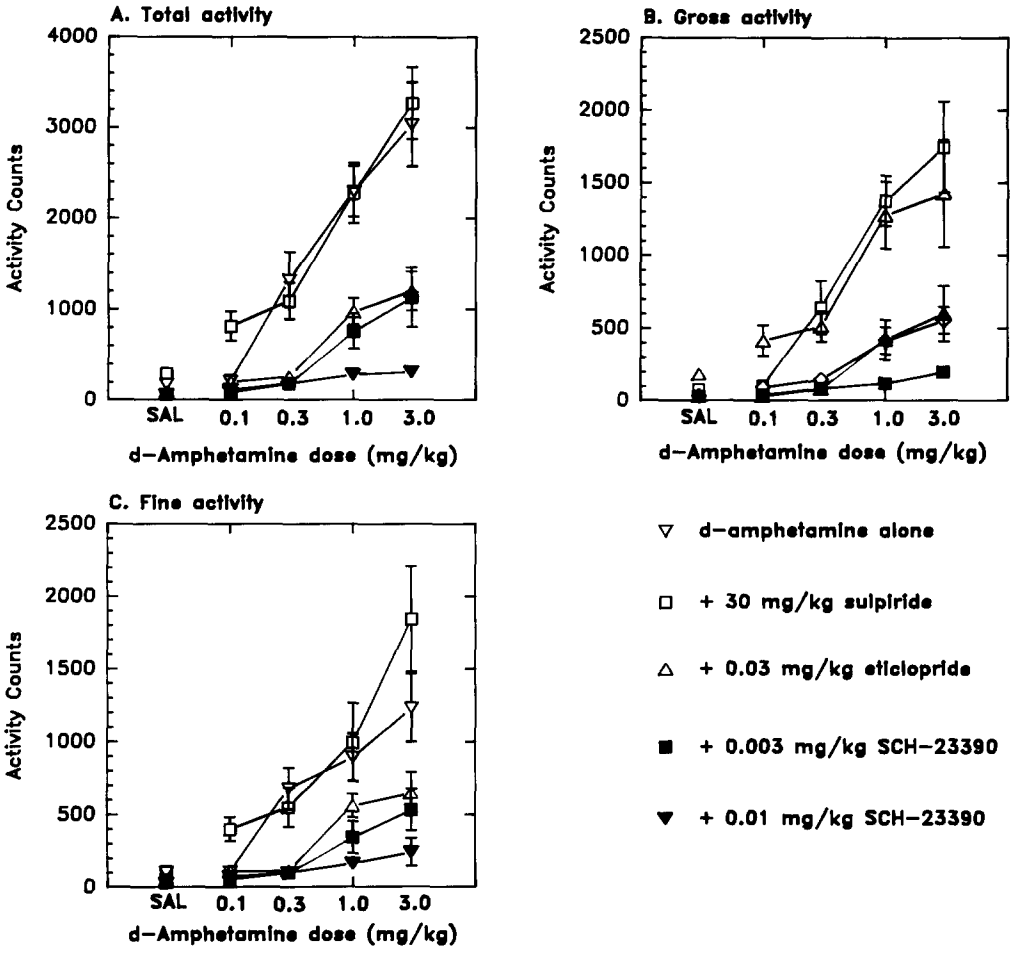

FIG. 2. *d*-Amphetamine dose dependently increased total (A), gross (B), and fine (C) activity counts in rats (n = 9). This effect was not antagonized by sulpiride (30 mg/kg). However, doses of eticlopride (0.03 mg/kg) and SCH23390 (0.003 mg/kg) markedly blocked *d*-amphetamine-induced increases in locomotor activity. Doses of *d*-amphetamine (0.1-3.0 mg/kg, IP) or saline (IP, points above SAL) were administered in random order alone or in combination with SC injections of antagonists 30 min prior to a 30-min test session. Pretreatment times for eticlopride and SCH23390 were 30 min and 60 min for sulpiride. Each point represents the mean activity counts ± SEM.

plain the reduction in response to the 30-mg/kg dose of caffeine in the presence of sulpiride because sulpiride did not block the locomotor response to *d*-amphetamine. This may suggest that caffeine is more sensitive to the antagonist effects of sulpiride and can be antagonized by smaller brain concentrations of sulpiride than can *d*-amphetamine. Another explanation is that the small antagonist effect of sulpiride was simply overwhelmed by the large amount of dopamine released by *d*-amphetamine, as reflected by the large increase in activity that the drug produced.

The mechanism of the behavioral stimulant effects of caffeine has been extensively studied. However, the results obtained from prior studies have been ambiguous and inconsistent. The three main actions of caffeine on the CNS have been described and all have been suggested to be involved in the stimulatory effects of caffeine. Antagonism of adenosine receptors has been generally favored as the mechanism of caffeine's behavioral stimulant effects. Caffeine inhibits adenosine receptors at concentrations that correlate to its potency as a behavioral stimulant (17), whereas other CNS effects of caffeine occur only at much higher concentrations of the drug (17). These concentrations are toxic and usually not found in the circulating blood upon oral ingestion of caffeine. Therefore, it seems unlikely that the pharmacological properties of caffeine are linked to its ability to inhibit phosphodiesterase or mobilize intracellular calcium.

Results with dopamine antagonists indicate that dopamine receptors mediate the stimulant effect of caffeine as well as that of *d*-amphetamine. However, caffeine must act differently from *d*-amphetamine because it neither releases dopamine nor inhibits dopamine reuptake. It has been proposed that caffeine acts indirectly at dopamine receptors by blockade of adenosine receptors. Adenosine is inhibitory to dopaminergic activity and its behavioral depressant effects may be a result of its ability to inhibit dopamine release from presynaptic terminals (17) or inhibit dopamine at the receptor level via receptor/receptor interactions (11). Because caffeine is a competitive antagonist at adenosine receptors, it may enhance

dopaminergic activity by removing the inhibitory influence of adenosine. Thus, increases in dopaminergic function may be, in part, the mechanism by which caffeine produces its behavioral stimulant effects.

The stimulant effect of caffeine is proposed to be mediated by the removal of inhibitory influences of adenosine from D_2 dopamine receptors; therefore, blockade of the stimulant effect of caffeine would be expected to occur only with an antagonist selective for D_2 dopamine receptors and not one selective for D_1 dopamine receptors. However, both D_1 and D_2 dopamine receptor antagonists blocked caffeine-induced stimulation of locomotor activity. Therefore, it was of interest to determine the receptor specificity of the antagonists used. To do this, the locomotor responses to the selective D_1 dopamine agonist SK&F38393 and the D_2 dopamine agonist quinpirole were determined alone and in the presence of either SCH23390 or eticlopride. Consistent with observations in previous studies, quinpirole stimulated locomotor activity with greater efficacy than did SK&F38393. Arnt (1) has shown that activation of the D_2 dopamine receptor by quinpirole or RU 24213 increases locomotor activity, sniffing, yawning, and rearing in rats. In contrast, the selective D_1 dopamine receptor agonists SK&F38393 and LU 24-040 produce slight increases in locomotor activity but have pronounced effects on grooming. Both antagonists markedly attenuated the locomotor response to SK&F38393 and quinpirole at doses that had no significant effect on locomotor activity alone. These findings are in agreement with those of other studies showing that selective D_1 dopamine antagonists block the locomotor stimulant effects of selective D_2 dopamine agonists and vice versa (2,16). This phenomenon could possibly be explained by evidence demonstrating a D_1/D_2 dopamine receptor interaction where concurrent administration of selective D_1 and D_2 dopamine agonists produce synergistic stimulatory effects on locomotor activity

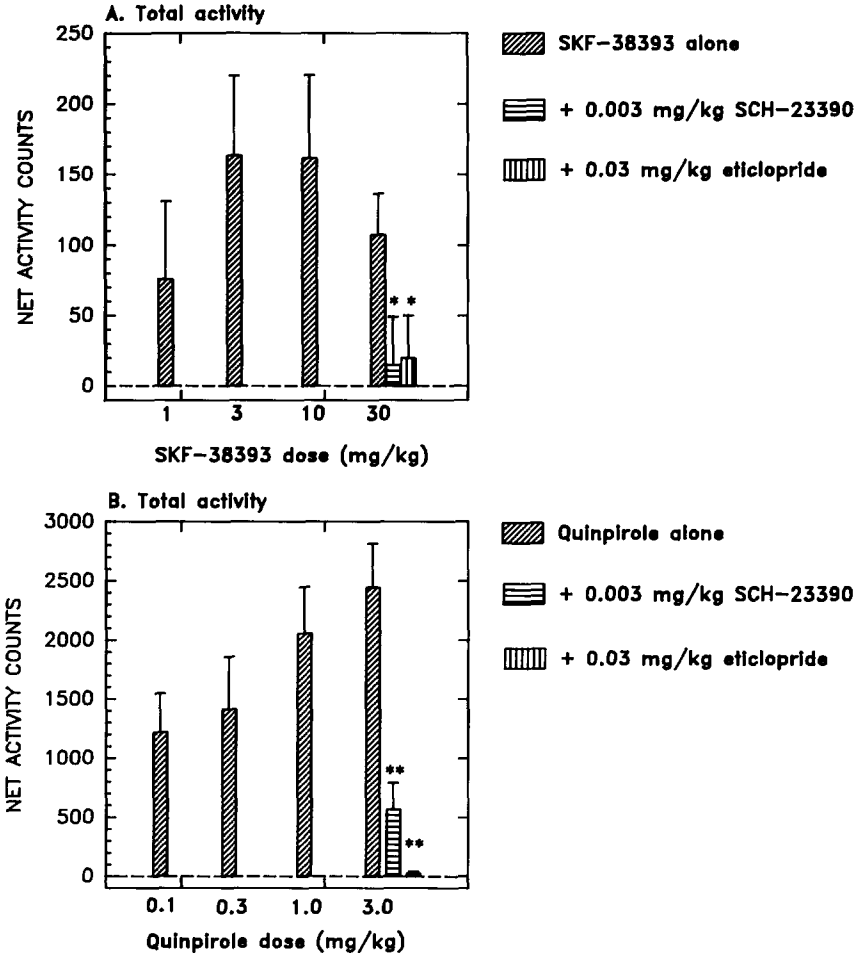

FIG. 3. Administration of eticlopride (0.03 mg/kg) and SCH23390 (0.003 mg/kg) attenuated the locomotor response to 30 mg/kg SK&F38393 (A) and 3.0 mg/kg quinpirole (B). Rats (n = 9) were injected in a random sequence with either saline (SC, points above SAL) or SK&F38393 (1.0–30 mg/kg, SC) or quinpirole (0.1–3.0 mg/kg) alone or in combination with SC injections of SCH23390 or eticlopride. Activity was recorded for 30 min, beginning 60 min after quinpirole administration and 30 min after SK&F38393 or antagonist administration. Activity counts are expressed as the net activity ± SEM. Significant differences from the corresponding point of rats administered 30 mg/kg SK&F38393 are indicated by an asterisk ($p < 0.05$) and of rats administered 3.0 mg/kg quinpirole by a double asterisk ($p < 0.01$).

(3,20). These findings suggest that the expression of dopamine agonist-induced behaviors is dependent upon the concurrent activation of D_1 and D_2 dopamine receptors and that blockade of either receptor subtype can attenuate the locomotor response to selective D_1 and D_2 dopamine receptor agonists. The present findings also suggest that caffeine-induced locomotor activity is dopamine dependent and both D_1 and D_2 dopamine receptors appear to be involved.

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