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The differential role of A1 and A2 adenosine receptor subtypes in locomotor activity and place conditioning in rats

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Previous studies have demonstrated that the non-specific adenosine antagonist caffeine possesses both motor activating and rewarding properties in a place-conditioning paradigm. The present experiments were designed to determine the relative contribution of A1 and A2 adenosine receptor subtypes to these effects. The A2 adenosine antagonist CGS 15943A (0.1–10.0 mg/kg) dose-dependently produced both a place preference and enhanced locomotor activity. In contrast, the A1 antagonist CPX (0.01–40.0 mg/kg) failed significantly to alter either behavioral measure. Both the A1 receptor agonist CPA (0.01–10.0 mg/kg) and the A2 receptor agonist CGS 21680 (0.01–1.0 mg/kg) reliably decreased activity but failed to produce significant place conditioning. The increased activity produced by the A2 antagonist CGS 15943A (1.0 mg/kg) was attenuated by behaviorally active doses of either CPA or CGS 21680. The place preference produced by CGS 15943A (1.0 mg/kg) was attenuated by CPA and CGS 21680, at agonist doses that failed to produce place conditioning when administered alone. In general, the results suggested that although it is the A2 receptor subtype that participates in the establishment of place conditioning and enhanced activity, both receptors participate in the diffuse depressant effects associated with adenosine.

Keywords: A1 and A2 receptors – Activity – Adenosine – CGS 15943A – CGS 21680 – CPA – CPX – Place conditioning – Rat

INTRODUCTION

Studies using a wide variety of behavioral methods have shown that some doses of caffeine consistently enhance locomotor activity (e.g. Snyder *et al.*, 1981). It has also been demonstrated previously that caffeine has rewarding properties as assessed by the place-conditioning paradigm (Brockwell *et al.*, 1991). It is now generally agreed that the behavioral effects produced by caffeine are due to its ability to antagonize the action of adenosine, a neuromodulator that decreases neuronal firing and neurotransmitter release in a variety of neurotransmitter systems within the brain (refer to Williams, 1989; Linden, 1994). However, pharmacological binding assays have revealed that caffeine is a relatively weak non-specific adenosine antagonist; it produces a comparable antagonism at both A1 and A2 adenosine receptor subtypes (e.g. Williams, 1989). To date, the role of each receptor subtype in the behavioral effects associated with caffeine remains unclear.

No study has demonstrated unequivocally the rewarding properties of a receptor-specific adenosine antagonist. In addition, relatively few studies have been conducted to investigate the ability of A1 and A2 adenosine receptor antagonists to enhance locomotor activity, and evidence from these studies remains largely equivocal. For example, although one early report suggested that the potency of caffeine and other methylxanthines for stimulating activity was correlated with their affinity for A1 receptors (Snyder *et al.*, 1981), it has subsequently been reported that at least one A1 antagonist, 8-cyclopentyltheophylline (CPT), failed to alter locomotor activity (Williams, 1989). In addition, Seale *et al.* (1988) reported that the A2-selective antagonist 3,7-dimethyl-1-propargylxanthine (DMPX) had greater motor stimulant effects than either of the nonspecific adenosine antagonists caffeine or theophylline.

An alternative strategy to examine the participation of each receptor subtype in motor activity and reward is to evaluate the ability of receptor-specific agonists to attenuate these caffeine-induced effects. Thus, for example, if the behavioral effects of caffeine are mediated solely by the A1 receptor subtype, it would be expected that enhanced activity and reward would be attenuated by the

co-administration of an A1, but not an A2, adenosine receptor agonist. An examination of the literature suggests that only one study has used this approach. Finn and Holtzman (1987) reported that in rats chronically treated with caffeine, the nonspecific agonist 5′N-ethylcarboxamidoadenosine (NECA) had a ten-fold greater ability than the A1 agonist N^6-R-(phenylisopropyl)adenosine (R-PIA) to shift the caffeine dose-effect curve for locomotor activity to the right. Since NECA is more potent than R-PIA at the A2 adenosine receptor but less potent than R-PIA at the A1 receptor, these data suggest a greater involvement of A2 receptors in the modulation of these effects.

The present study was conducted to determine the role of the A1 and A2 adenosine receptor subtypes in the production of motor activation and reward. It used both of the behavioral strategies outlined above. The first part of the study examined the ability of an A1 and an A2 adenosine receptor antagonist to produce motor activation and reward as assessed by the place-conditioning paradigm. The second part examined the ability of an A1 and an A2 adenosine receptor agonist to attenuate the behavioral effects produced by the receptor-specific antagonists.

METHOD

This research was undertaken with due regard for the Animals for Research Act and the Guidelines of the Canadian Council on Animal Care. The Queen's University Animal Care Committee reviewed and approved this protocol.

Subjects

Two hundred and thirty-two Wistar rats (Charles River, Canada) weighing 200–225 g upon arrival, were group housed ($n = 8$) in hanging wire cages, in a climatically controlled colony room, with a 12 h light (06.00–18.00 h)/dark cycle. Food and water were available continuously in the home cages. Rats were handled on a daily basis for several days during habituation to the colony room.

Apparatus

Place conditioning and locomotor activity were monitored in four similar rectangular boxes (84 × 27 × 36 cm high) constructed using wooden sides and removable Plexiglas covers. Each box consisted of two chambers joined by a small tunnel (8 × 8 × 6 cm high), which could be blocked by the insertion of two Plexiglas guillotine doors. The chambers differed in wall pattern and floor design. In two of the boxes, one chamber had brown walls and a wire mesh floor (1 × 1 cm), while the other chamber had black and white vertically striped walls (stripes were 1 cm wide) and a floor consisting of wire rods spaced 1 cm apart. In the other two chambers the floor and wall pairings were reversed. Each box was housed in an outer plywood shell that was insulated with sound-attenuating Styrofoam, illuminated by a 7.5 W light, and ventilated with a small fan. Each box was equipped with six pairs of infrared photosensors (two located 5 cm above the floor of each chamber and two located 3 cm above the floor of the tunnel), connected to an Experiment Controller, a single-board computer that recorded the number of sensor interruptions and the amount of time spent in each area of the conditioning box. For further details of the apparatus, see Brockwell *et al.* (1996).

Procedure

Behavioral testing consisted of three phases – preconditioning, conditioning, and test – conducted over a total of 12 days. Experimental sessions were conducted at 24 h intervals. The number of activity counts at each photosensor, and the amount of time spent in each area of the conditioning box, were recorded for each rat throughout all phases of the experiment.

Preconditioning. This phase was conducted to habituate the rats to the conditioning boxes and to provide a baseline measure of unconditioned chamber preference. During three 15 min sessions, rats were placed in one of the chambers (designated the start side) and allowed access to the entire box. The choice of start side was counterbalanced across rats, but remained the same for each rat throughout the experiment.

Conditioning. During each of the eight 30 min conditioning sessions, animals were confined to one chamber by blocking the entrance to the tunnel. On odd-numbered sessions, animals were administered the drug and confined to the non-start chamber. On even-numbered sessions, rats received the vehicle and were confined to the start chamber.

Two control groups ($n = 8$) were included in the study: One group received the vehicle exclusively, the other was conditioned with 2.0 mg/kg (+)-amphetamine, known to produce both a conditioned place preference and enhanced activity (Beninger *et al.*, 1985; Hoffman and Beninger, 1988).

Twelve groups received adenosine receptor antagonists to determine the dose of each compound that produced maximal effects on both motor activity and place conditioning. Six groups ($n = 8$) received the A1 antagonist

CPX (0.01, 0.1, 1.0, 10.0, 20.0, 40.0 mg/kg), and six groups ($n = 8$) were given the A2 antagonist CGS 15943A (0.1, 0.5, 1.0, 2.0, 5.0, 10.0 mg/kg). All rats were placed in the conditioning box immediately following injection.

Seven groups ($n = 8$) received the A2 antagonist CGS 15943A at 1.0 mg/kg, the antagonist dose found to produce maximal effects on both motor activity and place conditioning: four were pretreated with the A1 agonist CPA (0.01, 0.1, 1.0, 10.0 mg/kg); the remaining three were pretreated with the A2 agonist CGS 21680 (0.01, 0.1, 1.0 mg/kg). The agonists were administered 15 min prior to antagonist administration. On alternate conditioning sessions, rats were pretreated with the vehicle 15 min prior to a second vehicle injection. Groups receiving a combination of an agonist plus the A1 antagonist CPX were not included in the study due to the lack of significant behavioral effects when CPX was administered alone.

Seven groups ($n = 8$) received adenosine agonists alone to allow for a comparison with groups that received an agonist plus the A2 antagonist: four groups received the A1 agonist CPA (0.01, 0.1, 1.0, 10.0 mg/kg) and three received the A2 agonist CGS 21680 (0.01, 0.1, 1.0 mg/kg). These groups were injected 15 min prior to being placed in the conditioning box.

An additional control group ($n = 8$) was included to act as a partial replication of the group that received 1.0 mg/kg CGS 15943A alone. However, unlike the former group, the additional control group was injected with the vehicle (in effect, an agonist dose equal to 0.0 mg/kg) 15 min prior to the administration of 1.0 mg/kg CGS 15943A.

Test. During the single 15 min test session, animals in a drug-free state were placed in the start chamber and allowed access to the entire conditioning box.

Drugs

The adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX) [Research Biochemicals International (RBI)], the A2 receptor antagonist 9-chloro-2-(2-furanyl)-5,6-dihydro-1,2,4-triazolo[1,5-c]quinazolin-5-imine (CGS 15943A) [Ciba-Geigy Corp.], the A1 agonist N^6-cyclopentyladenosine (CPA) [RBI] and the A2 agonist 2-(carboxyethylphenylethylamino)adenosine-5′-carboxamide (CGS 21680) [Ciba-Geigy Corp.] were chosen from a variety of available compounds because they appeared to provide the best combination of receptor specificity and potency (see Williams, 1989). Each compound was suspended in a small quantity of the polymer polyoxyethylene sorbitan monooleate (Tween 80), and added to distilled water in an appropriate concentration to yield an injection volume of 1.0 ml/kg. The vehicle consisted of distilled water containing one drop of Tween 80 per ml of solution. The catecholamine agonist (+)-amphetamine (SmithKline Beecham Pharma Inc.) was dissolved in distilled water in an appropriate concentration to yield an injection volume of 1.0 ml/kg. All drugs were administered i.p.

Statistical analyses

All analyses were conducted according to the rationale outlined by Keppel (1982) using the BMDP 4V Statistical Software package. Motor activity was examined by comparing the number of sensor interruptions in the vehicle-paired and drug-paired chambers during the conditioning phase. The data were averaged across the four sessions of each Treatment condition. For each drug or drug combination, a two-way analysis of variance (ANOVA) was conducted with Dose as a between-subjects factor and Treatment (vehicle vs. drug) as a repeated measure. To analyze these data further, planned tests of simple main effects were conducted using one-way ANOVAs of the Treatment variable at each Dose.

Place conditioning was examined by comparing the amount of time spent on the drug-paired side of the conditioning box during the preconditioning and test phase. The data were averaged across the three sessions of the preconditioning phase. Two-way ANOVAs were conducted with Dose as a between-subjects factor and Phase (preconditioning vs. test) as a repeated measure. Planned tests of simple main effects were then conducted using one-way ANOVAs of the Phase variable at each dose. For clarity, the terms "place preference" and "place aversion" were used to denote the direction of the place conditioning.

Trend analyses of the Dose variable were conducted for each agonist or agonist–antagonist combination. Due to the repeated measures design, difference scores were calculated for each animal. For activity, scores were calculated by subtracting the average number of sensor interruptions produced in conditioning sessions following the vehicle from the average number of interruptions in sessions following the drug. For place conditioning, scores were calculated by subtracting the average amount of time spent in the drug-paired chamber during preconditioning from the time spent on that side during the test phase.

RESULTS

Activity

The control group that received the vehicle exclusively displayed an almost identical mean (± standard error)

number of sensor interruptions in both chambers during conditioning [start side = 184.0 (± 11.78); non-start side = 184.9 (± 16.75)]. In contrast, the group conditioned with amphetamine displayed a greater number of sensor interruptions following amphetamine than following vehicle treatment [vehicle-paired side = 192.4 (± 12.76); drug-paired side = 316.6 (± 40.1)]. As expected, one-way ANOVAs of the Treatment variable revealed that amphetamine significantly enhanced activity [$F(1,7) = 13.57$, $p < 0.01$], whereas the vehicle failed to alter activity levels significantly.

Antagonists. The A1 antagonist CPX appeared to produce little effect on activity (Fig. 1A). A two-way ANOVA failed to reveal any significant effects, as did all the one-way ANOVAs of the Treatment variable. On the other hand, each dose of the A2 antagonist CGS 15943A appeared to enhance activity (Fig. 1B). The greatest number of sensor interruptions occurred at a dose of 1.0 mg/kg CGS 15943A, with less pronounced activity occurring at lower doses. A two-way ANOVA revealed significant main effects for Dose [$F(5,42) = 7.25$, $p < 0.001$] and Treatment [$F(1,42) = 110.25$, $p < 0.001$], and a significant Dose × Treatment interaction [$F(5,42) = 2.43$, $p = 0.05$]. The results of one-way ANOVAs, with Treatment as a repeated measure, indicated that CGS 15943A enhanced activity at each dose [0.1 mg/kg: $F(1,7) = 5.60$, $p < 0.05$; 0.5 mg/kg: $F(1,7) = 6.95$, $p < 0.05$; 1.0 mg/kg: $F(1,7) = 56.33$, $p < 0.001$; 2.0 mg/kg: $F(1,7) = 49.13$, $p < 0.001$; 5.0 mg/kg: $F(1,7) = 8.16$, $p < 0.05$; 10.0 mg/kg: $F(1,7) = 37.42$, $p < 0.001$].

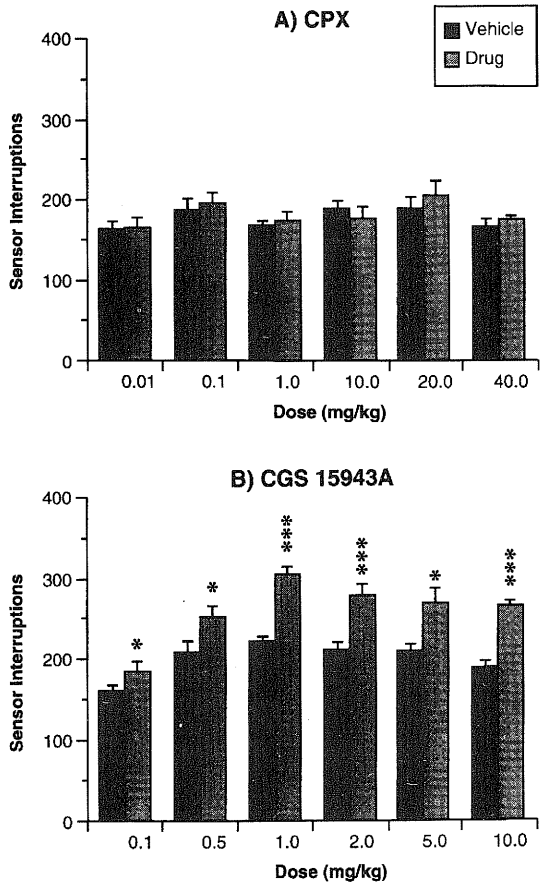

FIG. 1. Mean (+SEM) number of sensor interruptions during conditioning sessions following vehicle (black bar) and drug (grey bar) administration for groups ($n = 8$) receiving (A) the A1 antagonist CPX and (B) the A2 antagonist CGS 15943A. The data have been averaged across the four days of each treatment condition. $^{*}p < 0.05$, $^{***}p < 0.001$; differs significantly from vehicle treatment.

Agonists. The A1 agonist CPA produced a dose-dependent decrease in activity (Fig. 2A). A two-way ANOVA revealed significant main effects for Dose [$F(3,28) = 8.39$, $p < 0.001$] and Treatment [$F(1,28) = 115.12$, $p < 0.001$], and a significant Dose × Treatment interaction [$F(3,28) = 16.64$, $p < 0.001$]. One-way ANOVAs of the Treatment variable indicated that CPA significantly decreased activity at the three higher dose levels: [0.1 mg/kg: $F(1,7) = 14.35$, $p < 0.01$; 1.0 mg/kg: $F(1,7) = 38.77$, $p < 0.001$; 10.0 mg/kg: $F(1,7) = 396.40$, $p < 0.001$. Analyses of the Dose variable indicated the presence of a linear trend [$F(1,28) = 49.76$, $p < 0.001$], suggesting a dose-dependent decrease in motor activity.

Similarly, the A2 agonist CGS 21680 produced a dose-dependent decrease in activity (Fig. 2B). A two-way ANOVA revealed a significant main effect for Treatment [$F(1,21) = 38.38$, $p < 0.001$], and a significant Dose × Treatment interaction [$F(2,21) = 20.61$, $p < 0.001$]. One-way ANOVAs of the Treatment variable indicated that 1.0 mg/kg CGS 21680 significantly depressed locomotor activity [$F(1,7) = 70.74$, $p < 0.01$]. Trend analyses revealed both linear [$F(1,21) = 29.54$, $p < 0.001$] and quadratic Dose effects [$F(1,21) = 11.67$, $p < 0.01$], reflecting both the decrease in activity from 0.01 to 1.0 mg/kg CGS 21680 and the comparable small reduction of activity at the two lower agonist doses.

Antagonist-agonist combinations. The control group received the vehicle 15 min prior to CGS 15943A (1.0 mg/kg). As with the group that received CGS 15943A (1.0 mg/kg) alone, greater activity was seen following drug (330.6(± 47.0)) than vehicle injections (215.2(± 14.3)). A one-way ANOVA of the Treatment variable revealed enhanced activity following administration of the vehicle–CGS 15943A combination [$F(1,7) = 8.97$, $p < 0.05$]. A two-way ANOVA comparing

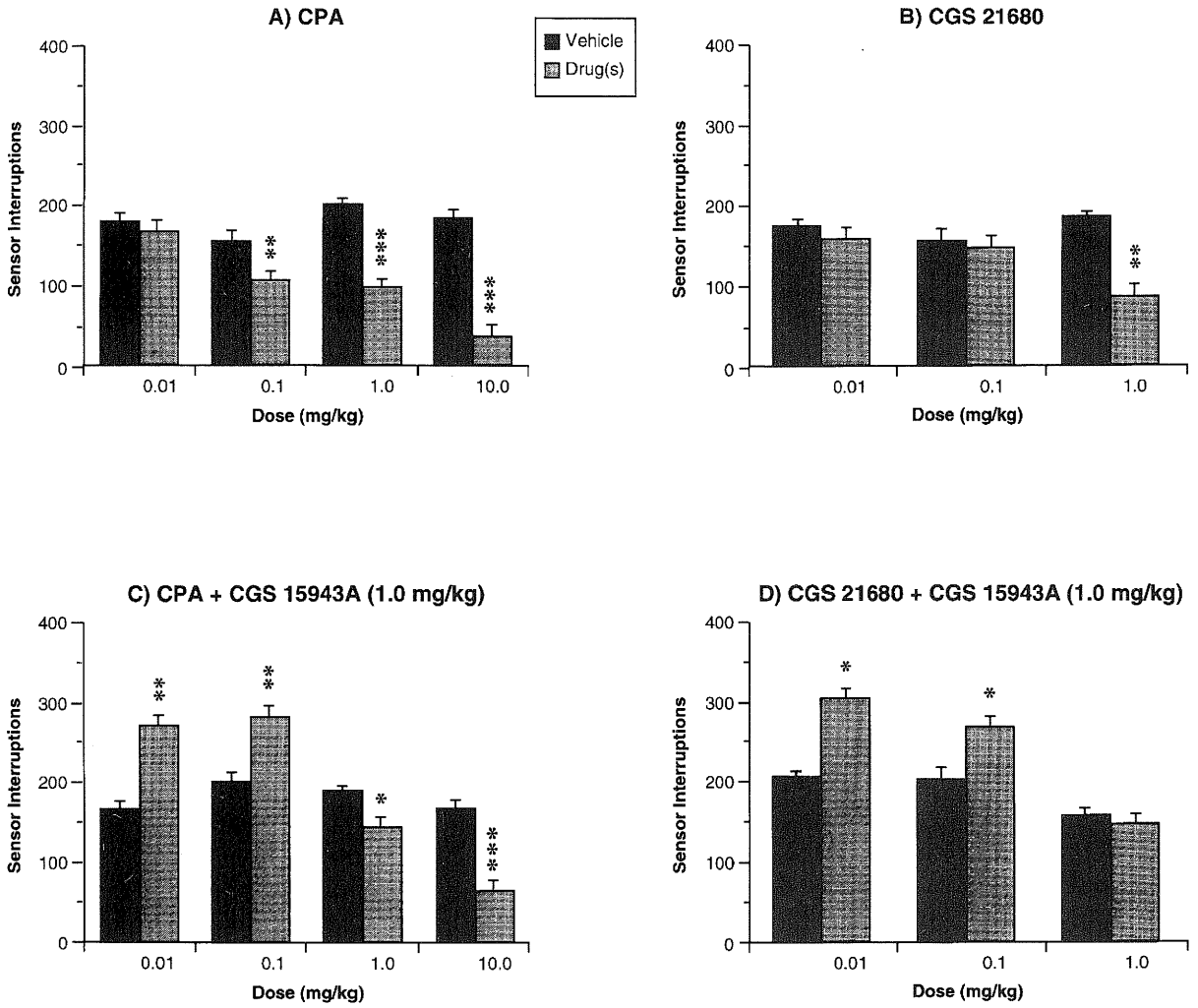

FIG. 2. Mean (+SEM) number of sensor interruptions during conditioning sessions following vehicle (black bar) and drug (grey bar) administration. Groups ($n=8$) received (A) the A1 agonist CPA, (B) the A2 agonist CGS 21680, (C) CPA followed by 1.0 mg/kg CGS 15943A, and (D) CGS 21680 followed by 1.0 mg/kg CGS 15943A. The data have been averaged across the four days of each treatment condition. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$; differs significantly from vehicle treatment.

the activity in this group to that of the group that received 1.0 mg/kg CGS 15943A alone revealed a significant main effect of Treatment [$F(1,14)=24.26$, $p<0.001$], but failed to reveal a significant main effect of Group or a significant interaction.

Treatment with CPA prior to CGS 15943A produced a dose-dependent reduction in the stimulant effect of CGS 15943A (Fig. 2C). A two-way ANOVA revealed a significant main effect of Dose [$F(3,28)=13.23$, $p<0.001$] and a significant Dose × Treatment interaction [$F(3,28)=26.51$, $p<0.001$]. One-way ANOVAs of the Treatment variable indicated enhanced activity for the two lowest doses of CPA [0.01 mg/kg: $F(1,7)=24.03$, $p<0.01$; and 0.1 mg/kg: $F(1,7)=16.69$, $p<0.01$]. In contrast, pretreatment with the two highest doses of CPA resulted in significant motor depression [1.0 mg/kg: $F(1,7)=9.82$, $p<0.05$; and 10.0 mg/kg: $F(1,7)=15.81$, $p<0.01$]. Analyses of the Dose variable revealed the presence of a significant linear trend [$F(1,28)=74.73$, $p<0.001$], suggesting a dose-dependent reduction of activity.

Animals pretreated with CGS 21680 followed by CGS 15943A also showed a dose-dependent reduction in the stimulant effect of CGS 15943A (Fig. 2D). A two-way ANOVA revealed significant main effects of Dose [$F(2,21)=15.90$, $p<0.001$] and Treatment [$F(1,21)=12.09$, $p<0.01$], and a significant Dose × Treatment interaction [$F(2,21)=5.08$, $p<0.05$]. One-way ANOVAs of the Treatment variable revealed elevated activity when animals were pretreated with 0.01 [$F(1,7)=7.75$, $p<0.05$] and 0.1 mg/kg CGS 21680 [$F(1,7)=11.87$, $p<0.05$], but not the 1.0 mg/kg dose.

Trend analyses indicated a linear Dose effect [$F(1,21) = 9.65$, $p < 0.01$], suggesting that the reduction of locomotor activity was dose-dependent.

Place conditioning

The control group that received the vehicle exclusively spent a comparable amount of time (s) on the drug-paired side during either phase [preconditioning = 415.7(±41.4); test = 416.2(±42.6)]. The group that received amphetamine displayed an increase in time spent on the drug-paired side during the test phase [preconditioning = 422.3(±17.6); test = 541.2(±36.0)]. As expected, (+)-amphetamine produced a significant place preference [$F(1,7) = 5.60$, $p < 0.05$].

Antagonists. Four of the six groups receiving CPX displayed a decrease, whereas two displayed an increase, in the amount of time spent on the drug-paired side during the test phase (Fig. 3A). However, a two-way ANOVA failed to reveal significant main effects of either Dose or Phase, or a significant interaction between these two factors, and one-way ANOVAs failed to reveal a significant main effect of Phase in any of the groups.

Animals receiving mid-range doses of the A2 antagonist CGS 15943A spent more time on the drug-paired side of the conditioning box during the test phase (Fig. 3B). A two-way ANOVA revealed a Phase effect which approached the critical level for significance [$F(1,42) = 3.42$, $p = 0.07$], suggesting that the A2 antagonist produced a trend towards a place preference when all doses of the drug were combined. Results of one-way ANOVAs of the Phase variable revealed that 1.0 mg/kg CGS 15943A produced a significant place preference [$F(1,7) = 5.56$, $p = 0.05$].

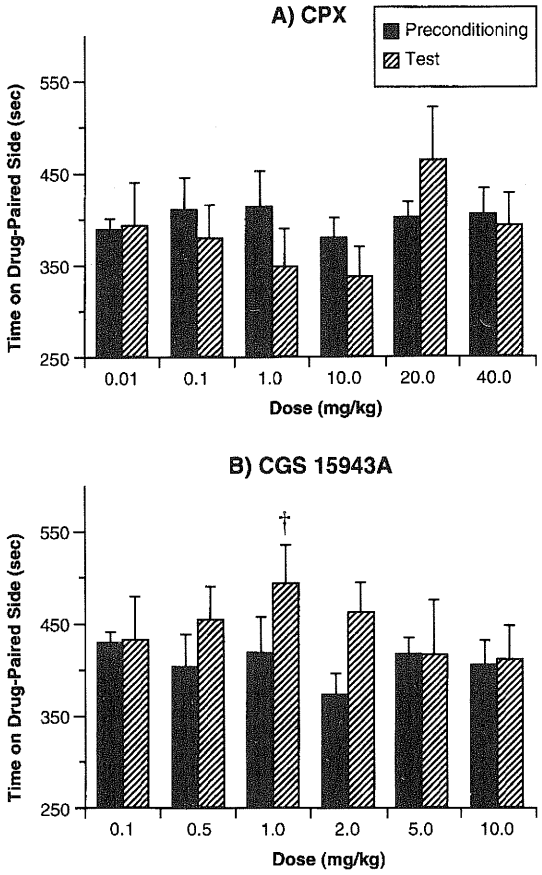

FIG. 3. Mean (+SEM) amount of time spent on the drug-paired side during the preconditioning (black bar) and test (cross-hatched bar) phases for groups ($n = 8$) receiving (A) the A1 antagonist CPX and (B) the A2 antagonist CGS 15943A. The data have been averaged across the three preconditioning sessions. †$p = 0.05$; differs significantly from preconditioning phase.

Agonists. The A1 agonist CPA appeared to produce a dose-dependent biphasic effect; animals given 0.01 and 0.1 mg/kg displayed a decrease, and animals given 1.0 and 10.0 mg/kg displayed an increase, in the amount of time spent on the drug-paired side following conditioning (Fig. 4A). However, a two-way ANOVA failed to reveal significant main effects of either Group or Phase, or a significant interaction between these two factors. One-way ANOVAs failed to reveal a significant Phase effect at any group. Trend analyses revealed a significant linear Dose effect [$F(1,28) = 4.47$, $p < 0.05$], reflecting the increase in time spent on the drug-paired side during the test phase from the two lower to the two higher doses of CPA.

Animals treated with the A2 agonist CGS 21680 spent less time on the drug-paired side of the conditioning box during the test phase (Fig. 4B). However, no statistically significant effects were found.

Antagonist–agonist combinations. The control group that received 1.0 mg/kg CGS 15943A pretreated with vehicle spent more time (s) on the drug-paired side of the conditioning box during the test phase [preconditioning = 373.9(±20.2); test = 487.6(±33.9)]. A one-way ANOVA of the Phase variable revealed a significant place preference [$F(1,7) = 11.67$, $p < 0.05$]. Statistical analyses were then conducted to compare the place conditioning produced by the control group with the group that received 1.0 mg/kg CGS 15943A alone. A two-way ANOVA, with Group as a between-measures factor and Phase as a repeated measure, revealed a significant main effect of Phase [$F(1,14) = 16.83$, $p < 0.01$], but failed to reveal either a significant main effect of Group or a significant Group × Phase interaction. Thus, the place preference produced by 1.0 mg/kg of CGS 15943A alone was replicated in the group receiving vehicle followed by CGS 15943A.

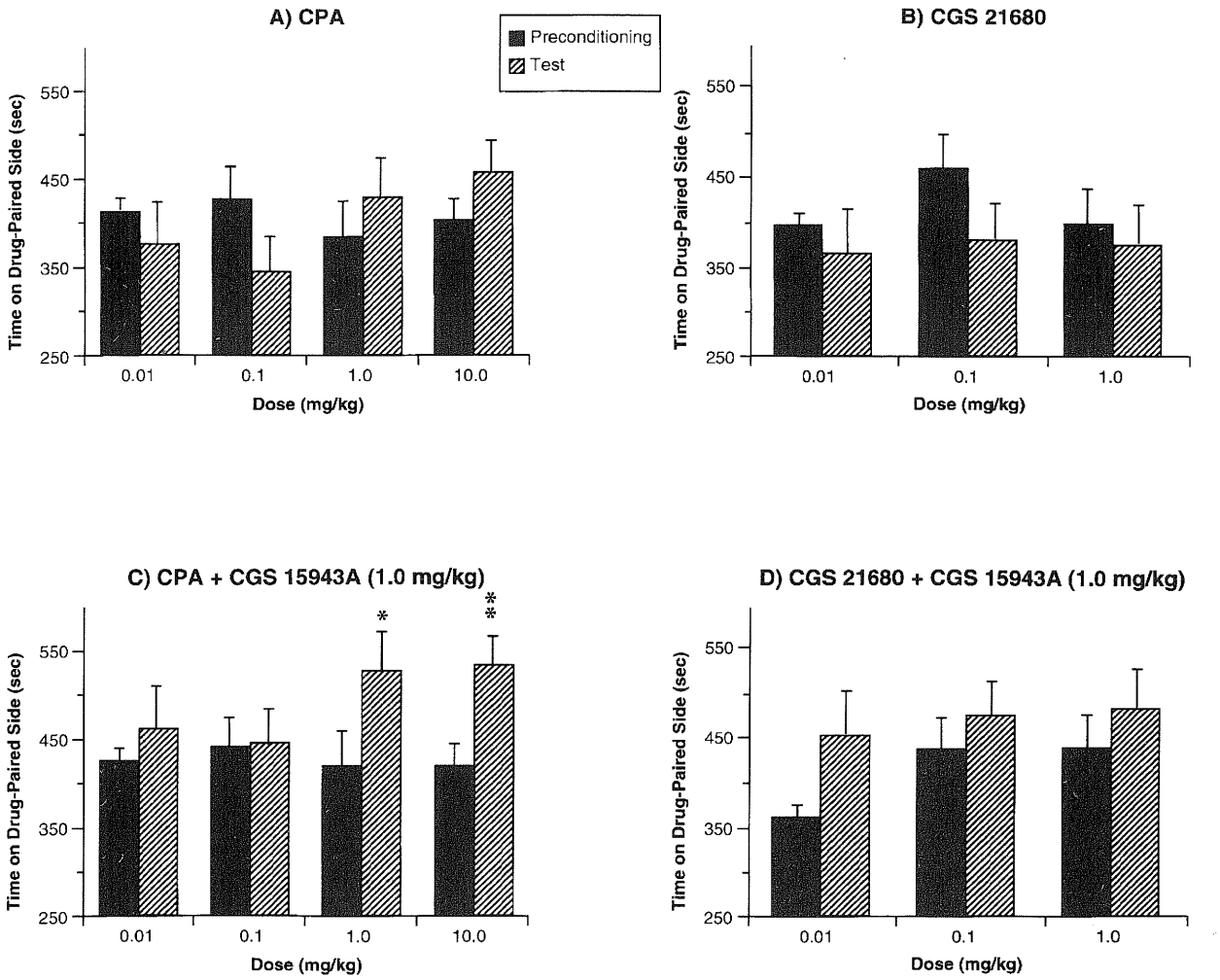

FIG. 4. Mean (+SEM) amount of time spent on the drug-paired side during the preconditioning (black bar) and test (cross-hatched bar) phases. Groups ($n=8$) received (A) the A1 agonist CPA, (B) the A2 agonist CGS 21680, (C) CPA followed by 1.0 mg/kg CGS 15943A, and (D) CGS 21680 followed by 1.0 mg/kg CGS 15943A. The data have been averaged across the three preconditioning sessions. $^{*}p<0.05$, $^{**}p<0.01$; differs significantly from preconditioning phase.

When CGS 15943A was preceded by the A1 agonist CPA, the amount of time spent on the drug-paired side appeared to be greater for the two higher CPA doses (Fig. 4C). A two-way ANOVA revealed a significant main effect of Phase [$F(1,28)=13.39$, $p<0.01$], indicating a place preference when the data from all of the groups were combined. One-way ANOVAs of the Phase variable revealed significant place preferences for groups pretreated with 1.0 [$F(1,7)=6.86$, $p<0.05$] and 10.0 mg/kg CPA [$F(1,7)=24.00$, $p<0.01$]. These results suggest that pretreatment with the two lower CPA doses (0.01 and 0.1 mg/kg) attenuated the place conditioning produced by the A2 antagonist. Trend analyses revealed a linear Dose effect [$F(1,28)=4.31$, $p<0.05$].

For groups pretreated with the A2 agonist prior to CGS 15943A, the time spent on the drug-paired side was greater in the test phase than in the preconditioning phase (Fig. 4D). However, statistical analyses failed to reveal any significant main effects or a significant interaction, and one-way ANOVAs of the Phase variable failed to reveal any significant effects. These results suggest that all doses of CGS 21680 attenuated the significant place conditioning produced by CGS 15943A. Trend analyses failed to reveal any significant Dose effects.

DISCUSSION

The results can be summarized as follows: (1) the A2 antagonist CGS 15943A enhanced locomotor activity across all doses tested (0.1 to 10.0 mg/kg). In contrast, the adenosine A1 receptor antagonist CPX failed to alter locomotor activity significantly across a wide dose range (0.1 to 40.0 mg/kg). Thus, the motor activating effects

consistently found with non-specific adenosine antagonists such as caffeine and theophylline appear to be mediated by the A2 receptor; (2) both the A1 and A2 receptors appear to participate in the depressant effects associated with adenosine. When administered alone, both the A1 agonist CPA and the A2 agonist CGS 21680 dose-dependently depressed motor activity; (3) the increased activity produced by the A2 antagonist CGS 15943A could be attenuated by motor depressant doses of either the A1 agonist CPA or the A2 agonist CGS 21680. However, in the case of the A1 agonist CPA, the minimum dose that attenuated activity when given alone was lower than the minimum dose found to depress CGS 15943A-induced motor activity. Thus, although 0.1 mg/kg CPA significantly reduced unconditioned activity when administered alone, this dose failed to attenuate the enhanced activity produced by CGS 15943A; (4) the A2 antagonist CGS 15943A produced a place preference when administered alone or in combination with a vehicle injection. In contrast, administration of the A1 antagonist CPX failed to produce place conditioning across a wide dose range. Thus, the rewarding effects associated with caffeine also appear to be mediated by the A2 receptor subtype; (5) The place preference produced by CGS 15943A could be attenuated by pretreatment with either the A1 agonist CPA or the A2 agonist CGS 21680, at agonist doses which did not themselves induce significant place conditioning. All doses of the A2 agonist CGS 21680, and two doses (0.01 and 0.1 mg/kg) of the A1 agonist CPA, attenuated the significant place preference produced by CGS 15943A.

Validity. As expected, saline failed to alter activity or to produce place conditioning, and amphetamine increased both activity and place preference. The technique used to measure locomotor activity in the present study was unusual in that it alternated vehicle and drug injections and measured activity in two visually and tactilely distinct environments, each of which was associated with one of the treatment conditions. Despite this difference, the present experiment appeared to provide a valid assessment of unconditioned motor activity. Thus, effects of amphetamine on activity (Costall and Naylor, 1979; Beninger, 1983; Harvey, 1987) and on place conditioning are consistent with previous data (Carr *et al.*, 1989; Hoffman, 1989).

Motor activity. The motor effects produced by the two adenosine antagonists are generally consistent with other studies. In agreement with our results, CGS 15943A dose-dependently increased motor activity in both rats (Holtzman, 1991) and mice (Griebel *et al.*, 1991). The failure of the A1 antagonist CPX to alter locomotor activity is consistent with the finding that CPT, a selective A1 antagonist with a similar pharmacological structure to CPX, failed to alter activity in rats (Williams, 1989). In contrast, Greibel *et al.* (1991) reported that although lower doses of CPX failed to alter locomotor activity in mice, doses in excess of 6.0 mg/kg significantly decreased locomotor activity and produced ataxia. The reasons for this discrepancy remain unknown but warrant further investigation.

The motor depression observed with the A1 agonist CPA is consistent with previous findings (Durcan and Morgan, 1988; 1989; Heffner *et al.*, 1989). The results produced by the A2 agonist are consistent with a report that CGS 21680 depressed locomotor activity in mice (Nikodijevic *et al.*, 1990). An amine derivative of CGS 21680, 2-[(2-aminoethylamino)carbonylethylphenylethylamino]-5′-N-ethylcarboxamidoadenosine (APEC), was also found to be a potent locomotor depressant in mice (Nikodijevic *et al.*, 1991). It is worth noting that several investigators have reported that A1 agonists such as R-PIA (Dunwiddie and Worth, 1982; Heffner *et al.*, 1989), N^{6-}cyclohexyladenosine (CHA) and CPA (Heffner *et al.*, 1989) produce significant ataxia. Although ataxia was not measured in the present study, observation suggested that both CPA and CGS 21680 produced ataxic symptoms. Animals generally became flaccid and malleable several minutes following the administration of a higher dose of either compound. They often remained immobile for several minutes at a time, but would respond to tactile stimulation by orienting towards the stimulus or initiating movement away from the stimulus. Movement was often uncoordinated, and animals frequently failed to support their weight completely on their legs or fell on their sides when attempting locomotion.

The results produced by pretreating animals with the A1 agonist CPA prior to the A2 antagonist are in accord with other studies that examined the effects of combinations of adenosine agonists and antagonists on locomotor activity (Barraco *et al.*, 1983; 1984; Finn and Holtzman, 1987; Bruns *et al.*, 1988; Durcan and Morgan, 1988; Seale *et al.*, 1988). These studies revealed that behaviorally active doses of adenosine agonists and antagonists produce motor effects that are mutually antagonistic regardless of the receptor subtypes involved. The results produced by pretreating animals with the A2 agonist CGS 21680 prior to the A2 antagonist are congruent with Seale *et al.* (1988), who reported a more potent antagonism between the A2 antagonist DMPX and the non-specific agonist NECA, than between DMPX and the A1 agonist CHA.

Place conditioning. The present study appears to provide the first unequivocal experimental documentation that antagonism of the action of adenosine at the A2

receptor can produce a rewarding effect. To date, only one other study has examined the rewarding properties of CGS 15943A. Mumford and Holtzman (1991) reported that the antagonist failed to alter the reinforcement threshold for intracranial self-stimulation (ICSS). A decrease in reinforcement threshold is generally taken as evidence that a drug has rewarding properties. Interestingly, the ICSS paradigm also failed to demonstrate the rewarding properties of other adenosine antagonists including caffeine, theophylline and a variety of other methylxanthines (e.g. Mumford *et al.*, 1988; Mumford and Holtzman, 1990). ICSS has effectively demonstrated the rewarding properties of a variety of substances including psychomotor stimulants. It remains unclear why ICSS has not proved sensitive to the rewarding properties of CGS 15943A or caffeine.

Dissociation of motor activity and reward. During the 1970s and 1980s a considerable amount of research was conducted to determine the neural substrates mediating the motor-activating and rewarding properties of a variety of substances (Beninger, 1983; Bozarth, 1986; Swerdlow *et al.*, 1986; Wise and Bozarth, 1987). Examination of the literature led some investigators (e.g. Wise and Bozarth, 1987) to propose that the motor activating and rewarding effects of many substances share a common neural circuitry or biological mechanism.

One of the most interesting findings of the present study is that under some conditions it is possible to produce a clear dissociation between behavioral measures of reward and motor activation. Comparison of Figs 2C and 4C indicates that the CPA–CGS 15943A combinations that produced significant place preferences also produced significant decreases in motor activity. Thus, it is possible to produce behavioral measures of reward in the absence of motor activation during conditioning; in fact, in the present study the two measures were in opposite directions. Similarly, as shown in Figs. 1 and 3, although all doses of CGS 15943A enhanced activity, only 1.0 mg/kg produced place conditioning. Thus it appears that, at some doses, CGS 15943A can enhance motor activity in the absence of rewarding effects.

Pharmacological antagonism and behavioral additivity. Pharmacological binding assays have revealed that the antagonist CPX and the agonist CPA have a greater than 700-fold selectivity for the A1 adenosine receptor subtype (Bruns *et al.*, 1986; Williams, 1989). The agonist CGS 21680 has a 140-fold selectivity for the A2 receptor (Hutchinson *et al.*, 1989; Jarvis *et al.*, 1989), whereas the A2 antagonist CGS 15943A has a selectivity of approximately 7-fold (Williams *et al.*, 1987). Therefore, at least in *in vitro* studies, the site of action of each of these compounds is well documented. With the exception of the A2 antagonist, the remaining antagonist CPX and both agonists bind to one receptor subtype in a highly selective manner. What remains unknown, however, is whether the behavioral effects produced by agonist–antagonist combinations are due to their interaction at specific adenosine receptors or result from the addition of non-specific behavioral effects.

In the present study, if an agonist–antagonist combination produced effects that were due to behavioral additivity, it would be expected that the measure of the drug combination would be fairly predictable; i.e., at each agonist dose tested, the effect produced by the combination would reflect the sum of the effects of the agonist and antagonist administered alone. On the other hand, if an agonist–antagonist combination produced a pharmacological interaction it would be expected that the effect produced by the combination could not be predicted by simply examining the effects produced by each compound administered alone.

The results of the trend analyses conducted on the data presented in Figs. 2 suggest that there was greater evidence for a pharmacological interaction between the two A2 compounds than between the A1 agonist CPA and CGS 15943A. The motor effects produced by CGS 15943A in combination with CPA appear to reflect behavioral additivity. Both this drug combination and CPA alone produced a linear dose-response function. On the other hand, the motor effects produced by the A2 agonist in combination with CGS 15943A support a pharmacological interaction. Trend analyses of the motor depression produced by CGS 21680 alone revealed both a linear and a quadratic function, but when combined with the A2 antagonist, CGS 21680 produced only a linear trend. Examination of Fig. 2B and 2D shows that 0.01 and 0.1 mg/kg CGS 21680 alone produced small, roughly equal activity decreases. However, for the groups receiving these doses of the A2 agonist in combination with CGS 15943A there was a dose-dependent reduction in activity, suggesting pharmacological antagonism.

The place-conditioning data presented in Fig. 4 also support a pharmacological interaction between the two A2 compounds, but not between the A1 agonist and the A2 antagonist. When administered alone or in combination with CGS 15943A, CPA produced a linear dose-response trend. Examination of Figs. 4A and 4C shows that doses of CPA alone that reduced the amount of time spent on the drug-paired side also reduced the magnitude of the preference produced by CGS 15943A when the two drugs were given in combination. Doses of CPA that increased the amount of time spent on the drug-paired side failed significantly to affect the magnitude of the place preference produced by CGS 15943A. Together,

these findings suggest that the motor effects produced by CPA in combination with the A2 antagonist reflect behavioral additivity. Although no significant trends were found for either CGS 21680 alone or in combination with the A2 antagonist, the results shown in Figs 4B and 4D might suggest pharmacological antagonism. The 0.01 and 1.0 mg/kg doses of CGS 21680 given alone appeared to produce roughly comparable decreases in the amount of time spent on the drug-paired side following conditioning. However, when CGS 21680 was given in combination with the A2 antagonist, the place preference produced by CGS 15943A appeared to be reduced to a greater extent by the higher agonist dose.

The results reviewed above suggest that there may have been a direct pharmacological antagonism between the adenosine agonist and antagonist that exerted their primary effects at the same (i.e. A2) receptor. In contrast, the behavioral effects produced by the co-administration of compounds that exerted their primary effects at different receptor subtypes appeared to be the result of behavioral additivity. Nevertheless, the fact that three of the four compounds tested were behaviorally active, and the ability of both the A1 and A2 agonists to disrupt the behavioral effects produced by CGS 15943A, suggest that the functional organization of the two adenosine receptor subtypes is complex. The neuromodulatory effects produced by adenosinergic compounds may ultimately depend on the balance of activity at both receptor subtypes.

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