Role of Adenosine A₂ Receptors in Brain Stimulation Reward under Baseline Conditions and during Cocaine Withdrawal in Rats

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The present experiments tested the hypothesis that adenosine A₂ receptors are involved in central reward function. Adenosine receptor agonists or antagonists were administered to animals that had been trained to self-stimulate in a rate-free brain stimulation reward (BSR) task that provides current thresholds as a measure of reward. The adenosine A_{2A} receptor-selective agonists 2-*p*-(2-carboxyethyl)phenethylamino-5'-*N*-ethylcarboxamido adenosine hydrochloride (CGS 21680) (0.1–1.0 mg/kg) and 2-[(2-aminoethylamino)carbonyl]ethyl phenylethylamino-5'-*N*-ethylcarboxamido adenosine (APEC) (0.003–0.03 mg/kg) elevated reward thresholds without increasing response latencies, a measure of performance. Specifically, CGS 21680 had no effect on response latency, whereas APEC shortened latencies. Bilateral infusion of CGS 21680 (3, 10, and 30 ng/side), directly into the nucleus accumbens, elevated thresholds but shortened latencies. The highly selective A_{2A} antagonist 8-(3-chlorostyryl)caffeine (0.01–10.0 mg/kg) and the A₂-preferring antagonist 3,7-dimethyl-1-propargylxanthine (DMPX) (0.3–10.0 mg/kg) did not

alter thresholds or latencies, but DMPX (1.0, 10.0 mg/kg) blocked the threshold-elevating effect of APEC (0.03 mg/kg). In another study, repeated administration of cocaine (eight cocaine injections of 15 mg/kg, i.p., administered over 9 hr) produced elevations in thresholds at 4, 8, and 12 hr after cocaine. DMPX (3 and 10 mg/kg), administered before both the 8 and 12 hr post-cocaine self-stimulation tests, reversed the threshold elevation produced by cocaine withdrawal. These results indicate that stimulating adenosine A_{2A} receptors diminishes BSR without producing performance deficits, whereas blocking adenosine receptors reverses the reward impairment produced by cocaine withdrawal or by an A_{2A} agonist. These findings indicate that adenosine, via A_{2A} receptors, may inhibit central reward processes, particularly during the neuroadaptations associated with chronic drug-induced neuronal activation.

Key words: adenosine; adenosine A_{2A} receptor; dopamine; brain stimulation reward; nucleus accumbens; cocaine; withdrawal; DMPX; CGS 21680; APEC; chlorostyryl-caffeine

Adenosine is a ubiquitous purine nucleoside that has a fundamental role in governing the physiological function of cells, including the processes underlying neurotransmission (for review, see Dunwiddie, 1985; Snyder, 1985; Dunwiddie and Fredholm, 1997). Many of the effects of adenosine are mediated by the four cell surface G-protein-linked receptors that have been identified: the A₁, A_{2A}, A_{2B}, and A₃ receptors (Collins and Hourani, 1993; Fredholm et al., 1994). The A_{2A} subtype is highly concentrated in the terminal regions of the ascending dopamine (DA) projections (Schiffmann et al., 1991; Fink et al., 1992; Dixon et al., 1996; Svenningsson et al., 1997). Within these regions, adenosine A_{2A}

receptor activation negatively modulates the postsynaptic effects of DA (Ferré et al., 1991b, 1993). Accordingly, adenosine A_{2A} agonists produce DA antagonist-like behavioral effects (Ferré, 1997); the nucleus accumbens (NAcc) and caudate putamen are particularly sensitive sites for these effects. For example, the adenosine A_{2A} receptor agonist 2-*p*-(2-carboxyethyl)phenethylamino-5'-*N*-ethylcarboxamido adenosine hydrochloride (CGS 21680) depressed locomotor activity when infused directly into the NAcc (Barraco et al., 1993; Hauber and Munkle, 1997) or the caudate putamen (Hauber and Munkle, 1997).

Although it is clear that adenosine A_{2A} receptors modulate the substrates subserving motor behaviors, relatively less is known about their role in central reward processes. There is evidence that DA neurotransmission, particularly within the NAcc, is involved in regulating the effects of reinforcers, such as food, sex, drugs of abuse, and brain stimulation reward (BSR) (Stellar and Corbett, 1989; Kornetsky and Bain, 1990; Koob, 1992; Robbins and Everitt, 1996; Wise, 1998; Fiorino and Phillips, 1999). Hence, given the dense concentration of adenosine A_{2A} receptors within the NAcc and their negative modulation of DA function, A_{2A} receptors may be involved in modulating the rewarding effects of BSR and other more "natural" stimuli.

In addition to their possible role in mediating the rewarding effects of electrical brain stimulation, adenosine receptors also could be involved in reward changes produced by drug withdrawal. During cocaine withdrawal, rats show reduced sensitivity to BSR (Markou and Koob, 1991). The withdrawal-related

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changes in BSR are thought to reflect, at least in part, alterations in DA-regulated neural substrates (Markou and Koob, 1991; Koob, 1992; Koob et al., 1993; Koob and Le Moal, 1997), particularly those within the NAcc (Weiss et al., 1992; Parsons et al., 1995). Because adenosine receptors within the NAcc alter DA transmission, those receptors might represent sites at which the effects of cocaine withdrawal could be modulated.

The purpose of this study was to explore the role of adenosine A₂ receptors in modulating BSR. First, the effects of various adenosine A₂ agonists and antagonists on BSR thresholds were assessed under baseline conditions in rats. Second, the effect of intra-NAcc infusion of an A_{2A} agonist was explored. Third, the effect of an adenosine A₂ receptor-preferring antagonist on cocaine withdrawal-induced BSR threshold elevations was investigated. In all experiments, the latency to respond for the rewarding electrical stimulation also was monitored to evaluate whether any of the manipulations produced nonspecific performance impairments.

MATERIALS AND METHODS

Subjects

Subjects were 69 male Wistar rats weighing 260–280 gm upon arrival in the laboratory. Rats were either obtained from Charles River Laboratories (Kingston, NY) or bred at The Scripps Research Institute from a stock originally derived from Charles River Laboratories. Rats were housed in groups of two in clear plastic cages with wood chip bedding. Food and water were available *ad libitum*. Animals were kept in a temperature-controlled vivarium under a 12 hr light/dark cycle (lights on at 10:00 P.M.). All animal facilities and procedures were maintained in accordance with the guidelines of the United States National Institutes of Health regarding the principles of animal care, approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute, and assessed by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Surgical placement of electrodes and cannulas

Stainless steel bipolar electrodes were implanted into the medial forebrain bundle at the level of the posterior lateral hypothalamus [anteroposterior (AP), –0.5 mm from bregma; mediolateral (ML), +1.7 mm; dorsoventral (DV), –8.3 mm from dura; tooth bar, 5.0 mm above the interaural line]. For the NAcc infusion experiment, indwelling bilateral stainless steel guide cannulas (23 gauge, 10 mm in length) aimed at the NAcc (AP, +3.2 mm from bregma; ML, ±1.7 mm; DV, –5.3 mm from skull surface; tooth bar, 5.0 mm above the interaural line) also were implanted. The electrode, cannulas, and three anchoring skull screws were cemented in place with dental acrylic (Co-Oral-Lite Manufacturing Co., Diamond Springs, CA). In the rats with NAcc cannulas, a 12.5 mm injector (30 gauge) was lowered once into the accumbens site during surgery and removed. The cannulas then were closed with wire obturators (33 gauge, 10 mm in length).

Apparatus

Training and testing took place in eight Plexiglas operant chambers [30 (length) × 30 (height) × 17 (width) cm] with wire grid floors contained within sound-attenuating cabinets. Within each operant chamber, a wheel manipulandum requiring a 0.2 N force to rotate it one-quarter turn protruded from one wall. Animals were connected to the stimulation circuit by gold-contact swivel commutators and bipolar leads (Plastics One, Roanoke, VA). Brain stimulation was administered by constant-current stimulators interfaced with a microcomputer. For the low-dose chlorostyryl-caffeine experiment, the electrical stimuli used were sinusoidal bursts of current with a frequency of 60 Hz and train duration of 100 msec. All other experiments were conducted using a different system (Stimtek model 1200; San Diego Instruments, San Diego, CA) in which 495 msec trains of cathodal square wave pulses (0.1 msec) with a frequency of 100 Hz were delivered. It should be noted that the baseline threshold values obtained with the original system were significantly lower than those obtained with the Stimtek equipment because of the different stimulation parameters (Markou and Koob, 1992a). The stimulation parameters chosen for both these systems were within the range

in which both threshold elevations and decreases could be reliably detected (see Results).

Brain stimulation reward threshold procedure

Animals were trained to respond according to a modification of the discrete-trial current-threshold procedure of Kornetsky and Esposito (1979), which has been described in detail previously (Markou and Koob, 1992a, 1993). Briefly, a trial was initiated by the delivery of a noncontingent electrical stimulus. A one-quarter wheel turn within 7.5 sec of the delivery of that noncontingent electrical stimulation resulted in the delivery of an electrical stimulus identical in all parameters to the noncontingent stimulus that initiated the trial. After a variable intertrial interval (7.5–12.5 sec, average of 10 sec), another trial was initiated with the delivery of noncontingent electrical stimulus. Failure to respond to the noncontingent stimulus within 7.5 sec resulted in the onset of the intertrial interval. Responding during the intertrial interval delayed the onset of the next trial by 12.5 sec. Current levels were varied in alternating descending and ascending series. A set of three trials was presented at each current intensity. Current intensities were altered in 5 μ A steps.

In each testing session, four alternating descending–ascending series were presented. The threshold for each series was defined as the midpoint between consecutive current intensities that yielded “positive scores” (animals responded for two or more of the three trials) and consecutive current intensities that yielded “negative scores” (animals did not respond for two or more of the three trials). The overall threshold of the session was defined as the mean of the thresholds for the four individual series. Each testing session was ~30 min in duration.

One of the features of the software operating the Stimtek system was that it would not deliver more than 15 levels of stimulation (i.e., 15 “steps”) in either the ascending or descending direction. As a result, there were a few instances in which the software did not allow the delivery of sufficiently high levels of current to obtain threshold estimates after adenosine agonist treatment because of the large threshold increases produced by those drugs. In those cases, a fifth series with descending current intensities was initiated with the starting current 50 μ A higher than the highest current level in the fourth series. This fifth series was initiated immediately after the completion of the fourth series and added an additional 5–7 min to the testing session. This procedure was used for only 8% of all the data points collected in these experiments, indicating that this fifth series threshold evaluation was not required frequently and was thus unlikely to have unduly influenced the results. Furthermore, to ensure that running a fifth series did not yield false threshold estimates, two animals were given saline injections and ran in five series. The thresholds for the fifth series were found to be almost identical to the thresholds for the previous four series, thus ruling out possible testing artifacts associated with testing subjects in a fifth series.

In addition to the threshold measure, a performance measure, response latency, was also assessed in this task (Markou and Koob, 1992a, 1993). Response latency was defined as the time elapsed between the presentation of the noncontingent stimulus (i.e., onset of the stimulus) and the one-quarter wheel turn response in a given trial. Mean latency for the session was defined as the mean latency of responding for all trials in which an animal responded within the 7.5 sec period (i.e., positive score responding).

Intracerebral injection procedure

All injections were given bilaterally in a volume of 1.0 μ l/site given over 2 min through 12.5 mm injectors. The injectors protruded 2.5 mm below the ends of the cannulas. After infusion, the injectors were kept in place for 1 min to allow for diffusion. Injectors then were removed and replaced with 10 mm wire obturators to keep the cannulas unblocked during testing and to avoid potential interference of the 12.5 mm obturators with drug activity in the tissue. Animals were tested immediately after injection, returned to their home cages after that first test, and tested again at 60 min after injection. After the second test, the 10 mm wire obturators were removed and replaced with 12.5 mm obturators.

Experimental design

Systemic dose–effect experiments. After training in the self-stimulation paradigm and achievement of stable baseline responding ($\leq 10\%$ variation in threshold for 3 consecutive days), subjects received saline injections preceding their daily testing sessions. Animals then were subjected to drug treatments, including vehicle treatments, administered in coun-

terbalanced orders according to Latin square designs. Drug testing days were separated by at least 3 d during which saline injections were administered before testing.

Six dose–effect experiments were performed, each in a separate group of rats. In the first two experiments, the effects of the adenosine agonists CGS 21680 ($n = 9$ rats) and 2-[(2-aminoethylamino)carbonyl ethyl phenylethylamino]-5'-N-ethylcarboxamid adenosine (APEC) ($n = 7$ rats) were assessed. Four of the rats from the CGS 21680 experiment also were used in the APEC experiment. These four rats continued to exhibit stable baseline thresholds and latencies throughout both experiments. Furthermore, two-factor ANOVAs (rats' treatment history $\times$ APEC dose) revealed that the four retested rats showed no difference in response to APEC compared with drug-naïve rats ($F < 1.0$ for the both the reward threshold measure and the response latency measure). In the next three experiments conducted in separate groups of drug-naïve rats, the effects of the adenosine antagonists 3,7-dimethyl-1-propargylxanthine (DMPX) ($n = 6$ rats) and 8-(3-chlorostyryl)caffeine or 1,3,7-trimethyl-8-(3-chlorostyryl)xanthine (CSC), were investigated. CSC was tested in two dose ranges, each in a separate group of rats ($n = 8$ rats for the low-dose range; $n = 4$ rats for the high-dose range). In the last experiment ($n = 6$ rats), the interaction between DMPX and APEC was explored.

Intra-nucleus accumbens CGS 21680 dose–effect experiment. After stable baseline BSR responding was achieved ($\leq 10\%$ variation in threshold for 3 consecutive days), animals ($n = 8$ rats) were given an intra-accumbens injection of saline, and the 10 mm wire obturators were replaced by 12.5 mm obturators. Animals again were tested until stable thresholds (3 d of $\leq 10\%$ variation in threshold) were observed, at which point all animals were given an intra-NAcc injection of saline and tested in the brain stimulation reward procedure. No sooner than 3 d later, injections of CGS 21680 were given (0, 3.0, 10.0, and 30.0 ng/site, dissolved in saline) were given according to a Latin square design. After the final treatment in the Latin square, six animals were given 10.0 ng/site CGS 21680 through 10.5 mm injectors (i.e., the drug was injected 2 mm above the original site). Injections were separated by at least 3 drug-free days on which animals were tested in the brain stimulation reward task.

On occasion, mild seizures were noted in animals that had received high levels of stimulation after CGS 21680 (10 or 30 ng/site) treatment. These seizures were mild, very brief in duration (10–15 min), and did not influence subsequent baseline levels of responding on the drug-free interim days. In addition, animals showing these seizures did not exhibit aberrant responses to saline infusions. Two animals showed strong seizures (lasting longer than 45 min) when tested in the brain stimulation procedure after CGS 21680 infusion (30 ng/site). Testing was immediately stopped, and data from those animals were not included in any of the analyses.

After the conclusion of this experiment, animals were deeply anesthetized with pentobarbital and perfused transcardially with a 4% paraformaldehyde solution. The brains were cut in 50 μ m sections, mounted onto slides, and stained with cresyl violet to evaluate the placements of the injectors and the electrodes.

Repeated cocaine injection regimens on brain stimulation reward thresholds at 4, 8, 12, and 24 hr after cocaine. Once stable BSR responding was achieved ($\leq 10\%$ variation in threshold for 3 consecutive days), saline injections preceding the daily testing sessions were administered. Animals then were divided into two groups that were balanced with regard to baseline reward thresholds. Rats in the repeated-cocaine group ($n = 6$ rats) and the repeated-saline group ($n = 5$ rats) underwent five consecutive cocaine or saline injection regimens and associated postinjection self-stimulation testing, as described below. The five cocaine injection regimens were separated by 5 d on which animals were tested after saline injections.

The cocaine or saline injection regimens consisted of eight intraperitoneal injections of 15 mg/kg cocaine (the repeated-cocaine group) or saline vehicle (the repeated-saline group) given over 9 hr in the vivarium, where the animals were housed except during testing. The injection regimen was started at 7:30 A.M. and was completed at 5:00 P.M. The first two injections were separated by an interval of 30 min, and the remaining injections were separated by 90 min intervals. This injection regimen was designed to mimic the overall pattern and amount of cocaine intake observed during a 12 hr intravenous cocaine self-administration session (Markou and Koob, 1991; Baldo et al., 1999). Withdrawal from this intraperitoneal injection regimen produces some behavioral effects that are similar to those observed during withdrawal

from self-administered cocaine (Baldo et al., 1999). Nevertheless, the amount and time course of cocaine penetration into the brain likely differs between the two procedures, because the pharmacokinetic profile of intraperitoneally administered cocaine differs from that of intravenously administered cocaine (Pettit and Pettit, 1994).

Rats were tested in the brain stimulation reward procedure at 4, 8, 12, and 24 hr after the cessation of each of the five injection regimens. These time points were chosen based on the time course of threshold elevations after cocaine self-administration (Markou and Koob, 1991). The time points generally corresponded to 9:00 P.M. on the same day of the injections, and 1:00 A.M., 5:00 A.M., and 5:00 P.M. on the subsequent day. At each of the four time points, animals were given an intraperitoneal injection of saline before testing. Animals were returned to the vivarium after each testing session. Four hours after the 24 hr time point (i.e., at 9:00 P.M.), the animals were tested again. The last time point approximately corresponded to the normal time-of-day for the animals' baseline testing (baseline testing occurred between 8:00 and 8:30 P.M.).

DMPX administration during cocaine withdrawal. Two groups of rats, which were balanced with regard to baseline reward thresholds, underwent five consecutive cocaine or saline injection regimens (cocaine-DMPX group, $n = 6$ rats; saline-DMPX group, $n = 8$ rats) with associated behavioral testing, as described for the previous experiment. For the second, third, and fourth injection regimens, animals in both groups received injections of 0, 3, or 10 mg/kg DMPX 20 min before both the 8 and 12 hr post-cocaine or post-saline time points. For the first and fifth injection regimens, animals received saline injections before the 8 and 12 hr post-cocaine or post-saline time points. The order of administration of those three DMPX doses was counterbalanced according to a Latin square design.

Drugs

The adenosine A_{2A} agonist CGS 21680, the A_{2A} antagonist CSC, and the A_2 -preferring antagonist DMPX were obtained from Research Biochemicals (Natick, MA). The A_{2A} agonist APEC was kindly provided by Research Biochemicals as part of the Chemical Synthesis Program of the National Institute of Mental Health, Contract N01MH30003. Gentamicin was obtained from Solo-Pak Laboratories (Elk Grove Village, IL).

CGS 21680 was dissolved in a hot saline solution that was allowed to cool to room temperature and was administered via intraperitoneal injection 15 min before the testing session in an injection volume of 1 ml/kg. APEC was initially dissolved in 100% DMSO at a concentration of 50 mg/ml and frozen. On drug injection days, thawed aliquots of that stock solution were mixed in ~ 0.05 ml of ethoxylated castor oil (Alkamuls EL-620, formerly Emulphor; Rhone-Poulenc Rorer, Cranbury, NJ), and the resulting mixture was diluted to the required concentration with saline. APEC was administered intraperitoneally 5 min before the testing session in a volume of 1 ml/kg. CSC was tested in two dose ranges. For the lower range (0.01–1.0 mg/kg), CSC was dissolved in dimethylsulfoxide and administered intraperitoneally 15 min before testing in an injection volume of 0.3 ml/kg. For the higher range (2.5–10.0 mg/kg), CSC was suspended in 0.15 ml dimethylsulfoxide and 0.7 ml Alkamuls (see above) and diluted to volume with saline (Jacobson et al., 1993). The resulting suspension was injected intraperitoneally through a 25 $\frac{1}{2}$ gauge needle 15 min before the testing session in a volume of 1 ml/kg. DMPX was dissolved in a hot saline solution that was allowed to cool to room temperature. Intraperitoneal injections of DMPX were given 20 min before behavioral testing in a volume of 2 ml/kg.

In the DMPX–APEC interaction study, DMPX was injected intraperitoneally 20 min before the testing session, and APEC was administered intraperitoneally 15 min after DMPX (i.e., 5 min before the testing session). Cocaine HCl was dissolved in saline and injected intraperitoneally in a volume of 1 ml/kg.

Statistical analyses

Dose–effect experiments. For each separate dose–effect experiment, percentage change from baseline threshold was calculated by expressing the drug-influenced threshold scores as a percentage of the mean threshold for the previous 3 baseline testing days (predrug baseline thresholds). These percentage of baseline scores were subjected to one-factor ANOVA with repeated measures. For the DMPX–APEC interaction experiment, percentage of baseline scores were subjected to a two-factor repeated-measures ANOVA (DMPX treatment $\times$ APEC treatment). To evaluate the effect of 10 ng/site CGS 21680 injected 2 mm above the NAcc site (through 10.5 mm injectors), a repeated-measures ANOVA (site $\times$ time point) was performed on percentage of baseline threshold

scores. To test for possible confounding effects of the order of drug administration, the repeated-measures ANOVAs were recalculated with the order of the treatments as the independent variable. There were a few instances in which animals did not respond for the electrical brain stimulation after treatment with adenosine agonists. In those cases, missing data points were estimated by a procedure proposed by Yates (1933).

Cocaine withdrawal experiments. Percentage of baseline threshold was calculated two ways: by expressing the treatment-influenced threshold scores as a percentage of the mean threshold for the previous 3 baseline testing days that preceded each injection regimen ("local baselines") or by expressing the treatment-influenced threshold scores as a percentage of the pretreatment baseline established before any injection regimens were initiated ("initial baseline"). Separate analyses were conducted on the values obtained by those two different methods. However, because those two analyses yielded almost identical results, for the sake of brevity, only the local baseline data are reported.

For the repeated cocaine withdrawal experiment, percentage change data were analyzed in a mixed-design ANOVA (treatment $\times$ regimen $\times$ time point), with repeated measures for the regimen and time point factors.

For the DMPX–cocaine withdrawal experiment, percentage change data were analyzed in a mixed-design ANOVA (treatment $\times$ time point $\times$ DMPX dose), with repeated measures on the time point and dose factors. To test for possible confounding effects of the order of DMPX administration, the ANOVA for experiment 2 was recalculated with the order of the treatments as an independent variable.

Baseline thresholds (i.e., the means of each set of baseline scores for the 3 d immediately preceding each of the five injection regimens) also were subjected to statistical analysis to assess the stability of baseline responding throughout the course of the study. Because the point of this analysis was to determine whether baseline responding varied for any of the groups in the entire study, the data from both cocaine withdrawal experiments were analyzed together with a two-factor ANOVA (group $\times$ regimen). For all experiments, latency data were analyzed in the same manner as the threshold data.

After significance in the ANOVAs, *post hoc* comparisons among means were conducted with the Newman–Keuls test (Winer, 1971). The level of statistical significance (α level) was set at $p \leq 0.05$.

RESULTS

Order effects

In the DMPX–cocaine withdrawal experiment, there was a main effect of DMPX dose order on the response latency measure ($F_{(2,24)} = 3.8$; $p < 0.04$), but no interaction of dose order with either the treatment or time point factors ($F = 0.24$ – 0.54 ; NS). The main effect of dose order was a result of a small decrease in the mean latency value for the third DMPX manipulation relative to the other two, on data collapsed across the treatment and time point factors. The biggest difference between means was 7.7% of baseline latency, or ~ 0.23 sec. No effect of dose order was noted for either the reward threshold measure ($F = 0.36$ – 1.59 ; NS) or the latency measure ($F = 0.04$ – 1.76 ; NS), in any of the other experiments.

Pretreatment baseline threshold and latency

For the Stimtek equipment, the mean $\pm$ SEM baseline current intensity threshold for all subjects in all experiments was 110 ± 7.85 μ A with a range of 66.4–256.4 μ A. For the equipment used for the low-dose CSC study, the mean $\pm$ SEM baseline current intensity threshold was 19.8 ± 1.5 μ A with a range of 10.5–25.0 μ A. There was only one effect in any of the experiments of pretreatment baseline values (i.e., the mean score for the 3 d immediately preceding each treatment). In the DMPX–cocaine withdrawal experiment, there was an interaction between baseline period and group ($F_{(12,84)} = 1.9$; $p < 0.05$) for the response latency measure. The values for the third and fourth pretreatment baselines in the cocaine–DMPX group were significantly higher than the value for the first baseline in that group, as well as all the

Figure 1. Data are presented as mean percentage change from baseline threshold or latency (see Results). Error bars represent one SEM. *A*, Effects of the adenosine A_{2A} receptor agonist CGS 21680 on brain stimulation reward thresholds and response latencies. * $p < 0.05$, different from vehicle. *B*, Effects of the adenosine A_{2A} receptor agonist APEC on brain stimulation reward thresholds and response latencies. * $p < 0.05$, different from vehicle; # $p < 0.05$, different from 0.003 mg/kg.

values for all the other treatment groups. The largest difference between means was 0.6 sec. In all other experiments, baseline responding was unaltered over the course of testing for both the reward threshold and the response latency measures ($F = 0.06$ – 2.94 ; NS).

Adenosine receptor agonists (CGS 21680 and APEC)

Intraperitoneal administration of the adenosine A_{2A} receptor agonist CGS 21680 elevated brain stimulation reward thresholds ($F_{(3,24)} = 3.72$; $p < 0.03$) (Fig. 1*A*, left panel). Treatment with the 0.3 or 1.0 mg/kg dose produced higher threshold values compared with the vehicle value ($p < 0.05$). In contrast to its effects on reward thresholds, CGS 21680 did not alter response latencies ($F_{(3,24)} = 0.67$; NS) (Fig. 1*A*, right panel).

The adenosine A_{2A} receptor agonist APEC also produced a significant elevation of reward threshold ($F_{(3,18)} = 5.4$; $p < 0.01$) (Fig. 1*B*, left panel). The highest dose (0.03 mg/kg) significantly elevated thresholds relative to vehicle or the 0.003 mg/kg dose ($p < 0.05$). Nevertheless, unlike CGS 21680, which had no effect on the latency measure, APEC reduced response latency ($F_{(3,18)} = 7.49$; $p < 0.002$); the effect of the 0.03 mg/kg dose was significantly different from the effect of the 0.003 mg/kg dose and vehicle ($p < 0.05$) (Fig. 1*B*, right panel).

Administration of CGS 21680 directly into the NAcc elevated brain stimulation reward thresholds at the 60 min time point (main effect of dose, $F_{(3,21)} = 7.8$; $p < 0.001$; main effect of time,

Figure 2. Top, Effect of CGS 21680 injected into the nucleus accumbens on brain stimulation reward thresholds (*A*, *B*) and response latencies (*C*, *D*). Error bars indicate one SEM. * $p < 0.05$, different from all other values obtained in the nucleus accumbens site at both time points; + $p < 0.05$, different from 0 ng/side at 0–30 and 60–90 min; # $p < 0.05$, different from 3 ng/side at 0–30 min; $^ap < 0.05$, different from vehicle [with regard to data collapsed across the two time points (see Results)]. Bottom, Placements of nucleus accumbens injectors (*E*) and medial forebrain bundle–lateral hypothalamus electrodes (*F*). Sections are arranged in rostrocaudal order; numbers indicate distance from bregma in millimeters. Filled ovals represent injector or electrode tips. Bilateral accumbens placements are shown only on one side for clarity. The left–right placement of the unilateral electrodes alternated among animals; again, placements are shown on one side for the sake of clarity. Figures were adapted from the atlas of Pellegrino et al. (1979).

$F_{(1,7)} = 33.88$; $p < 0.0007$; dose $\times$ time interaction, $F_{(3,21)} = 12.92$; $p < 0.0002$) (Fig. 2*A,B*). The threshold value associated with the 30 ng/side dose at the 60 min time point was significantly higher than the threshold values for all other treatments at all time points ($p < 0.05$). Furthermore, the 10 ng/side dose at the 60 min time point produced a threshold elevation that was significantly higher than the effects of vehicle at 0 and 60 min and the effects of the 3 ng/side dose at 0 min ($p < 0.05$). For the latency measure, there was a main effect of dose ($F_{(3,21)} = 4.37$; $p < 0.02$) but no effect of time ($F_{(1,7)} = 1.18$; NS) or dose $\times$ time interaction ($F_{(3,21)} = 0.35$; NS). *Post hoc* analyses performed on latency means collapsed across the time factor revealed that the effect of the 0.03 ng/side dose was significantly lower than the effect of vehicle (Fig. 2*C,D*).

Injection of the 10 ng/side dose of CGS 21680 at a site 2 mm dorsal to the nucleus accumbens did not significantly change reward thresholds (site, $F_{(1,5)} = 4.5$; $p < 0.09$; time, $F_{(1,5)} = 5.0$; $p < 0.08$; and site $\times$ time interaction, $F_{(1,5)} = 1.8$; $p < 0.2$). The differences in effect sizes were 37% at 0 min and 13% at 60 min (data not shown). There were no statistically significant differ-

ences in the effects of the 10 ng/side dose on latency when injected through 12.5 versus 10.5 mm injectors (i.e., directly into the nucleus accumbens or dorsal to the nucleus accumbens, respectively) ($F = 0.43$ – 2.3 ; NS).

As shown in Figure 2 (bottom), the injector tips were located medial to the anterior commissure, within the nucleus accumbens. Electrodes were located within the medial forebrain bundle at the level of the lateral hypothalamus.

Adenosine receptor antagonists (CSC and DMPX)

Brain stimulation reward thresholds were not altered by the highly selective adenosine A_{2A} receptor antagonist CSC at either of the two dose ranges tested (0.01–1.0 mg/kg dose range, $F_{(3,21)} = 1.28$; NS; 2.5–10.0 mg/kg dose range, $F_{(3,9)} = 0.74$; NS) (Fig. 3*A*). In addition, CSC did not produce any statistically significant effects on response latency at either of the two dose ranges ($F_{(3,21)} = 1.86$; NS; and $F_{(3,9)} = 1.17$; NS, for the low- and high-dose ranges, respectively) (Fig. 3*A*). The adenosine A_2 receptor-preferring antagonist DMPX also did not significantly alter brain stimulation reward thresholds ($F_{(4,20)} = 2.77$; $p < 0.06$)

Figure 3. *A*, Effects of the adenosine A_{2A} receptor antagonist CSC or the A_2 receptor-preferring antagonist DMPX on brain stimulation reward thresholds and response latencies. CSC was tested in two dose ranges in separate groups of rats: a low-dose range (open squares) and a high-dose range (filled squares). Data are presented as mean percentage change from baseline threshold or latency (see Results). Error bars represent one SEM.

(Fig. 3*B*, left panel) or response latencies ($F_{(4,20)} = 1.66$; NS) (Fig. 3*B*, right panel).

DMPX–APEC interaction

As shown in Figure 4 (left panel), pretreatment with the adenosine A_2 receptor-preferring antagonist DMPX dose-dependently reversed the threshold-elevating effect of the selective adenosine A_{2A} receptor agonist APEC (main effect of DMPX pretreatment, $F_{(3,15)} = 11.32$; $p < 0.0005$; APEC treatment, $F_{(1,5)} = 16.35$; $p < 0.01$; and pretreatment $\times$ treatment interaction, $F_{(3,15)} = 6.22$; $p < 0.006$). The DMPX 0–APEC 0.03 treatment resulted in significant threshold elevations relative to all of the DMPX–APEC 0 combinations, as well as the DMPX 10.0–APEC 0.03 combination. Furthermore, the threshold value associated with the DMPX 0.3–APEC 0.03 combination was significantly higher than the values for the DMPX 1.0–APEC 0.03 combination, the values for the DMPX 10.0–APEC 0.03 combination, and the values for all the DMPX–APEC 0 combinations. Finally, the value for the DMPX 1.0–APEC 0.03 combination was significantly higher than the values for the DMPX 0.3–APEC 0, DMPX 1.0–APEC 0, DMPX 10.0–APEC 0, and DMPX 10.0–APEC 0.03 combinations. For the latency measure, there was a significant main effect of pretreatment ($F_{(3,15)} = 4.83$; $p < 0.02$) but no main effect of treatment and no pretreatment $\times$ treatment interaction ($F = 0.95$ – 1.28 ; NS) (Fig. 4, right panel).

Repeated cocaine injection regimens on BSR thresholds at 4, 8, 12, and 24 hr after cocaine

Regimens of repeated intraperitoneal cocaine injections produced elevations in brain stimulation reward thresholds at the

Figure 4. Interaction between DMPX and APEC on brain stimulation reward thresholds and response latencies. Data are presented as mean percent change from baseline threshold (see Results). Error bars represent one SEM. * $p < 0.05$, different from corresponding DMPX–APEC 0 combination; # $p < 0.05$, different from DMPX 10–APEC 0.03; + $p < 0.05$, different from DMPX 1.0–APEC 0.03. For additional comparisons, see Results.

post-cocaine time points assayed (main effect of treatment, $F_{(1,9)} = 21.4$; $p < 0.002$). The effects were consistent across each of the five injection regimens, as indicated by a lack of a main effect of the regimen factor ($F_{(4,36)} = 1.3$; NS). There was a tendency for thresholds to increase across the first three time points in both the cocaine and saline-treated animals (main effect of time point, $F_{(3,27)} = 11.1$; $p < 0.0001$). Withdrawal effects did not occur equally across time points, as indicated by a treatment $\times$ time point interaction ($F_{(3,27)} = 3.7$; $p < 0.03$). There was also a regimen $\times$ time point interaction ($F_{(12,108)} = 2.3$; $p < 0.02$); however, there was no treatment $\times$ regimen $\times$ time point interaction ($F_{(12,108)} = 0.73$; NS). Given multiple main effects and interactions, and an experimental design strongly driven by our *a priori* hypothesis, *post hoc* tests were performed on means that were not collapsed across any factor. As displayed in Figure 5*A*, these *post hoc* comparisons revealed that thresholds were elevated relative to saline-treated animals, at the time points indicated by asterisks. For each of the first four injection regimens, the threshold values at the 12 hr time points were significantly higher than the threshold values at the corresponding 24 hr post-treatment time points, suggesting that thresholds began returning toward baseline values after 12 hr after cocaine. For the response latency measure, there was a main effect of post-treatment time point ($F_{(3,27)} = 7.3$; $p < 0.002$) but no effect of treatment or regimen and no interactions among those factors ($F = 0.36$ – 1.6 ; NS) (Fig. 5*B*). The main effect of time was a result of a small, progressive decrease in response latency of 5% of baseline values (equivalent to ~ 0.15 sec) between the 4 hr time point and the 24 hr time point on data collapsed across the treatment and regimen factors.

Effects of DMPX during cocaine withdrawal

The adenosine A_2 receptor-preferring antagonist DMPX reversed the brain stimulation reward threshold elevation observed at the 8 hr post-cocaine time point. An ANOVA revealed a main effect of DMPX dose ($F_{(2,24)} = 10.9$; $p < 0.0005$), a main effect of time point ($F_{(1,12)} = 28.9$; $p < 0.0003$), no main effect of cocaine treatment ($F_{(1,12)} = 0.6$; NS), a cocaine treatment $\times$ DMPX dose interaction ($F_{(2,24)} = 3.5$; $p < 0.05$), a DMPX dose $\times$ time point

Figure 5. Effects of withdrawal from five consecutive regimens of intraperitoneal cocaine injections (see Results) on reward thresholds (*A*) and response latencies (*B*). Animals were tested at 4, 8, 12, and 24 hr after treatment. Error bars indicate one SEM. * $p < 0.05$, different from corresponding saline value.

interaction ($F_{(2,24)} = 5.0$; $p < 0.02$), and a DMPX dose $\times$ cocaine treatment $\times$ time point interaction ($F_{(2,24)} = 4.8$; $p < 0.02$). For the 8 hr post-treatment time point (Fig. 6*A*), *post hoc* comparisons among means revealed that the mean threshold value for animals subjected to a cocaine injection regimen and then treated with 0 mg/kg DMPX was significantly higher than all other means ($p < 0.05$). Furthermore, the mean threshold value for the cocaine-treated animals given 10 mg/kg DMPX was significantly lower than the value for the saline-treated animals given 0 mg/kg DMPX ($p < 0.05$). Thus, both 3 and 10 mg/kg DMPX reversed the threshold elevation produced by cocaine withdrawal; however, those doses had no significant effects in saline-treated animals. For the 12 hr post-treatment time point (Fig. 6*B*), *post hoc* tests indicated that the rats treated with either cocaine or saline and then injected with 3 mg/kg DMPX yielded significantly lower threshold scores than animals treated with either cocaine–DMPX 0 mg/kg, or saline–DMPX 0 mg/kg ($p < 0.05$). Also, animals treated with saline–DMPX 3 mg/kg yielded significantly lower scores than animals treated with cocaine or saline, and then injected with 10 mg/kg DMPX ($p < 0.05$). There were no effects on response latency produced by any of the manipulations in this experiment ($F = 0.006$ – 4.2 ; NS) (Fig. 6, *insets*).

Figure 6. Effects of DMPX during cocaine withdrawal at 8 hr (*A*) and 12 hr (*B*) after cocaine or saline treatment. Graphs depict effects on reward thresholds; *insets* depict effects on response latency. Error bars indicate one SEM. * $p < 0.05$, different from all other means at 8 hr time point; # $p < 0.05$, different from post-saline–DMPX 0 at 8 hr time point; $p < 0.05$, different corresponding group given 3 mg/kg DMPX. For additional comparisons, see Results.

DISCUSSION

Effects of adenosine receptor agonists and antagonists on BSR under baseline conditions

Systemic administration of the adenosine A_{2A} receptor-selective agonists CGS 21680 and APEC elevated BSR thresholds, and systemic administration of APEC significantly decreased the latency to respond for the electrical stimulation. Furthermore, intra-NAcc infusion of CGS 21680 elevated BSR thresholds and reduced response latencies, although neither the highly selective adenosine A_{2A} receptor antagonist CSC nor the A_2 -preferring antagonist DMPX influenced BSR thresholds or response latencies. Nevertheless, DMPX dose-dependently antagonized the threshold-elevating effect of APEC. Elevations in BSR thresholds are thought to reflect a decrease in the rewarding effects of the stimulation (Edmonds and Gallistel, 1977; Bird and Kornetsky, 1990; Markou and Koob, 1992a, 1993). Therefore, the reward threshold-elevating effects of the adenosine A_{2A} receptor agonists CGS 21680 and APEC likely reflect attenuation of stimulation-derived reward. The absence of increases in response latency rules out nonspecific performance impairments as a factor contributing to the BSR threshold elevations.

The reduction of response latency (i.e., increase in response speed) by systemic APEC or intra-NAcc CGS 21680 was an unexpected finding, given the well known motor depressant effects of adenosine A_{2A} agonists (Ferré et al., 1991a; Barraco et

al., 1993; Hauber and Munkle, 1997). One mechanism that might explain this paradox is that the adenosine agonists reduced the adjunctive exploratory behavior often manifested in the BSR paradigm but left responses directed at obtaining the stimulation intact, thus allowing the subject to remain close to the wheel manipulandum, ready to respond.

Although intra-NAcc CGS 21680 elevated reward thresholds, the effect was most pronounced at 60 min after infusion. Other studies investigating the behavioral effects of centrally administered CGS 21680 also reported delayed peak drug effects, whether the site of injection was the lateral ventricle (Ferré et al., 1991a), the NAcc (Hauber and Munkle, 1997), or the dorsal striatum (Hauber and Munkle, 1997). Moreover, in the present study, infusion of CGS 21680 into a site 2 mm above the NAcc, which is closer than the NAcc is to the lateral ventricles, failed to produce a significant threshold elevation. For these reasons, it is unlikely that diffusion accounts for the effects observed, although the mechanism responsible for the delayed onset of centrally administered CGS 21680 remains to be determined.

The precise mechanism by which adenosine A_{2A} receptor stimulation inhibits BSR is presently unknown. Rewarding electrical brain stimulation increases extracellular levels of DA in the NAcc (Fiorino et al., 1993) (but see Garriss et al., 1999 discussed below), and blockade of DA receptors in the NAcc reduces the effects of BSR (Stellar and Corbett, 1989). Furthermore, it has been shown that stimulation of adenosine A_{2A} receptors opposes the effect of DA at striatal output cells. Specifically, A_{2A} receptor stimulation reduces the affinity of dopamine receptors for DA agonists *in vitro* (Ferré et al., 1991b), diminishes the effects of intrastriatal infusion of a dopamine agonist on pallidal GABA efflux *in vivo* (Ferré et al., 1993), opposes the effects of dopamine D2 receptor-mediated signal transduction in cultured cells (Yang et al., 1995), reduces the effects of dopamine on GABA efflux in punches of striatum and globus pallidus (Mayfield et al., 1996), disinhibits the firing of striatal output cells that are the targets of the dopaminergic projections (Mori et al., 1996), and opposes the effects of dopamine receptor stimulation on immediate early gene expression in the striatum (Morelli et al., 1994; Svenningsson et al., 1999). Accordingly, adenosine A_{2A} receptor agonists produce behavioral effects reminiscent of the effects of DA receptor antagonists (Heffner et al., 1989; Brown et al., 1991; Barraco et al., 1993; Zarrindast et al., 1993; Rimondini et al., 1997). Thus, one might hypothesize that the stimulation of adenosine A_{2A} receptors decreases the effects of BSR by reducing the postsynaptic effects of DA neurotransmission within the NAcc. Nevertheless, it should be noted that the question of whether dopamine directly mediates the rewarding properties of electrical stimulation or modulates reward-related arousal and anticipation is under debate (Damsma et al., 1992; Fiorino et al., 1993; Mirenowicz and Schultz, 1996; Garriss et al., 1999). Because both anticipatory and reward processes are involved in responding for BSR, one would predict that reducing DA neurotransmission via adenosine A_{2A} receptor stimulation would elevate BSR thresholds, as does pharmacological blockade of DA receptors (Fouriez and Wise, 1976; Gallistel and Davis, 1983; Stellar and Corbett, 1989; Bird and Kornetsky, 1990).

Whereas adenosine A_{2A} receptor agonists produced elevations in reward thresholds, the adenosine receptor antagonists DMPX and CSC had little or no effect on either reward thresholds or response latencies. Nevertheless, DMPX dose-dependently blocked the threshold elevation produced by the A_{2A} receptor agonist APEC, indicating that at the doses used in the present

study, DMPX antagonized adenosine A_{2A} receptors. In addition, the doses of CSC used in the present study have been shown previously to elicit hyperactivity and to block the effects of APEC on locomotor activity (Jacobson et al., 1993). The lack of effects of the two antagonists, when given alone in the present study, could be interpreted as evidence that the neural substrates underlying BSR are not under tonic control by endogenous adenosine acting through A_{2A} receptors under baseline conditions. Interestingly, earlier work showed that self-stimulation thresholds are elevated by various methylxanthine adenosine receptor antagonists (Mumford and Holtzman, 1990), although the non-xanthine A_2 adenosine receptor-preferring antagonist CGS 15943 did not influence BSR thresholds (Mumford and Holtzman, 1991a). Hence, it is possible that the previously reported effects of methylxanthines on BSR are because of pharmacological effects other than adenosine receptor blockade, a hypothesis supported by the observation that the discriminative stimulus produced by high doses of methylxanthines is qualitatively different from the discriminative stimulus associated with lower methylxanthine doses that generalize to the non-xanthine adenosine antagonist CGS 15943 (Mumford and Holtzman, 1991b).

Effects of adenosine agonists and antagonists on BSR during cocaine withdrawal

Withdrawal from a regimen of experimenter-administered cocaine injections elevated BSR thresholds but did not increase response latency, suggesting that cocaine withdrawal reduced the effects of BSR without producing performance impairments, similarly to previously published results (Markou and Koob, 1991). In addition, the adenosine A_2 receptor-preferring antagonist DMPX reversed the threshold elevation associated with cocaine withdrawal at 8 hr after cocaine with no effect in control subjects. Thus, blockade of adenosine receptors reverses at least some of the impairments in central reward function induced by cocaine withdrawal.

There are several observations that support the hypothesis that cocaine withdrawal-induced deficits in DA function are associated with, and are at least partially responsible for, the behavioral effects of withdrawal. First, the direct DA receptor agonist bromocriptine reversed the threshold elevation produced by withdrawal from prolonged self-administration sessions (Markou and Koob, 1992b). Second, extracellular levels of DA were depressed relative to prebinge baselines in the NAcc during cocaine withdrawal (Rossetti et al., 1992; Weiss et al., 1992; Parsons et al., 1995). Third, sensitivity to the locomotor depressant effect of the DA receptor antagonist cis-flupenthixol is increased during withdrawal from self-administered or experimenter-administered cocaine (Baldo et al., 1999).

Considering that adenosine A_{2A} receptor agonists elevated thresholds, that an adenosine antagonist reversed the threshold-elevating effect of cocaine withdrawal, and that the DA system is involved in withdrawal, one might hypothesize that dysregulation of endogenous adenosine systems contributes to the effects of cocaine withdrawal. Specifically, increased activity of adenosine at NAcc-localized A_{2A} receptors, which negatively modulate the postsynaptic effects of DA (Ferré et al., 1991b, 1993; Svenningsson et al., 1999), could underlie withdrawal-induced BSR attenuation. Thus, the adenosine system might not modulate the self-stimulation substrate under baseline conditions (see above) but might become overactive in response to a challenge, such as cocaine exposure. Adenosine levels in the brain increase in response to conditions of heightened energy demand on neurons

(Rudolphi et al., 1992), an effect that is thought to represent a biological response to excessive metabolic challenge to cells (Dunwiddie and Fredholm, 1997; Williams and Burnstock, 1997). Hence, if prolonged exposure to cocaine in rats can be conceptualized as a chemical challenge to the brain that produces excessive neuronal energy use (Porrino, 1993), there could be a compensatory increase in adenosinergic neuromodulation that persists into the early post-cocaine withdrawal period and produces the BSR deficit observed in the present study. Interestingly, there is some evidence that A_{2A} receptor stimulation and cocaine withdrawal produce similar neuronal effects. For example, both adenosine A_{2A} receptor stimulation and cocaine withdrawal reduce metabolic activity in limbic structures, such as the NAcc (Hammer et al., 1993; Nehlig et al., 1994). Moreover, recent studies have provided evidence for counteradaptations consistent with elevated adenosine levels, such as upregulation of adenosine uptake (Manzoni et al., 1998) and transporter sites (Kaplan and Leite-Morris, 1997) after discontinuation of chronic treatment with drugs of abuse, such as cocaine or morphine. Hence, it is possible that the adenosine system is recruited as part of the response of the brain to extended exposure to drugs of abuse and that adenosine receptor-mediated processes contribute to the reward deficits associated with drug withdrawal.

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