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# Role of central and peripheral adenosine receptors in the cardiovascular responses to intraperitoneal injections of adenosine $A_1$ and $A_{2A}$ subtype receptor agonists

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**1** The cardiovascular effects of the adenosine $A_1$ receptor agonist $N^6$-cyclopentyladenosine (CPA) and the adenosine $A_{2A}$ receptor agonist 2-*p*-(2-carboxyethyl)phenethylamino-5'-*N*-ethylcarboxamidoadenosine (CGS 21680) were investigated in rats implanted with telemetry transmitters for the measurement of blood pressure and heart rate.

**2** Intraperitoneal (i.p.) injections of the adenosine $A_1$ receptor agonist CPA led to dose-dependent decreases in both blood pressure and heart rate. These effects of 0.3 mg kg$^{-1}$ CPA were antagonized by i.p. injections of the adenosine $A_1$ receptor antagonist 8-cyclopentyl-1,3-dimethyl-xanthine (CPT), but not by i.p. injections of the adenosine $A_{2A}$ receptor antagonist 3-(3-hydroxypropyl)-8-(*m*-methoxystyryl)-7-methyl-1-propargylxanthine phosphate disodium salt (MSX-3). Injections (i.p.) of the peripherally acting nonselective adenosine antagonist 8-sulfophenyltheophylline (8-SPT) and the purported nonselective adenosine antagonist caffeine also antagonized the cardiovascular effects of CPA.

**3** The adenosine $A_{2A}$ agonist CGS 21680 given i.p. produced a dose-dependent decrease in blood pressure and an increase in heart rate. These effects of 0.5 mg kg$^{-1}$ CGS 21680 were antagonized by i.p. injections of the adenosine $A_{2A}$ receptor antagonist MSX-3, but not by i.p. injections of the antagonists CPT, 8-SPT or caffeine.

**4** Central administration (intracerebral ventricular) of CGS 21680 produced an increase in heart rate, but no change in blood pressure. MSX-3 given i.p. antagonized the effects of the central injection of CGS 21680.

**5** These results suggest that adenosine $A_1$ receptor agonists produce decreases in blood pressure and heart rate that are mediated by $A_1$ receptors in the periphery, with little or no contribution of central adenosine $A_1$ receptors to those effects.

**6** The heart rate increasing effect of adenosine $A_{2A}$ agonists appears to be mediated by adenosine $A_{2A}$ receptors in the central nervous system. The blood pressure decreasing effect of adenosine $A_{2A}$ agonists is most probably mediated in the periphery.
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**Keywords:** CGS 21680; CPA; $A_1$ receptors; $A_{2A}$ receptors; blood pressure; heart rate

**Abbreviations:** CGS 21680, 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine; CPT, 8-cyclopentyl-1,3-dimethyl-xanthine; CPA, $N^6$-cyclopentyladenosine; CV-1808, 5′-*N*-ethylcarboxamide adenosine; i.c.v., intracerebral ventricular; i.p., intraperitoneal; KF17837, (E)-1,3-dipropyl-7-methyl-8-(3,4-dimethoxystyryl)xanthine; MSX-3, 3-(3-hydroxypropyl)-8-(*m*-methoxystyryl)-7-methyl-1-propargylxanthine phosphate disodium salt; NTS, nucleus tractus solitarius; SCH 58261, 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine; 8-SPT, 8-sulfophenyltheophylline

## Introduction

Adenosine is a potent modulator of cardiovascular function. When administered systemically, adenosine produces hypotension and bradycardia (Barraco *et al*., 1987; Evoniuk *et al*., 1987). These effects are thought to be mediated at adenosine receptors localized centrally (central nervous system) and in the periphery (heart and vasculature) through different receptor subtypes, particularly the adenosine $A_1$ and $A_{2A}$ subtypes (Shryock & Belardinelli, 1997; Spyer & Thomas, 2000; Tabrizchi & Bedi, 2001; Dhalla *et al*., 2003). In the periphery, $A_1$ receptors are located primarily in the heart and mediate negative inotropic and chronotropic effects (Shryock & Belardinelli, 1997). Adenosine $A_{2A}$ receptors are located primarily in the vasculature and mediate vasodilation (Tabrizchi & Bedi, 2001). In the central nervous system, adenosine $A_1$ receptors are widely distributed, while

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adenosine $A_{2A}$ receptors are found in limited regions of the brain, most prominently in the striatum (Dunwiddie & Masino, 2001). However, high levels of $A_{2A}$ receptors are also found in the cardiovascular regulation regions of the hindbrain, including the nucleus tractus solitarius and the rostral ventral lateral medulla (Thomas *et al*., 2000). In fact, adenosine $A_{2A}$ receptors are thought to play a neuromodulatory role in baroreceptor reflex control (Barraco *et al*., 1988; Thomas *et al*., 2000).

Thus, an action at both adenosine $A_1$ and $A_{2A}$ receptors at both central and peripheral levels could play a role in the cardiovascular effects of systemically administered adenosine agonists. The purpose of the current experiments was to further delineate the central *versus* peripheral cardiovascular effects of two specific adenosine receptor subtype agonists, the adenosine $A_1$ agonist CPA and the adenosine $A_{2A}$ agonist CGS 21680. When given systemically to conscious animals, CPA produces large decreases in both blood pressure and heart rate (Appel *et al*., 1995; Schindler *et al*., 2004). An action in the periphery is most likely for CPA as current evidence suggests that administration of adenosine $A_1$ agonists in the central nervous system produces effects opposite from those seen after systemic administration (Barraco & Phillis, 1991, Scislo & O'Leary, 2002). The adenosine $A_{2A}$ agonist CGS 21680 produces a decrease in blood pressure and an increase in heart rate when given systemically to conscious animals (Alberti *et al*., 1997; Schindler *et al*., 2004). Central administration of adenosine $A_{2A}$ agonists produces blood pressure decreases similar to those seen with systemic administration, but effects on heart rate are different, with decreases in heart rate most commonly seen (Barraco & Phillis, 1991; Scislo & O'Leary, 1998; Kitchen *et al*., 2000). In fact, the heart rate increase seen following systemic administration is often attributed to a reflex tachycardia resulting from the observed decreases in blood pressure (Bonizzoni *et al*., 1995; Webb *et al*., 1990; Alberti *et al*., 1997).

Most of the studies using central administration of adenosine agonists have been performed in anesthetized animals. Lappe *et al*. (1992) showed that systemic administration of the $A_2$ agonist *N*6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl adenosine produced large decreases in blood pressure in anesthetized animals, with virtually no change in blood pressure in conscious animals. Heart rate was increased to a greater extent in conscious animals when compared to anesthetized animals. Similar differences have been noted following central administration. Barraco *et al*. (1988) found that adenosine injected into the NTS of anesthetized rats produced only decreases in blood pressure and heart rate. In contrast, De Paula & Machado (2001) found that in conscious rats adenosine, injected over a similar dose range, produced increases in both blood pressure and heart rate at the higher doses. Therefore, anesthesia may have affected the results of those studies using central administration.

In the current study, only conscious animals were used. The peripherally acting nonselective adenosine antagonist 8-SPT and central administration of the adenosine $A_{2A}$ agonist CGS 21680 were used to determine where the cardiovascular effects were mediated. Most drugs were injected intraperitoneal (i.p.) and cardiovascular parameters were monitored *via* telemetry. This procedure and injection route has been used successfully to study the cardiovascular effects of adenosine agonists previously (Bonizzoni *et al*., 1995; Casati *et al*., 1995; Monopoli *et al*., 1998; Schindler *et al*., 2004).

## Methods

### *Subjects*

Adult male, Sprague–Dawley rats, weighing 300–350 g, were used in the following experiments. They were individually housed in a temperature- and humidity-controlled room with a 12 h light/dark cycle (lights on at 07:00). All testing occurred in a room separate from the housing room. Testing was typically performed 5 days per week at approximately the same time each day for any individual animal. All animals used in this study were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All procedures were conducted in accordance with the guidelines of the Institutional Care and Use Committee of the NIDA/IRP and the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

### *Cardiovascular procedures*

Following arrival in the laboratory, five rats were given 1 week to adapt. Surgery was then performed to implant telemetry transmitters (Data Sciences International, St Paul, MN, U.S.A.) for the measurement of systemic blood pressure. Details of the surgery are given elsewhere (Tella *et al*., 1999). Briefly, under isoflurane anesthesia, a 4–5 cm long incision was made on the midline of abdomen. The descending aorta was exposed below the bifurcation of the renal arteries. A vascular clamp was placed immediately posterior to the renal artery and a curved 21-gauge needle was used to puncture the vessel anterior to the bifurcation. The catheter of the transmitter was inserted about 2 cm into the aorta, the area was dried and a drop of adhesive (Vet Bond) was applied to the catheter entry point. The transmitter was then sutured to the abdominal musculature and the abdominal incision and the skin were closed. An i.p. injection of 50,000 U $kg^{-1}$ dual penicillin was given to safeguard against infections.

At 1 to 2 weeks following surgery, experimental procedures began. During the experimental session, the animal's entire home cage (with food and water removed) was placed on top of the telemetry receiver. Three telemetry receivers were located in three separate but identical sound-attenuation chambers. Mean arterial blood pressure and heart rate were sampled every minute and were then monitored for up to 2 h. Testing continued daily (Monday–Friday) until the cardiovascular parameters were stable from session to session. Animals were then given an i.p. injection of saline just prior to placement of the cage on the telemetry receiver, at least 2 times per week, until cardiovascular parameters following saline injection remained stable. The cardiovascular response to the i.p. injection of saline was indistinguishable from that observed when the animals were placed in the telemetry chamber with no prior injection. Testing with the adenosine drugs was then started, with test drugs given i.p. no more frequently than 2 times per week, usually on Tuesdays and Fridays, with control saline injections given on Thursdays. Testing continued for up to 5 months for any individual rat and up to 30 drug

treatments were tested over that period (not all animals received every drug treatment and not all drug treatments are reported here).

Six additional rats were tested with CGS 21680 (10 $\mu$g) given i.c.v. Following the telemetry implant, these animals had a second surgery to implant guide cannula in the lateral ventricle approximately 2 weeks after the cardiovascular surgery. The animals were stereotaxically implanted with stainless-steel guide cannulae (22 G, Plastic ONE) in the right ventricle under Equithesin (3 ml kg$^{-1}$ i.p., 9.72 mg ml$^{-1}$ sodium pentobarbital, 44.4 mg ml$^{-1}$ chloral hydrate, NIDA Pharmacy, Baltimore, MD, U.S.A.) anesthesia (coordinates respect to bregma: A −0.8, L −1.3, V −4.5). Guide cannulae were fixed with dental acrylic and stainless-steel screws to the skull surface. Stainless-steel stylets were inserted into the cannulae to prevent occlusion. A recovery period of 6–7 days was allowed before testing. Testing for these rats lasted for less than 1 month. For i.c.v. administration, injection needles (28 G) extending 0.5 mm below the guide were inserted into the cannulae. CGS 21680 (10 $\mu$g) was administered i.c.v. at a rate of 1 $\mu$l min$^{-1}$ by means of a microdrive pump (final injection volume: 10 $\mu$l). The dose of the $A_{2A}$ receptor agonist was chosen according to previously published studies (Ferré *et al*., 1991). The needle was then left in place for an additional 5 min. Correct cannula placement was ascertained immediately after the animals were killed (overdose of Equithesin) by injecting ink into the ventricular system.

### *Drugs*

The adenosine $A_1$ receptor agonist $N^6$-cyclopentyladenosine (CPA), the adenosine $A_{2A}$ receptor agonist 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine (CGS 21680), the adenosine $A_1$ receptor antagonist 8-cyclopentyl-1,3-dimethyl-xanthine (CPT) and the nonselective adenosine receptor antagonists 8-sulfophenyltheophylline (8-SPT) and caffeine (anhydrate base) were purchased from Sigma Chemical Company (St Louis, MO, U.S.A.). The adenosine $A_{2A}$ receptor antagonist 3-(3-hydroxypropyl)-8-(*m*-methoxystyryl)-7-methyl-1-propargylxanthine phosphate disodium salt (MSX-3) was synthesized at the Pharmaceutical Institute, University of Bonn, Germany (Sauer *et al*., 2000).

All drugs were dissolved in sterile saline (with a few drops of 0.1 N NaOH for CPT and MSX-3; final pH adjusted to 7.4) and administered i.p. (except as noted below) in a volume of 2 ml kg$^{-1}$ of body weight (3 ml kg$^{-1}$ for MSX-3). MSX-3, CPT, caffeine and 8-SPT were administered 10 min before CGS 21680, CPA or saline. CGS 21680 was also administered *via* the i.c.v. route. For that study CGS 21680 was dissolved in saline and administered in a volume of 10 $\mu$l. For all drugs tested, baseline values of blood pressure and heart rate had recovered prior to the administration of any other treatment. Further, occasionally treatments or doses were repeated in the same animal and the results of those tests were always in close agreement.

### *Data analysis*

Data for statistical comparisons for the cardiovascular experiments are presented as the mean blood pressure or heart rate for the entire 2 h session, unless otherwise specified. Statistical comparisons among different treatments were made using analysis-of-variance, followed by Fisher's protected least-significant-difference tests. For time course analysis, blood pressure and heart rate were averaged over 10- or 5-min periods.

## Results

The adenosine $A_1$ agonist CPA given i.p. produced a dose-dependent decrease in both blood pressure and heart rate (Figure 1). These effects were clearly evident at a dose of 0.1 mg kg$^{-1}$. The adenosine $A_{2A}$ agonist CGS 21680 given i.p.

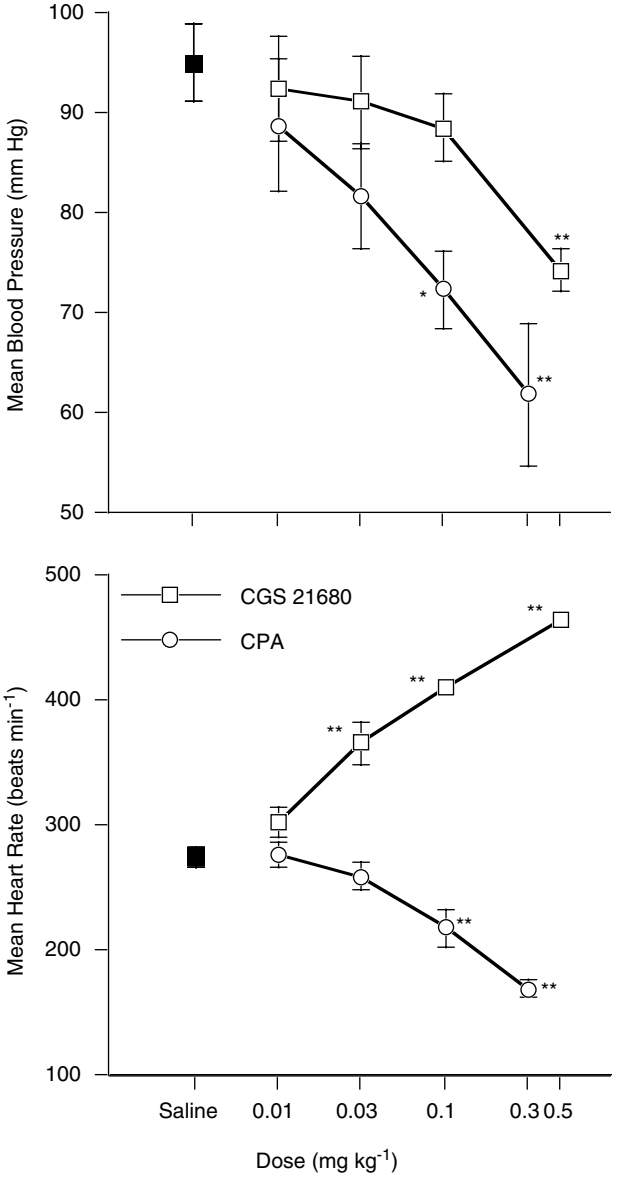


**Figure 1** Dose–effect functions for the effects of the adenosine $A_1$ receptor agonist CPA and the adenosine $A_{2A}$ agonist CGS 21680 on blood pressure (top panel) and heart rate (bottom panel). Drugs were administered i.p. just prior to placement in a sound-attenuation chamber. Cardiovascular parameters were measured for 2 h. The average blood pressure and heart rate were calculated for the entire 2-h period. The point above saline represents multiple determinations for the saline control. Each point is the mean ± s.e.m. of three rats. $*P<0.05$, $**P<0.01$ from saline control.

also produced a decrease in blood pressure, but this effect was significant only at the highest dose tested (0.5 mg kg$^{-1}$). In contrast to the $A_1$ agonist CPA, CGS 21680 produced a dose-dependent increase in heart rate. Here, significant increases were seen with doses as low as 0.03 mg kg$^{-1}$, well below the doses of CGS 21680 that produced decreases in blood pressure.

From the beginning to the end of the entire testing period, baseline measures of blood pressure and heart rate were stable (blood pressure, 97.3±4.0 to 98.6±7.9 mmHg; heart rate 277.6±4.9 to 275.5±9.2 beats min$^{-1}$; each value is the mean of 3 baseline days following the first test and the mean of 3 baseline days following the last test). Drug responses remained stable over this period as well. Three rats were tested a total of 3 times with 0.3 mg kg$^{-1}$ CPA and 0.5 mg kg$^{-1}$ CGS. For 0.3 mg kg$^{-1}$ CPA, both the blood pressure (first test 63.4±3.4 mmHg, second test 61.8±7.2 mmHg, third test 58.7±8.0 mmHg) and heart rate (first test 168.0±10.1 beats min$^{-1}$, second test 168.0±7.8 beats min$^{-1}$, third test 169.5±9.5 beats min$^{-1}$) responses were very stable across time. For 0.5 mg kg$^{-1}$ CGS also, the blood pressure (first test 77.1±2.0 mmHg, second test 74.2±2.1 mmHg, third test 63.5±6.9 mmHg) and heart rate (first test 475.6±4.7 beats min$^{-1}$, second test 463.7±1.9 beats min$^{-1}$, third test 468.3±9.5 beats min$^{-1}$) responses over this period of time were similar.

Four separate adenosine antagonists were injected i.p. prior to CPA and CGS 21680 and tested for their ability to antagonize the cardiovascular effects of the adenosine agonists. For these studies, the highest doses of CPA and CGS 21680 were chosen as previous studies have shown these are the minimal doses to produce maximal locomotor depressant effects (Karcz-Kubicha *et al*., 2003), an effect primarily involving the central nervous system. The adenosine $A_1$ antagonist CPT (4.8 mg kg$^{-1}$), the adenosine $A_{2A}$ antagonist MSX-3 (3.0 mg kg$^{-1}$) and the peripherally acting adenosine antagonist 8-SPT (25 mg kg$^{-1}$) had no significant effects on either blood pressure or heart rate at the doses tested (Figure 2, left hand set of bars). These doses of CPT and MSX-3 were chosen based on previous work showing selective blockade for other effects of CPA and CGS 21680 (Karcz-Kubicha *et al*., 2003). The dose of 8-SPT was chosen based on preliminary work showing it to be free of cardiovascular effects on its own. In contrast to CPT, MSX-3 and 8-SPT, the nonselective adenosine antagonist caffeine at 30 mg kg$^{-1}$, slightly increased both blood pressure and heart rate. This dose of caffeine (30 mg kg$^{-1}$) was chosen based on its maximal effect on locomotor activity (Karcz-Kubicha *et al*., 2003).

The adenosine $A_1$ antagonist CPT was able to block completely the effects of 0.3 mg kg$^{-1}$ CPA on both blood pressure and heart rate (Figure 2, middle set of bars). In contrast, the adenosine $A_{2A}$ antagonist MSX-3 had no effect when given as a pretreatment before CPA. The nonselective adenosine antagonist caffeine was able to antagonize partially the effect of CPA on both blood pressure and heart rate, while the peripherally acting antagonist 8-SPT completely antagonized the effects of CPA. In contrast to its effect as a pretreatment before CPA, CPT did not alter the effects of the adenosine $A_{2A}$ agonist CGS 21680 (Figure 2, right-hand set of bars). However, the adenosine $A_{2A}$ antagonist MSX-3 did antagonize both the blood pressure decreasing and heart rate increasing effects of 0.5 mg kg$^{-1}$ CGS 21680. Unlike for CPA, neither caffeine nor 8-SPT was able to antagonize significantly the cardiovascular effects of 0.5 mg kg$^{-1}$ CGS 21680.

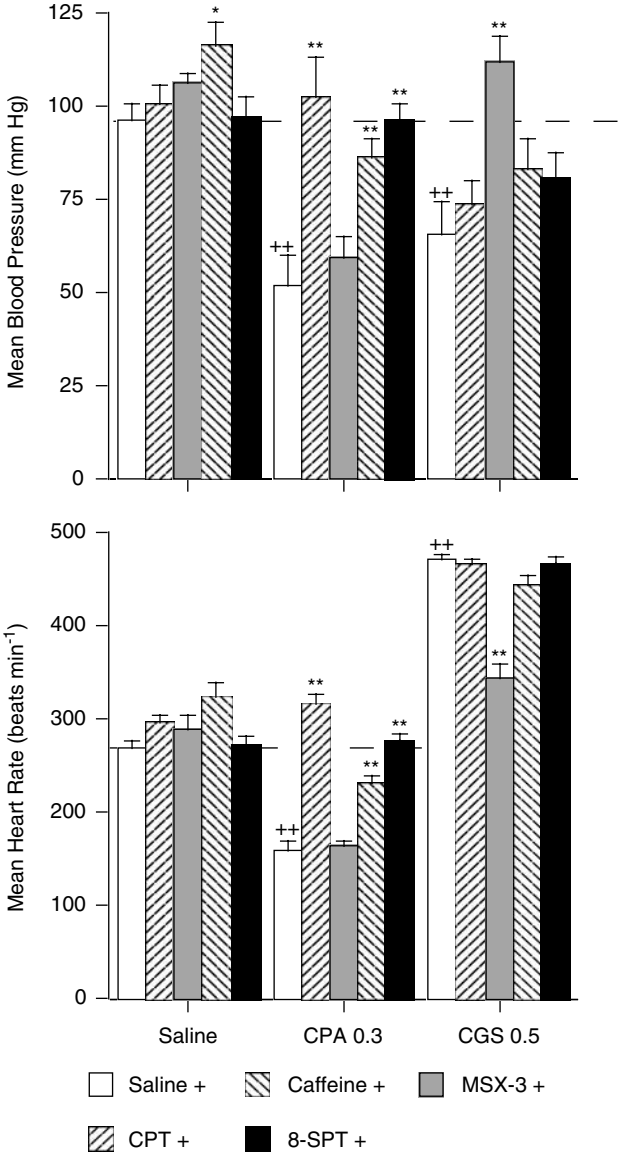


**Figure 2** Effects of the adenosine $A_1$ antagonist CPT (4.8 mg kg$^{-1}$), the adenosine $A_{2A}$ antagonist MSX-3 (3.0 mg kg$^{-1}$), the peripherally acting adenosine antagonist 8-SPT (25 mg kg$^{-1}$) and the nonselective adenosine antagonist caffeine (30 mg kg$^{-1}$) on the cardiovascular effects of 0.3 mg kg$^{-1}$ CPA and 0.5 mg kg$^{-1}$ CGS 21680. All drugs were administered i.p. The antagonists were given as pretreatments 10 min prior to saline, CPA or CGS 21680. All bars above saline represent the effects of the pretreatments followed by saline administration. All bars above CPA 0.3 and CGS 0.5 represent the effects of all the pretreatments followed by 0.3 mg kg$^{-1}$ CPA and 0.5 mg kg$^{-1}$CGS 21680, respectively. There were multiple determinations for the saline control. Each point represents the mean±s.e.m. of 4–5 rats. *Significant difference from respective control (open bars), +significant difference from saline control (left most open bar). One symbol, $P<0.05$; two symbols $P<0.01$.

The time course for selected groups presented as means in Figure 2 are presented in Figure 3. The left-hand panels show the effects of the adenosine $A_1$ agonist CPA alone and in combination with selected antagonists. For comparison, saline control is also shown. After placement in the chamber, both blood pressure and heart rate were slightly elevated. By

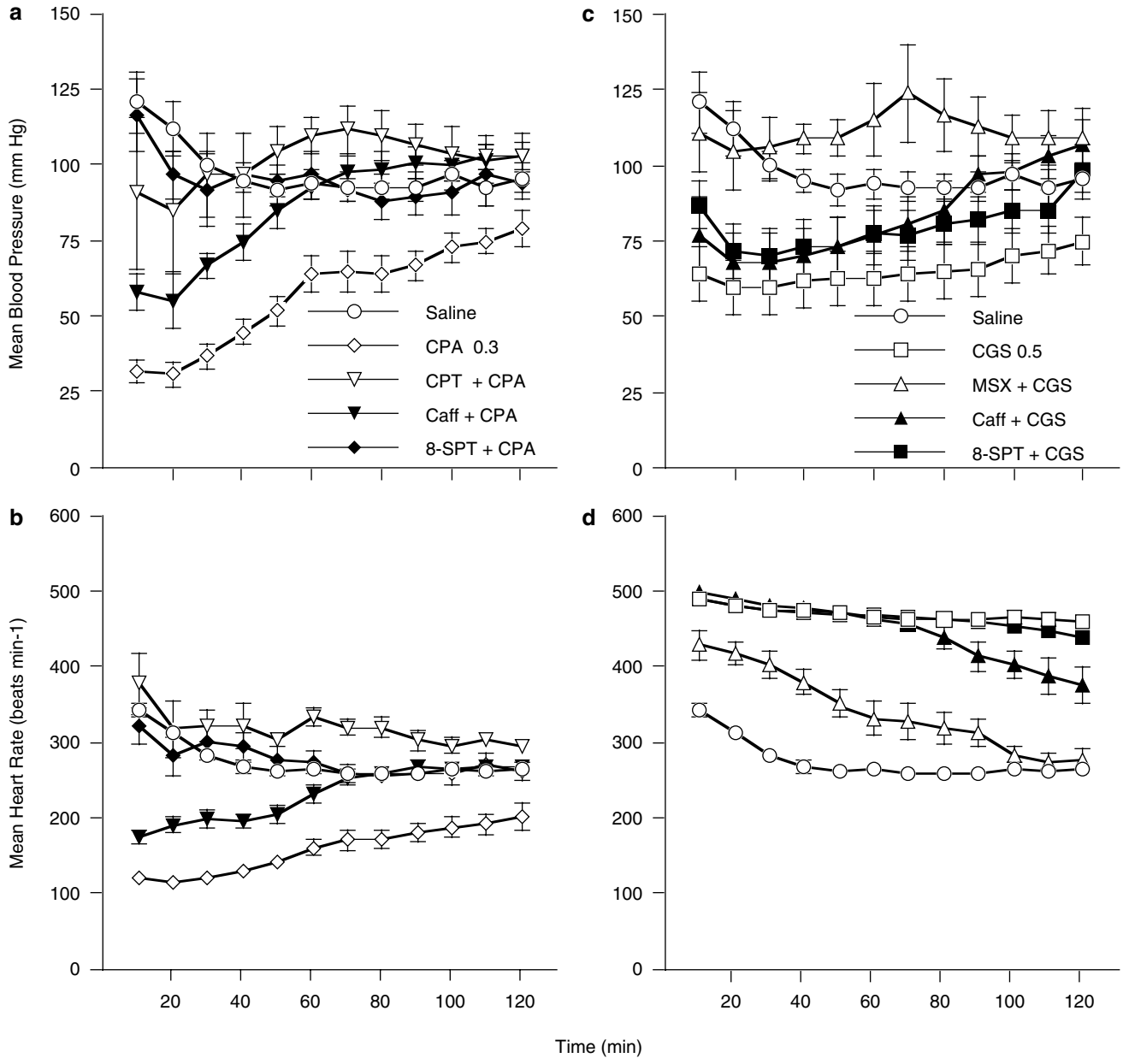


**Figure 3** Time course for the effects of the adenosine $A_1$ agonist CPA (a and c) and the adenosine $A_{2A}$ agonist CGS 21680 (CGS, c and d) on both blood pressure (a and c) and heart rate (b and d). Also shown are the effects of CPA and CGS 21680 following pretreatment with selected antagonists (doses as in Figure 2) and saline control. The saline control is the same for both the left- and right-hand panels. Blood pressure and heart rate were averaged over 10-min blocks for presentation. The entire 120-min period following the i.p. injections is shown. Each point represents the mean ± s.e.m. of 4–5 rats. Error bars are s.e.m. Where no error bars are seen, the error falls within the range covered by the symbol.

30–40 min, those values had stabilized. These effects observed following saline were not different then if the rats had been placed in the chamber without the saline injection. A profound decrease in blood pressure (Figure 3a) was observed following an i.p. injection of CPA ($0.3\,mg\,kg^{-1}$). Heart rate was also decreased (Figure 3b). Both blood pressure and heart rate returned toward baseline by the end of the session, but neither parameter recovered fully. Both the adenosine $A_1$ antagonist CPT and the peripherally acting antagonist 8-SPT were able to completely reverse the effects of CPA. The nonselective antagonist caffeine partially antagonized the effects of CPA.

The right-hand panels of Figure 3 show the effects of the adenosine $A_{2A}$ agonist CGS 21680 alone and in combination with selected antagonists. CGS 21680 ($0.5\,mg\,kg^{-1}$) produced a decrease in blood pressure (Figure 3c) and an increase in heart rate (Figure 3d) that slowly recovered toward baseline by the end of the session. The adenosine $A_{2A}$ antagonist MSX-3 antagonized the effects of CGS 21680, although the antagonism of heart rate was not complete. Neither the nonselective antagonist caffeine nor the peripherally acting antagonist 8-SPT was able to antagonize the cardiovascular effects of CGS 21680, although the decrease in blood pressure was not as pronounced following antagonist administration.

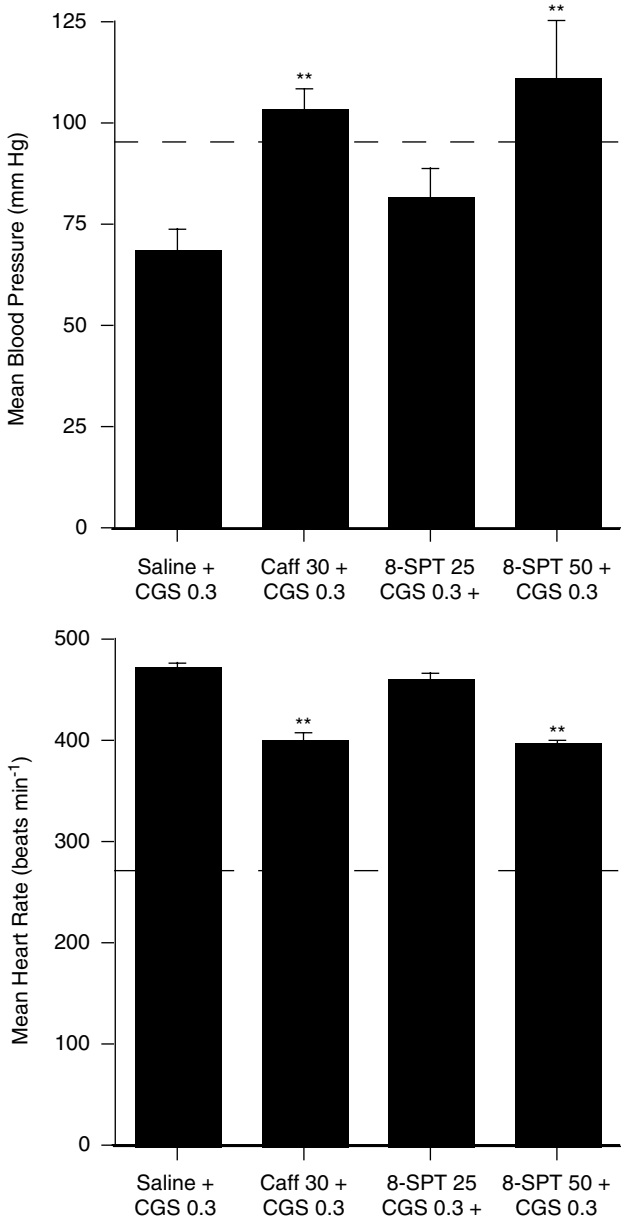


**Figure 4** Effects of the adenosine antagonists caffeine and 8-SPT (25 and 50 mg kg$^{-1}$) as pretreatments before 0.3 mg kg$^{-1}$ CGS 21680 (CGS 0.3). All drugs were administered i.p. The antagonists were given 10 min prior to CGS 21680. Each bar represents the mean ± s.e.m. of 3–4 rats. $^{**}P<0.01$ from CGS 21680 alone.

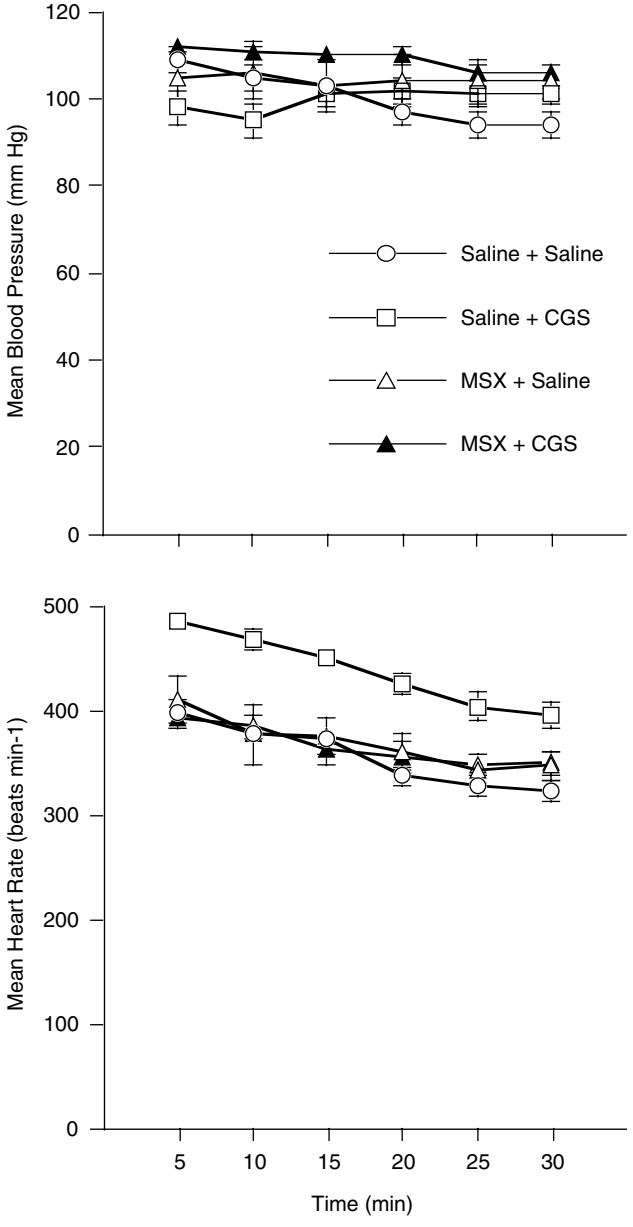


**Figure 5** Effects of CGS 21680 given centrally (i.c.v.). The second drug noted for each condition (10 $\mu$g CGS 21680 or saline) was given *via* a cannula implanted in the lateral ventricle. The first drurg noted for each condition (MSX-3, 3.0 mg kg$^{-1}$, or saline) was given i.p. 10 min prior to CGS 21680 or saline. Blood pressure and heart rate were averaged over 5-min blocks for presentation. The 30-min recording period following the i.c.v. injection is shown. Each curve is the mean of six rats. Error bars are s.e.m. Where no error bars are seen, the error falls within the range covered by the symbol.

As both caffeine and 8-SPT have been reported to be nonselective adenosine antagonists, their failure to antagonize the effects of CGS 21680 at a dose that was completely antagonized by MSX-3 was surprising. In order to further test their ability to antagonize adenosine $A_{2A}$ receptors, the dose of CGS 21680 was lowered to 0.3 mg kg$^{-1}$. The results of this study are shown in Figure 4. Here, caffeine clearly blocked the effects of CGS 21680 on blood pressure and partially antagonized the heart rate increasing effect. However, even at this lower CGS 21680 dose, 25 mg kg$^{-1}$ 8-SPT had no effect when given as a pretreatment before CGS 21680. The dose of 8-SPT was then increased to 50 mg kg$^{-1}$. This dose did have a small effect on its own on both blood pressure and heart rate (blood pressure 115.6 ± 11.0 mmHg; heart rate 307.6 ± 12.4 beats min$^{-1}$). The higher dose of 8-SPT was able to completely reverse the hypotension following 0.3 mg kg$^{-1}$ CGS, but only slightly reduced the heart rate increase.

The failure of 8-SPT to antagonize the heart rate effects of CGS 21680 could result from central effects of the adenosine $A_{2A}$ agonist. To further test this possibility, CGS 21680 was injected directly into the central nervous system *via* the lateral ventricle. CGS 21680, at a dose of 10 $\mu$g, failed to affect blood pressure, but did increase heart rate. Figure 5 shows the results for the first 30 min of the session averaged over 5-min blocks. CGS 21680 had no effect on blood pressure, but clearly

increased heart rate during this period of time. When the averaged heart rate was analyzed, this heart rate increase following CGS 21680 was significant ($P<0.05$). No other groups differed from saline control and no effect on blood pressure was significant. When given alone, the $A_{2A}$ antagonist MSX-3 ($3.0\,mg\,kg^{-1}$) given peripherally (i.p.) again had no effect on either blood pressure or heart rate. However, as a pretreatment before i.c.v. CGS 21680, MSX-3 antagonized the increase in heart rate produced by the adenosine $A_{2A}$ agonist.

## Discussion

The effects of the adenosine $A_1$ agonist CPA and the adenosine $A_{2A}$ agonist CGS 21680 on blood pressure and heart rate are in full agreement with previous results. A number of investigators have shown that systemic administration of adenosine $A_{2A}$ agonists produces decreases in blood pressure and increases in heart rate. This includes CGS 21680 (Jackson *et al*., 1993; Casati *et al*., 1995; Mathot *et al*., 1995a; Alberti *et al*., 1997; Tabrizchi, 1997; Schindler *et al*., 2004) as well as other adenosine $A_{2A}$ agonists (e.g., 2-hexynyl-*N*-ethylcarboxyamidoadenosine: Bonizzoni *et al*., 1995; Casati *et al*., 1995; Alberti *et al*., 1997). Webb *et al*. (1990) also showed that the adenosine $A_2$ agonist CV-1808 produced hypotension and tachycardia. Likewise, it has previously been shown that CPA (Jackson *et al*., 1993; Appel *et al*., 1995; Schindler *et al*., 2004) as well as other $A_1$ agonists (CCPA: Bonizzoni *et al*., 1995; Casati *et al*., 1995; 2-chloroadenosine: Webb *et al*., 1990) decrease both blood pressure and heart rate. Nonselective compounds given systemically, such as *N*-ethylcarboxyamidoadenosine and adenosine itself, produce effects similar to the adenosine $A_1$ agonists (Barraco *et al*., 1987; Evoniuk *et al*., 1987; Webb *et al*., 1990; Bonizzoni *et al*., 1995).

The specific adenosine $A_1$ antagonist CPT and the nonselective adenosine antagonist caffeine antagonized the effects of the adenosine $A_1$ agonist CPA. The peripherally acting nonselective adenosine antagonist 8-SPT also antagonized the cardiovascular effects of CPA. These results indicate that the cardiovascular effects of CPA were mediated by adenosine $A_1$ receptors in the periphery. The peripheral action of 8-SPT has been confirmed by Evoniuk *et al*. (1987), where a dose of up to $50\,mg\,kg^{-1}$ 8-SPT failed to produce detectable levels of the drug in brain, yet still antagonized the cardiovascular effects of the adenosine agonists adenosine, 2-chloroadenosine, R-phenylisopropyladenosine and *N*-ethylcarboxyamidoadenosine. Webb *et al*. (1990) also showed that 8-SPT was an effective antagonist of adenosine $A_1$ cardiovascular effects. While these results point to adenosine $A_1$ receptors in the periphery, they do not pinpoint the location of those receptors. Likely targets are the adenosine $A_1$ receptors in the heart that can mediate negative inotropic and chronotropic effects (Shryock & Belardinelli, 1997).

The specific adenosine $A_{2A}$ antagonist MSX-3 completely blocked the cardiovascular effects of the adenosine $A_{2A}$ agonist CGS 21680, but failed to alter the effects of the $A_1$ agonist CPA. This result shows that the cardiovascular effects of CGS 21680 are mediated by adenosine $A_{2A}$ receptors. Previous investigators have not always shown blockade of the tachycardia produced by CGS 21680 using different adenosine $A_{2A}$ antagonists. Jackson *et al*. (1993) showed that KF17837 could block the hypotension following CGS 21680, but not the tachycardia. Likewise, Mathot *et al*. (1995b) found that 8-(3-chlorostyryl)caffeine could block the blood pressure decrease but not the heart rate increase following CGS 21680. Lappe *et al*. (1992) did show that the nonselective adenosine antagonist CGS 15943 could block the hypotension and tachycardia following administration of the adenosine $A_2$ receptor agonist *N*6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl adenosine.

The tachycardia following administration of the adenosine $A_{2A}$ agonist CGS 21680 has often been assumed to result from a reflex response to the hypotension produced by peripheral vasodilation (Bonizzoni *et al*., 1995; Webb *et al*., 1990; Alberti *et al*., 1997). However, the results of this and previous studies do not support this interpretation. First, the tachycardia was evident at doses lower than those that produced hypotension (Figure 1). Second, blood pressure typically recovers faster than heart rate (Mathot *et al*., 1995a; Schindler *et al*., 2004). Finally, as noted above, some antagonists can block the decrease in blood pressure, but not the increase in heart rate following CGS 21680.

The results of the 8-SPT antagonist studies seem to suggest a central mechanism in the tachycardia following CGS 21680. However, 8-SPT is more potent as an adenosine $A_1$ antagonist than as an adenosine $A_{2A}$ antagonist (Gao *et al*., 2001) confounding the interpretation of the results. Nevertheless, central mediation of this effect still appears most likely. The adenosine $A_{2A}$ antagonist KF17837 does not readily cross the blood–brain barrier (Stone-Elander *et al*., 1997) and does not antagonize the tachycardia following CGS 21680. The adenosine $A_{2A}$ antagonist 8-(3-chlorostyryl)caffeine is taken up by the brain more rapidly than KF17837, but is rapidly washed out (Ishiwata *et al*., 2000). In contrast, 3-(3-hydroxypropyl)-7-methyl-8(*m*-methoxystyryl)-1-propargylxanthine, the active drug to which the pro-drug MSX-3 is converted (Sauer *et al*., 2000), has a high degree of brain penetration and is metabolically stable (Müller, 2000). MSX-3 also antagonizes the tachycardia following CGS 21680. To provide further support that it is a central action of adenosine $A_{2A}$ that produces tachycardia, CGS 21680 was administered centrally (i.c.v.). As with peripheral administration, CGS 21680 given i.c.v. also produced tachycardia, but with little change in blood pressure. All of these results taken together suggest a central mechanism of action for the tachycardia following the administration of CGS 21680. The slight decrease in tachycardia observed following pretreatment with $50\,mg\,kg^{-1}$ 8-SPT could indicate that there is a small contribution of reflex tachycardia to the overall effect of CGS. That is, when the blood pressure response was completely blocked, we would expect to negate any contribution of reflex tachycardia. The fact that tachycardia was only slightly reduced indicates that the primary effect of CGS on heart rate is not through reflex mechanisms.

The specific region of the brain responsible for the tachycardia following CGS 21680 administration is not clear. Adenosine $A_{2A}$ receptors are not widely distributed in the central nervous system. Adenosine $A_{2A}$ receptors are most prominent in the striatum, but are also found in cardiovascular control regions such as the nucleus tractus solitarius and rostral ventral lateral medulla (Thomas *et al*., 2000). Most evidence with central administration of drug suggests that agonist action at adenosine $A_{2A}$ receptors in the nucleus tractus solitarius produces primarily bradycardia (Barraco &

Phillis, 1991; Scislo & O'Leary, 1998). However, bardycardia is also observed when adenosine is injected into the NTS of anesthetized animals (Barraco *et al*., 1988). When adenosine is given into the NTS of conscious animals (De Paula & Machado, 2001), heart rate increases can be observed. Other brain areas have not been as extensively studied.

While the tachycardia following CGS 21680 appears to result from central mechanisms, the mechanisms for the hypotension are less clear. Since 8-SPT is not as potent at adenosine $A_{2A}$ receptors, the failure of 25 mg kg$^{-1}$ 8-SPT to antagonize the blood pressure effect is not as informative as could be expected. When the dose of 8-SPT was increased to 50 mg kg$^{-1}$, blockade of the hypotension was observed. Therefore, a peripheral site of action appears most likely. Action at adenosine $A_{2A}$ receptors is well known to produce vasodilation in the periphery. In the current study, CGS 21680 administered centrally had little effect on blood pressure, further supporting a peripheral site of action. Further, KF17837, an adenosine $A_{2A}$ antagonist that does not readily cross the blood–brain barrier (Stone-Elander *et al*., 1997), does not antagonize the tachycardia following CGS 21680.

The adenosine antagonists MSX-3 and CPT had little effect on either blood pressure or heart rate when given alone. These results suggest that there is little tonic control of these cardiovascular parameters by adenosine. However, other investigators have shown some effects of adenosine antagonists on their own. For example, Monopoli *et al*. (1998) showed that the adenosine $A_{2A}$ antagonist SCH 58261 increased both blood pressure and heart rate following a high dose. In the current study, the nonselective antagonist caffeine and the high dose of 8-SPT also produced small increases in blood pressure and heart rate. Thus, some tonic control by adenosine may be evident.

While caffeine clearly antagonized the cardiovascular effects of the adenosine $A_1$ agonist CPA, it did not significantly alter the effects of 0.5 mg kg$^{-1}$ of the adenosine $A_{2A}$ agonist CGS 21680. Likewise, 25 mg kg$^{-1}$ 8-SPT failed to antagonize the cardiovascular effects of CGS 21680. These results suggest that caffeine and 25 mg kg$^{-1}$ 8-SPT are most prominently adenosine $A_1$ antagonists. Decreasing the dose of CGS 21680 to 0.3 mg kg$^{-1}$ allowed caffeine to antagonize the blood pressure decrease following CGS 21680 and to produce some antagonism of the heart rate increase. In contrast, decreasing the dose of CGS 21680 did not improve 25 mg kg$^{-1}$ 8-SPT's ability to antagonize the cardiovascular effects of CGS 21680. Increasing the dose of 8-SPT to 50 mg kg$^{-1}$ did lead to antagonism of the blood pressure response of CGS 21680. As noted above, 8-SPT clearly is more potent at adenosine $A_1$ receptors than at adenosine $A_{2A}$ receptors (Gao *et al*., 2001). The effects observed here support the classification of 8-SPT as primarily an adenosine $A_1$ antagonist. Similarly, although *in vitro* radioligand binding experiments suggest that $A_1$ and $A_{2A}$ receptors have similar affinities for caffeine (Abo Salem *et al*., 2004), the present results indicate *in vivo* caffeine acts primarily as an adenosine $A_1$ antagonist, as recently suggested (Karcz-Kubicha *et al*., 2003).

In summary, the adenosine $A_1$ agonist CPA produced dose-dependent decreases in both blood pressure and heart rate. These effects are mediated primarily in the periphery at specific adenosine $A_1$ receptor sites. The adenosine $A_{2A}$ agonist CGS 21680 produced dose-dependent decreases in blood pressure, but increases in heart rate. The heart rate increasing effects of CGS 21680 appear to be mediated in the central nervous system at adenosine $A_{2A}$ receptors, and do not result from reflex tachycardia following the decrease in blood pressure. The blood pressure decreasing effect of CGS 21680 is most probably mediated in the periphery. Finally, both the peripherally acting nonselective adenosine antagonist 8-SPT and the purported nonselective adenosine antagonist caffeine appear to be primarily adenosine $A_1$ antagonists, as both were clearly more effective at antagonizing CPA than CGS 21680.

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