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Cardiovascular Pharmacology of the A_{2A} Adenosine Receptor Antagonist, SCH 58261, in the Rat

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ABSTRACT

We characterized the *in vivo* cardiovascular profile of SCH 58261, 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c] pyrimidine, a selective A_{2A} adenosine receptor antagonist, in conscious, freely moving rats by use of the telemetry system. In normotensive rats, SCH 58261, at 10 mg/kg i.p., significantly ($P < .05$) inhibited hypotension and tachycardia induced by the A_{2A} receptor agonist 2-hexynyl-5′-N-ethylcarboxamidoadenosine (0.01 mg/kg i.p.), but not the bradycardic effect caused by the A_1 receptor agonist 2-chloro-N^6-cyclopentyladenosine (0.03 mg/kg i.p.). SCH 58261, when administered alone, at 0.1 and 1 mg/kg i.p., did not induce significant hemodynamic changes, but at 10 mg/kg i.p., it slightly increased both systolic blood pressure (SBP) and diastolic blood pressure (DBP) ($+19 \pm 3$ and $+16 \pm 2$ mm Hg, respectively; $P < .01$) and heart rate (HR) ($+85 \pm 5$ beats/min; $P < .01$). These effects were inhibited by adrenergic blockade with propranolol (30 mg/kg i.p.) and phentolamine (10 mg/kg i.p.): -5 ± 3 mm Hg on DBP and -12 ± 11 beats/min on HR ($P < .01$). In spontaneously hypertensive rats, SCH 58261, at 3 and 10 mg/kg i.p., increased weakly both SBP ($+19 \pm 5$ mm Hg and $+25 \pm 4$ mm Hg) and DBP ($+14 \pm 4$ mm Hg and $+23 \pm 4$ mm Hg) *vs*. vehicle ($P < .01$) and HR ($+45 \pm 17$ and $+64 \pm 18$ beats/min *vs*. vehicle, respectively; $P < .01$). The data indicate that SCH 58261 retains A_{2A} selective receptor antagonist properties *in vivo.* Its effect on cardiovascular sympathetic outflow further suggests that endogenous adenosine exerts a tonic vascular regulation through A_{2A} receptors. Therefore, SCH 58261 can be a useful pharmacological tool for clarifying A_{2A}-mediated cardiovascular actions of adenosine.

Adenosine modulates a variety of physiological processes in mammals. Many of the responses mediated by adenosine are caused by its interaction with specific membrane-bound receptors. From pharmacological and molecular biology studies, four adenosine receptor subtypes have been characterized, namely A_1, A_{2A}, A_{2B} and A_3 (Fredholm *et al.*, 1994). These receptors belong to the large family of G protein-coupled receptors. Activation of A_1 and A_3 receptors leads to the inhibition of adenylate cyclase by a G_i protein, whereas A_{2A} and A_{2B} receptors stimulate the enzyme through a G_s protein (Olah and Stiles, 1995). In the cardiovascular system, activation of the A_1 receptor subtype produces an inhibitory action on the heart, which accounts for the decrease in blood pressure, bradycardia and reduction in cardiac output (Olsson and Pearson, 1990; Webb *et al.*, 1990). Stimulation of the A_{2A} receptor subtype elicits a variety of effects including vasodilation, inhibition of both platelet aggregation and neutrophil adhesion and reduction in generation of oxygen free radicals, all of which account for most beneficial effects of adenosine in reperfusion injury (Olsson and Pearson, 1990; Schlack *et al.*, 1993).

In the past decade, many adenosine receptor agonists and antagonists with different degrees of selectivity for A_1 and A_{2A} receptors have been synthesized. Adenosine analogs acted as selective agonists for either the A_1 or A_{2A} receptors (Fredholm *et al.*, 1994). Regarding adenosine receptor antagonists, many xanthines which are derivatives of the natural compounds caffeine and theophylline have been found to be potent and selective A_1 receptor antagonists. More recently, the discovery that 8-styrylxanthines and other heterocyclic compounds are selective A_{2A} antagonists has made a better understanding of the biology of A_{2A} receptors possible (Ongini and Fredholm, 1996). One such compound, SCH 58261, is a potent antagonist at the A_{2A} receptors as shown by the results of a variety of *in vitro* assays ranging from receptor binding to isolated tissue preparations. Therefore, SCH 58261 has been shown to have high affinity for the A_{2A} receptor in brain striatal membranes and to antagonize the typical A_{2A} receptor-mediated responses, such as adenosine receptor agonist-induced vasodilation in porcine and bovine isolated arteries, platelet aggregation inhibition (Zocchi *et*

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ABBREVIATIONS: SCH 58261, 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine; 2HE-NECA, 2-hexynyl-5′-N-ethylcarboxamidoadenosine; CCPA, 2-chloro-N^6-cyclopentyladenosine; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SHRs, spontaneously hypertensive rats; NTS, nucleus tractus solitarius; DMSO, dimethyl sulfoxide.

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al., 1996a) and increase in vascular conductance in the guinea pig isolated heart (Belardinelli *et al.,* in press). Moreover, the radioligand [^{3}H]SCH 58261 has been found to label A_{2A} receptors in membranes and slices from rat brain striatum (Zocchi *et al.*, 1996b; Fredholm *et al.*, 1998), human platelets (Dionisotti *et al.*, 1996), porcine coronary arteries (Belardinelli *et al.*, 1996) and Chinese hamster ovary cells expressing the human cloned A_{2A} receptors (Dionisotti *et al.*, 1997). So far, however, there are no data describing the *in vivo* cardiovascular profile of SCH 58261.

With this background we have designed experiments with SCH 58261 to assess its A_{2A} receptor antagonist properties on hemodynamic responses to selective A_{2A} and A_1 adenosine agonists and its own cardiovascular effects. Blood pressure and heart rate were monitored in conscious, freely moving rats implanted with the telemetry system, as described previously (Casati *et al.*, 1995). The hemodynamic profiles were analyzed by use of a curve fitting model (Bonizzoni *et al.*, 1995). We have found that SCH 58261, at a dose which selectively antagonizes A_{2A} receptors, has an effect *per se* by increasing blood pressure and heart rate. This action is inhibited by adrenergic blockade. From these findings it appears that endogenous adenosine, acting through A_{2A} receptor stimulation, may exert a tonic regulation on cardiovascular sympathetic activity.

Materials and Methods

Male Sprague-Dawley rats and SHRs were supplied by Charles River, Calco, Como, Italy. They were acclimatized to standard conditions and housed in individual cages for 1 week before the surgical operation, with free access to food and water.

Blood pressure and heart rate were recorded by using the telemetry system (Data Sciences, St. Paul, MN), as described previously (Casati *et al.*, 1995). Rats were anaesthetized with pentobarbital (30 mg/kg i.p.), a tract of the abdominal aorta was isolated, the catheter tip was inserted in the descending aorta above the iliac bifurcation and the sensor was affixed to the muscles. After recovery from anesthesia, rats were housed individually in cages placed on the radio-frequency receivers.

Hemodynamic recordings were taken every 5 min, starting 2 h before administration of drugs and continuing up to 24 h thereafter. Each recording lasted for 10 s and the haemodynamic values of all cardiac cycles within this period (about 50 at base-line) were averaged.

Experimental Protocols

Effects of SCH 58261 on BP and HR in rats. SCH 58261 at 0.1, 1 and 10 mg/kg i.p., or vehicle (Tween 80 aqueous suspension, 5 ml/kg i.p.) were given to a group of normotensive rats ($n = 6$), according to a latin square design. Between the different treatments, there was a 72-hr wash-out period. In an additional set of experiments, a group of SHRs ($n = 8$) were administered SCH 58261 at 3 and 10 mg/kg i.p. or vehicle with the same experimental design.

Effects of SCH 58261 on A_1 and A_{2A} receptor agonist-mediated cardiovascular responses. The selective A_{2A} receptor agonist 2HE-NECA (0.01 mg/kg i.p.) and the selective A_1 receptor agonist CCPA (0.03 mg/kg i.p.) dissolved in DMSO 2% were used to investigate the A_{2A} selectivity of SCH 58261 (10 mg/kg i.p.). The doses of agonists were chosen as those inducing submaximal hemodynamic effects, based on preliminary experiments. A group of normotensive rats ($n = 10$) received the following treatments, each consisting of the administration of two different compounds separated by a 30-min interval: SCH 58261 + CCPA; vehicle (Tween 80) + CCPA; SCH 58261 + 2HE-NECA; vehicle (Tween 80) + 2HE-NECA; SCH 58261 + DMSO; vehicle (Tween 80) + DMSO. All treatments were given to each rat, according to a latin square design. Between the different treatments, there was a 72-hr wash-out period.

Interaction between sympathetic outflow and SCH 58261-induced responses on BP and HR. The role of sympathetic activity in the effects of SCH 58261 (10 mg/kg i.p.) was examined by investigating the effects of adrenergic blockade on BP and HR. Propranolol (30 mg/kg i.p.) and phentolamine (10 mg/kg i.p.) were dissolved in physiologic solution. A group of normotensive rats ($n = 10$) received the following treatments, each consisting in the administration of two different compounds separated by a 30-min time interval: propranolol and phentolamine + SCH 58261; propranolol and phentolamine + vehicle (Tween 80); saline + SCH 58261; saline + vehicle (Tween 80). All treatments were given to each rat, according to a latin square design. Between the different treatments, there was a 72 h wash-out period.

Statistical Analysis

Hemodynamic activity of SCH 58261. As for the dose-related hemodynamic activity, peak effects were calculated directly from raw data, considering values recorded around t_{peak} (50 min). Areas over the curves were obtained as the differences between vehicle and SCH 58261 profiles in the 180 min after drug administration.

The hemodynamic effects induced by SCH 58261 at the highest dose (10 mg/kg i.p.) were characterized further in a separate group of normotensive and hypertensive rats and analyzed by use of the curve-fitting model proposed by Bonizzoni *et al.* (1995). The following family of 4-constant exponential functions was used to fit experimental data.

$$E[y(t)] = \alpha \cdot \exp[-\beta \cdot [g(t + \theta) - g(\tau + \theta)]^2]$$

where $E[y(t)]$ is the expected value of the effect $y(t)$ recorded at time t from drug administration, $g()$ is any monotonic function of t, α is the maximum intensity of the effect (peak) and τ is the time at peak. The shape of the curve depends on function $g()$ and constant θ ($\theta > 0$), whereas β expresses width of peak: for given $g()$ and θ, the larger β the narrower the peak. Least square estimates of the constants of the above models were obtained by PROC NLIN (SAS Institute Inc., 1989). BP and HR profiles were analyzed with this model after subtraction of vehicle profile.

As for the dose-related hemodynamic activity, peak effects were calculated directly from raw data, considering values recorded around t_{peak} (50 min). Areas over the curves were obtained as the differences between vehicle and SCH 58261 profiles in the 180 min after drug administration. Statistical comparisons were performed considering 95% ($P < .05$) and 99% ($P < .01$) confidence limits.

A_{2A} antagonist properties of SCH 58261. Because SCH 58261, at 10 mg/kg i.p., administered to normotensive rats induces hemodynamic activity *per se*, the net hemodynamic effects of adenosine receptor agonists in the presence and in the absence of the antagonist were obtained by subtracting the changes induced by SCH 58261 and its vehicle. Statistical comparisons on peak effects, which occurred from 15 to 30 min after agonist administration, were performed considering 95% ($P < .05$) and 99% ($P < .01$) confidence limits.

Role of sympathetic activation in the hemodynamic response of SCH 58261. The net hemodynamic activity of SCH 58261 (10 mg/kg i.p.), either in the absence or in the presence of adrenergic blockade, were obtained by subtracting the effects induced by the pretreatment with either the *alpha* and *beta* adrenoceptor blockers or vehicle *per se*. Therefore, BP and HR profiles of group *alpha* and *beta* adrenoceptor blockers + vehicle were subtracted from that of group *alpha* and *beta* adrenoceptor blockers + SCH 58261, and profiles of group vehicle + vehicle were subtracted from that of group vehicle + SCH 58261. Statistical comparisons on peak effects, which occurred from 30 to 60 min after SCH 58261 administration, were performed considering 95% ($P < .05$) and 99% ($P < .01$) confidence limits.

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Drugs. SCH 58261 was synthesized at the Dept. of Pharmaceutical Sciences, University of Ferrara (Baraldi *et al.*, 1994). 2HE-NECA was synthesized at the Department of Chemical Sciences, University of Camerino (Prof. G. Cristalli). CCPA was purchased from Research Biochemical International, Natick, MA.

Results

Hemodynamic activity of SCH 58261. The base-line of the hemodynamic parameters was obtained from each rat by averaging all recordings taken for 2 hr before treatment. In normotensive rats, baseline values for SBP, DBP and HR were 120 ± 3 mm Hg, 85 ± 3 mm Hg and 300 ± 2 beats/min, respectively. SCH 58261 given at 0.1 mg/kg i.p. did not induce any change on BP and HR. A slight increase in BP and HR was observed at 1 mg/kg, although no significant differences were found *vs*. vehicle: peak effects were +9 ± 4 mm Hg and + 9 ± 5 beats/min for DBP and HR, respectively. At 10 mg/kg i.p., the effect on DBP was + 12 ± 4 mm Hg ($P < .05$ *vs*. vehicle) and on HR the effect was +25 ± 12 beats/min, which was not statistically significant as compared with vehicle. Also the area over the curve for DBP (+1779 ± 458 mm Hg for 180 min) was significantly different than vehicle ($P < .05$), whereas that calculated for HR (+4422 ± 2198 beats for 180 min) was not.

In a separate group of normotensive rats we further investigated the hemodynamic profile of SCH 58261 at 10 mg/kg i.p. by analyzing the experimental traces with the curve-fitting model. In these rats the base-line values for SBP, DBP and HR were 130 ± 2 mm Hg, 90 ± 2 mm Hg and 305 ± 4 beats/min, respectively. The vehicle administration produced a prompt increase in BP (about +20 mm Hg) and HR (about +100 beats/min). These effects recovered completely to base line in 30 to 60 min (fig. 1). After administration of SCH 58261 the initial rise in hemodynamic parameters was similar, but recovery was markedly slower (fig. 1). The subtraction of vehicle profile allowed the characterization of the net effects induced by SCH 58261: peak effects for SBP, DBP and HR were +19 ± 3 mm Hg, +16 ± 2 mm Hg and +85 ± 5 beats/min, respectively ($P < .01$ *vs*. vehicle; fig. 1). These values were reached about 50 min after administration of the compound, and declined with a $t_{1/2}$ of about 60 min (table 1).

In SHRs, base-line values for SBP, DBP and HR were 183 ± 5 mm Hg, 128 ± 5 mm Hg and 300 ± 2 beats/min, respectively. SCH 58261 given at 3 and 10 mg/kg i.p. significantly increased BP and HR ($P < .01$ *vs*. vehicle). Peak effects (*i.e.,* +19 ± 5 mm Hg, +14 ± 4 mm Hg and +45 ± 17 beats/min on SBP, DBP and HR, respectively, at 3 mg/kg; +25 ± 4 mm Hg, +23 ± 4 mm Hg and +64 ± 18 beats/min on SBP, DBP and HR, respectively, at 10 mg/kg) were reached about 30 min after administration of the compound, and declined with a $t_{1/2}$ of about 30 to 60 min, except for HR at 10 mg/kg, which lasted about 2 hr (table 1).

Selective A_{2A} antagonism by SCH 58261 of agonist-mediated responses. As expected, both adenosine receptor agonists, 2HE-NECA and CCPA, were readily effective and induced hemodynamic effects, peaking about 15 to 30 min after injection (fig. 2 and table 2). The A_{2A} selective agonist 2HE-NECA (0.01 mg/kg i.p.) decreased DBP to 60 ± 3 mm

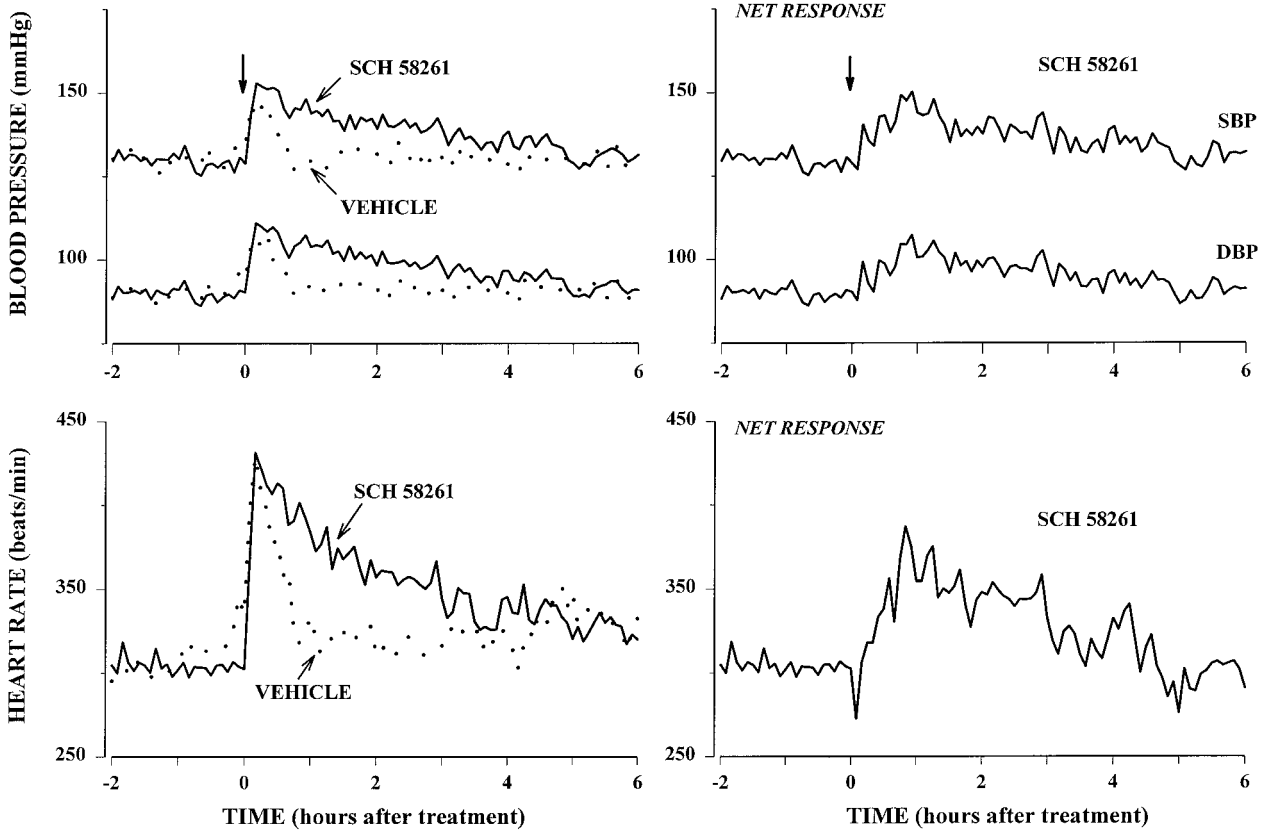

Fig. 1. Time course of the effects of SCH 58261 (10 mg/kg i.p.) on BP and HR in conscious, freely moving normotensive rats with use of the telemetry system. The net hemodynamic effects induced by SCH 58261 are indicated in the right panels. Each profile is the mean of nine rats.

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TABLE 1
Time course of the effects on BP and HR induced by SCH 58261 in conscious rats[a]

Rat strain	Dose	t_{peak}			$t_{1/2}$		
		SBP	DBP	HR	SBP	DBP	HR
	mg/kg i.p.		*min*			*min*	
Normotensive	10	51 (38–68)	51 (37–70)	55 (44–69)	67 (40–114)	77 (49–124)	58 (37–90)
Hypertensive (SHR)	3	13 (2–72)	28 (20–38)	22 (15–33)	57 (28–116)	46 (18–122)	41 (20–86)
Hypertensive (SHR)	10	17 (5–60)	39 (23–68)	33 (15–73)	80 (21–307)	36 (19–71)	117 (19–159)

[a] Data are geometric means with 95% confidence limits (n = 8–9).

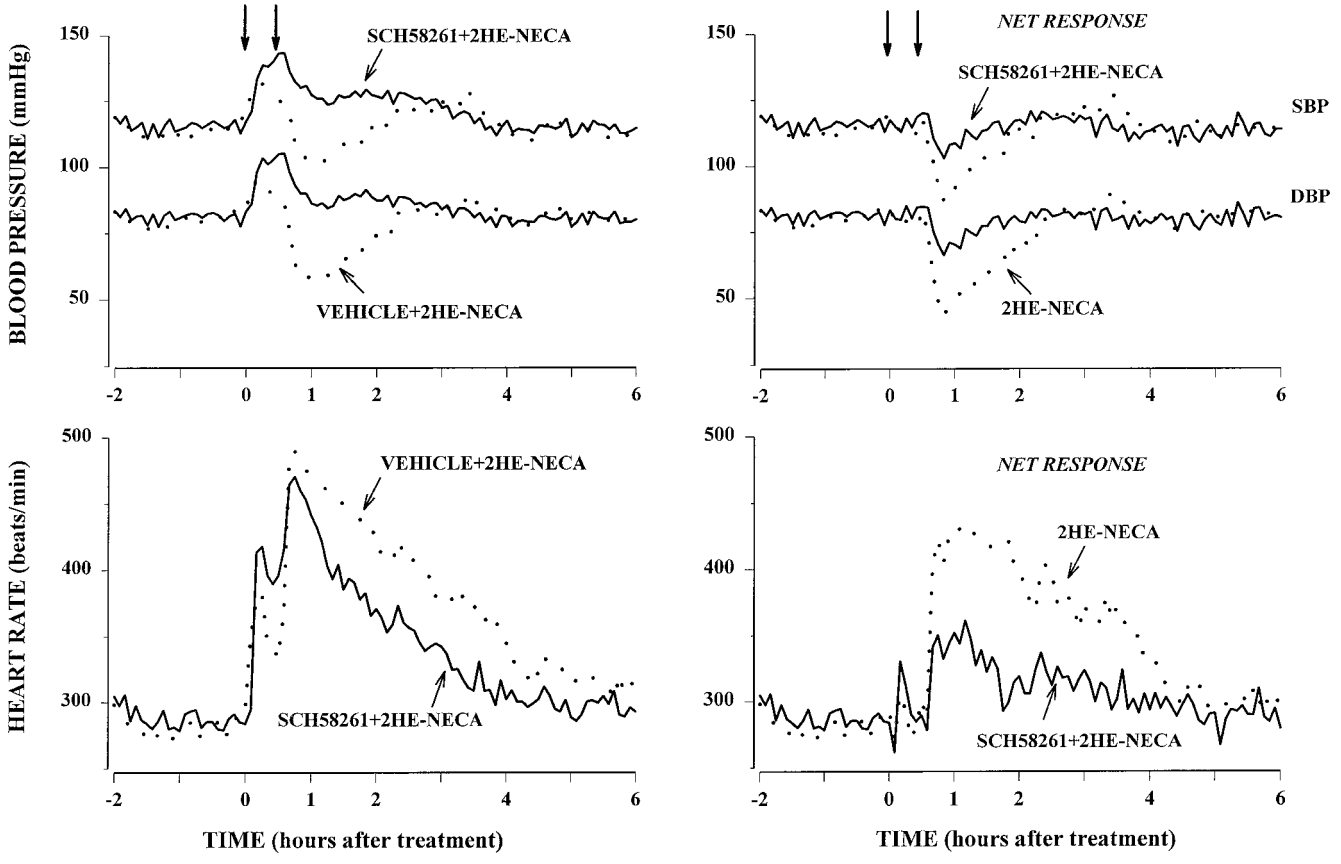

Fig. 2. Hemodynamic effects of 2HE-NECA (0.01 mg/kg i.p.) either in the absence or in the presence of pretreatment with SCH 58261 (10 mg/kg i.p.) in conscious normotensive rats. Each profile is the mean of 10 rats. Hypotension and reflex tachycardia induced by 2HE-NECA are expressed as net effects after subtraction of changes induced by vehicle (dotted line, right panels). SCH 58261 markedly inhibited the cardiovascular effects of 2HE-NECA (left panels). This response was also evident after subtraction of the cardiovascular changes produced by SCH 58261 (solid lines, right panels). The rats received two treatments: SCH 58261 or vehicle (first arrow), and after 30 min, 2HE-NECA (second arrow).

Hg. This effect was accompanied by reflex tachycardia, which reached 478 ± 5 beats/min (fig. 2). Pretreatment with SCH 58261 (10 mg/kg i.p.) prevented the effects of 2HE-NECA on DBP and slightly affected HR (peak effects were 90 ± 4 mm Hg and 443 ± 13 beats/min, for DBP and HR, respectively; fig. 2). Since SCH 58261 exerts hemodynamic effects *per se*, we calculated the net effect and found that both inhibition of 2HE-NECA action on DBP and reflex tachycardia are reduced significantly ($P < .05$; fig. 2 and table 2). The A_1 selective agonist CCPA (0.03 mg/kg i.p.) decreased DBP and HR up to 41 ± 6 mm Hg and 172 ± 3 beats/min, respectively. Pretreatment with SCH 58261 did not induce significant changes on the response to CCPA (peak effects were 49 ± 3 mm Hg and 181 ± 7 beats/min on DBP and HR, respectively). The net effect, after subtraction of the hemodynamic changes produced by SCH 58261 *per se*, confirmed that CCPA-induced bradycardia was not affected (table 2).

Effects of SCH 58261 after adrenergic blockade. As expected, pretreatment with propranolol (30 mg/kg i.p.) and phentolamine (10 mg/kg i.p.) to normotensive rats reduced BP and HR (fig. 3). In the group treated with SCH 58261 (10 mg/kg i.p.) we observed an increase in BP and HR (peak effects were +18 ± 3 mm Hg on DBP, +21 ± 3 mm Hg on SBP and +80 ± 16 beats/min on HR). These hemodynamic effects were abolished by adrenergic blockade (fig. 3). The response was also confirmed by subtracting the hemodynamic changes induced by the *alpha* and *beta* adrenergic blocking agents (fig 3, bottom panels). In fact, peak effects of SCH 58261 were −5 ± 3 mm Hg on DBP, −5 ± 3 mm Hg on SBP and −12 ± 11 beats/min on HR after adrenergic blockade ($P < .01$).

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TABLE 2
Hemodynamic activity of selective adenosine receptor agonists either in the absence or in the presence of pretreatment with SCH 58261 (SCH; 10 mg/kg i.p.)[a]

Adenosine agonist	Dose	DBP		HR	
		Without SCH	With SCH	Without SCH	With SCH
	mg/kg i.p.	*mm Hg*		*beats/min*	
2HE-NECA (A_{2A} selective)	0.01	−32 ± 2	−11 ± 4*	+140 ± 9	+48 ± 20*
CCPA (A_1 selective)	0.03	−52 ± 5	−54 ± 3	−167 ± 15	−223 ± 22

[a] Data are means ± S.E. of peak effects obtained after subtraction of the hemodynamic changes induced by SCH 58261 or vehicle (n = 10).
* $P < .05$ *vs.* rats not receiving SCH 58261.

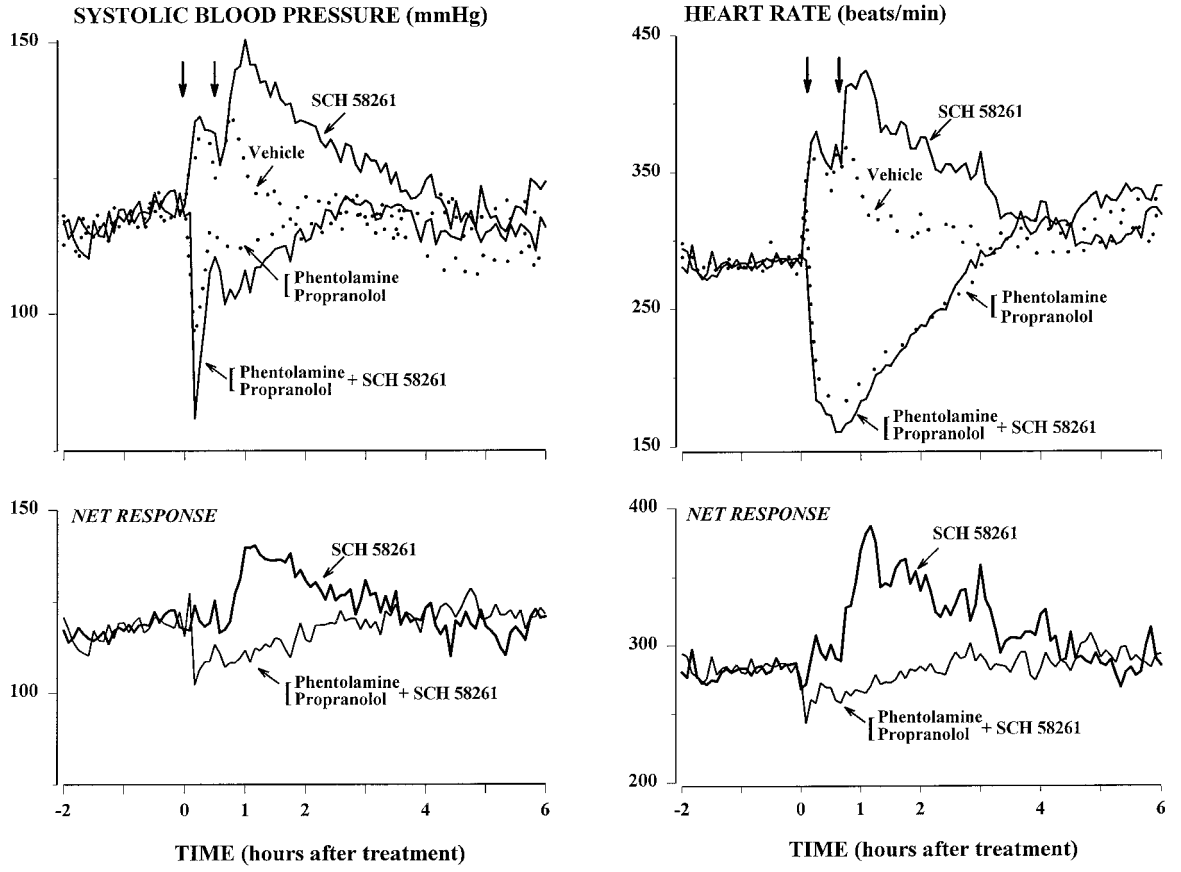

Fig. 3. Hemodynamic effects of SCH 58261 (10 mg/kg i.p.) and propranolol + phentolamine (30 + 10 mg/kg i.p.) in conscious normotensive rats. Both treatments were given either alone or in combination. Each profile is the mean of 10 rats. Pressor and chronotropic effects of SCH 58261 are expressed as net effects after subtraction of changes induced by vehicle (bottom panels). Note that adrenergic blockade completely abolished the hemodynamic changes produced by SCH 58261 (bottom panels). The rats received two treatments: propranolol + phentolamine or vehicle (first arrow), and after 30 min, SCH 58261 or vehicle (second arrow).

Discussion

This study shows that the new non-xanthine A_{2A} adenosine receptor antagonist, SCH 58261, retains its receptor selectivity after *in vivo* administration. The compound is able to block BP and HR changes induced by the A_{2A} receptor agonist, 2HE-NECA, but does not affect the responses evoked by the A_1 receptor agonist, CCPA. Moreover, SCH 58261 alone has been found to increase both BP and HR at a dose which showed A_{2A} receptor antagonist activity. This effect is prevented by adrenergic blockade, which indicates a possible modulatory role for adenosine A_{2A} receptors on sympathetic outflow.

In previous studies we demonstrated that 2HE-NECA is a potent A_{2A} adenosine receptor agonist (Monopoli *et al.*, 1994a), whereas CCPA is a selective agonist on A_1 receptors (Monopoli *et al.*, 1994b). We found that in conscious SHRs, 2HE-NECA given intraperitoneally causes a dose-dependent decrease in BP and is 15-fold more potent than the reference A_{2A} receptor agonist, CGS 21680 (Casati *et al.*, 1995). The hypotensive response is short-lasting and, as expected, is accompanied by reflex tachycardia. Moreover, an increase in plasma renin activity was observed (Sala *et al.*, 1996). On the other hand, CCPA produces dose-dependent decreases in both BP and HR (Casati *et al.*, 1995). In the present study, both adenosine receptor agonists were administered at doses inducing submaximal hemodynamic effects, based on preliminary experiments carried out in normotensive rats (data not shown). Against these effects, we evaluated the antagonist

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properties of SCH 58261. The compound was effective in antagonizing the A_{2A} agonist-induced fall in BP and the reflex increase in HR, whereas no inhibition was shown on A_1-mediated responses. The selectivity of SCH 58261 for A_{2A} *vs*. A_1 receptors previously had been reported to be 50- to 100-fold in binding studies using membrane homogenates (Zocchi *et al.*, 1996a) or 750-fold in autoradiography studies in rat brain (Fredholm *et al.*, 1998). Thus, in agreement with *in vitro* studies, SCH 58261 retains its A_{2A} receptor selectivity under *in vivo* conditions. Consistent with these data, in a separate study we also have found that SCH 58261 inhibits hypotension induced by CGS 21680 in the anesthetized rabbit, whereas it does not affect N^6-cyclopentyladenosine-induced bradycardia (Monopoli *et al.*, 1996).

Other compounds have been claimed to be selective A_{2A} adenosine receptor antagonists *in vivo*. The triazoloquinazoline CGS 15943 originally was described as a potent A_{2A} receptor antagonist (Williams *et al.*, 1987). However, the drug subsequently has been found to interact potently with A_1 receptors as well as to be active on A_{2B} receptors (Zocchi *et al.*, 1996a) and A_3 receptors (Kim *et al*., 1996). Moreover, in some *in vitro* assays involving A_{2A}-mediated responses, CGS 15943 does not show antagonist properties (Dionisotti *et al.*, 1994).

Although CGS 15943 often has been used as a reference A_{2A} antagonist, it is now clear that to understand the biology of A_{2A} receptors it is necessary to rely on the more selective compounds which recently have been described (Ongini and Fredholm, 1996). One such selective A_{2A} antagonist is the non-xanthine heterocycle ZM 241385 (Poucher *et al.*, 1995). This compound has been reported to have high affinity (in the low nanomolar range) at A_{2A} receptors with a selectivity of 400- to 1000-fold for A_{2A} *vs*. A_1 receptors and low affinity for A_3 receptors. However, as for A_{2B} receptors, ZM 241385 has been found to have a rather low A_{2A} *vs*. A_{2B} selectivity (30- to 80-fold) in the guinea pig aorta model (Poucher *et al.*, 1995), a finding confirmed in Chinese hamster ovary cells expressing the human A_{2B} receptor (Fredholm *et al*., personal communication). *In vivo* studies indicate that ZM 241385 blocks hypotension, but not bradycardia, induced by adenosine (Keddie *et al.*, 1996; Poucher *et al.*, 1996). The compound also was effective after intraduodenal administration in anesthetized dogs and cats, in which it induces a rapid and prolonged attenuation of the vasodilating responses to adenosine (Poucher *et al.*, 1996). However, all these studies were conducted with adenosine as a stimulating agent which has no receptor selectivity and is metabolized rapidly, whereas there are no studies available which use selective adenosine receptor agonists.

Another compound of interest is the 8-styrylxanthine KF 17837, which is relatively A_{2A}-selective *in vitro* (Nonaka *et al.*, 1994) and retains A_{2A} antagonist properties in the anesthetized rat (Jackson *et al.*, 1993). Thus, hemodynamic changes induced by the A_{2A} receptor agonist, CGS 21680, are blocked by KF 17837, but it does not affect bradycardia and BP reduction caused by the selective A_1 receptor agonist, N^6-cyclopentyladenosine (Jackson *et al.*, 1993). However, its marked affinity for A_{2A} receptors was not observed in other studies conducted in rat and bovine brain (Jacobson *et al.*, 1993; Dionisotti *et al.*, 1994). Moreover, KF 17837 failed to show antagonist properties in bovine coronary arteries and in rabbit platelets, which are functional models specific for A_{2A}-mediated responses (Dionisotti *et al.*, 1994).

In the present study, SCH 58261 administration in both normotensive and hypertensive rats resulted in a transient raise in BP and HR. These responses were evident in SHRs already at the dose of 3 mg/kg i.p., which suggests a greater sensitivity of this strain to hemodynamic changes possibly because of the higher level of sympathetic activity. Our findings agree with the recent data on A_{2A} receptor knockout mice, in which the lack of functional A_{2A} receptors leads to high arterial pressure levels and abolishes the hemodynamic responses to the selective A_{2A} receptor agonist CGS 21680 (Ledent *et al.*, 1997). The fact that either selective blockade of the A_{2A} receptor or the absence of this receptor subtype induces cardiovascular effects, gives further evidence for the physiological role of adenosine in the control of BP occurring through A_{2A} receptors. The hemodynamic changes induced by SCH 58261 at 10 mg/kg i.p. in normotensive rats were prevented completely by giving the adrenergic blocking agents propranolol and phentolamine, which indicates an interplay between SCH 58261 and the sympathetic nervous system. The question of how A_{2A} receptor inhibition can result in stimulation of sympathetic outflow is still to be investigated. There is evidence that adenosine exerts a key neuromodulatory role in the NTS-mediated mechanisms of baroreflex control of BP (Barraco *et al.*, 1991). Cardiovascular and neuronal responses to adenosine injected into the rat subpostremal NTS have mimicked the effects of baroreceptor activation (Tao and Abdel Rahman, 1993). The presence of A_{2A} receptors in the rat NTS was demonstrated at first by autoradiography with [^{3}H-NECA] (Bisserbe *et al.*, 1985), and more recently, it was characterized in binding studies with the selective A_{2A} agonist radioligand CGS 21680 (Barraco *et al.*, 1995). Moreover, microinjections of CGS 21680 in the NTS elicit cardiovascular depressor responses which are blocked by pretreatment with CGS 15943. Altogether these findings support the notion that presynaptic A_{2A} receptors in the NTS are located predominantly on baroreflex afferent terminals. The mechanisms underlying the cardiovascular responses mediated by adenosine in the NTS involve the release of different neurotransmitters such as glutamate, norepinephrine, acetylcholine, 5-HT *via* selective activation of A_{2A} receptors. This release is evoked with low nanomolar concentrations of CGS 21680 and is blocked by CGS 15943, CSC, but not by DPCPX. (Barraco *et al.*, 1995, 1996; Mayfield *et al.*, 1993). Based on our present findings, we can hypothesize that selective blockade of A_{2A} receptors by SCH 58261 would have an inhibitory effect on NTS. The inhibition of NTS activity could lead to excitatory hemodynamic responses which are prevented by *alpha* and *beta* blockers. However, this study *per se* can not confirm or reject this hypothesis for SCH 58261.

In previous studies, we found that SCH 58261 induces behavioral stimulating action in conscious rats (Bertorelli *et al.*, 1996). Like caffeine, the compound has been reported to increase wakefulness. This central effect also may be responsible for the general state of arousal, which would also account for the sympathetic activation. However, whether sympathetic activation is mediated reflexly or whether it is induced directly through central nervous system stimulation, the results of the present study suggest that endogenous adenosine released under normal physiological conditions

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may exert a tonic regulation on sympathetic outflow through the A_{2A} receptor activation.

It has been reported that the natural methylxanthines, caffeine and theophylline, exert marked actions on the cardiovascular system through complex mechanisms (Fredholm, 1984). Their effects on BP depend on both dose and route of administration. Intravenous injection of large doses induces an initial fall in BP, followed by a secondary rise. After oral administration, the net effect is a moderate increase in BP, which most likely involves the activation of the sympathoadrenal system. In fact, this response is not present in reserpine-treated animals. However, the major problem in defining the mechanims which underlie the cardiovascular effects induced by xanthines, is their lack of selectivity for adenosine receptor subtypes and their significant phosphodiesterase inhibitory activity. Because tolerance to the cardiovascular effects of caffeine develops rapidly (Robertson *et al.*, 1981), it would be interesting to investigate whether the effects of SCH 58261 alone undergo tolerance after a repeated-dose regimen. It would also be of interest to determine whether other A_{2A} receptor antagonists, such as KF 17837 or ZM 241385, produce similar cardiovascular responses in conscious animals.

In conclusion, the present study shows that SCH 58261 is a selective A_{2A} receptor antagonist *in vivo*. Moreover, the finding that blockade of A_{2A} receptors by SCH 58261 induces pressor effects further supports the notion that endogenous adenosine can exert a tonic regulation on sympathetic outflow through A_{2A} receptors. Although much still needs to be investigated to elucidate the biological mechanisms underlying its effects, SCH 58261 can be regarded as a reliable pharmacological tool for use in further elucidating the function of A_{2A} receptors in the cardiovascular actions of adenosine.

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