

Tachycardia Caused by A_{2A} Adenosine Receptor Agonists is Mediated by Direct Sympathoexcitation in Awake Rats

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Running Title: Sympathoexcitation by A_{2A} adenosine receptor agonist

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# of Text pages	27
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List of nonstandard abbreviations:

Regadenoson/ CVT-3146: (adenosine, 2-[4-(methylamino)carbonyl]-1*H*-pyrazol-1-yl])

ZM 241385: (4-(2-[7-Amino-2-(2-furyl)-[1,2,4]-triazolo-[2,3-*a*]-[1,3,5]-triazin-5-ylamino]ethyl)phenol)

CGS 21860: 2-*p*-(2-carboxyethyl)phenethylamino-5'-*N*-ethylcarboxamidoadenosine

NECA: 5'-*N*-Ethylcarboxamido-adenosine

SNS: Sympathetic Nervous System

SNP: Sodium Nitroprusside

MAP: Mean Arterial Pressure

Recommended section: Cardiovascular

Abstract

Adenosine-induced tachycardia is suggested to be mediated via A_{2A} receptors however the exact mechanism for this effect remains to be understood. The present study was carried out using regadenoson, a selective A_{2A} adenosine receptor agonist, to determine the role of the A_{2A} receptor subtype in adenosine-induced tachycardia. Regadenoson (0.3 μ g/kg-50 μ g/kg) given as a rapid iv bolus to awake rats caused a dose-dependent increase in heart rate (HR). Mean arterial pressure (MAP) increased at lower doses while at higher doses there was a decrease in MAP. The increase in HR was evident at the lowest dose (0.3 μ g/kg) of regadenoson at which there was no appreciable decrease in MAP. Pretreatment with ZM241385 (30 μ g/kg), an A_{2A} receptor antagonist, attenuated the decrease in MAP and the increase in HR caused by Regadenoson. Pretreatment with metoprolol (1 mg/kg), a beta-blocker, attenuated the increase in HR, but had no effect on the hypotension caused by regadenoson. In the presence of hexamethonium (10 mg/kg), a ganglionic blocker, the tachycardia was completely prevented even though MAP was further reduced. Regadenoson treatment (10 μ g/kg) significantly ($p < 0.05$) increased plasma norepinephrine levels almost 2-fold above baseline. The dissociation of HR and MAP effects by dose, time and pharmacological interventions provides evidence that tachycardia caused by regadenoson is independent of the decrease in MAP and may not entirely be baroreflex mediated, suggesting that regadenoson may cause a direct stimulation of the sympathetic nervous system (SNS) via activation of A_{2A} adenosine receptors.

Introduction

Adenosine elicits a wide variety of cardiovascular effects that are mediated by four distinct subtypes (A_1 , A_{2A} , A_{2B} and A_3) of G-protein coupled receptors (Belardinelli et al., 1989; Fredholm et al., 2001; Dhalla et al., 2003). Coronary vasodilation caused by adenosine, which is primarily mediated by the A_{2A} receptor subtype, led to the use of this nucleoside in myocardial perfusion imaging studies with radionuclide agents (Verani, 1991; Iskandrian et al., 1994). Myocardial perfusion imaging using adenosine or A_{2A} receptor agonists, as coronary vasodilators, is accompanied by a significant sinus tachycardia (Johnston et al., 1995; Udelson et al., 2004; Hendel et al., 2005). The adenosine receptor subtype responsible for the tachycardia has been proposed to be the A_{2A} receptor (Koos et al., 1993; Alberti et al., 1997), however this remains to be conclusively established, as well as the mechanism of the acceleration of the heart rate.

A_{2A} adenosine receptor agonists cause vasodilation in most vascular beds by a direct action on A_{2A} receptors on endothelial and vascular smooth muscle cells (Belardinelli et al., 1998; Hein et al., 1999). The peripheral vasodilation causes a decrease in arterial pressure, which in turn elicits a baroreflex-mediated activation of the sympathetic nervous system (SNS) and thereby an increase in heart rate. This has been proposed to be the mechanism underlying the increase in HR caused by adenosine and A_{2A} receptor agonists (Nekooeian and Tabrizchi, 1996; Alberti et al., 1997). However, it has been shown that adenosine elicits a direct positive chronotropic effect (Koos et al., 1993), and that activation of A_{2A} receptors leads to direct stimulation of the autonomic nervous system (Koos and Chau, 1998). In this regard, adenosine

has also been shown to cause stimulation of carotid body chemoreceptors (McQueen and Ribeiro, 1981; Biaggioni et al., 1987). The adenosine receptor subtype responsible for chemoreceptor activation by adenosine and stable analogs has been proposed to be the A_2 receptor (McQueen and Ribeiro, 1986).

To further establish the role of A_{2A} receptors and the mechanism of the tachycardia caused by adenosine and A_{2A} receptor agonists we used regadenoson (also known as CVT-3146), a novel A_{2A} adenosine receptor agonist (Gao et al., 2001). Regadenoson has been shown to be selective for the A_{2A} adenosine receptors versus the A_1 , A_{2B} and A_3 receptor subtypes in radioligand binding and functional studies (Gao et al., 2001). This A_{2A} receptor agonist is currently being developed as a pharmacological “stress” agent (coronary vasodilator) for myocardial perfusion imaging using radionuclides (Hendel et al., 2005). Clinical data show that rapid iv injections of regadenoson cause a transient (2-5 min) near-maximal increase in coronary blood flow velocity which is accompanied by a sinus tachycardia of approximately 20 ± 2 bpm (Kerensky et al., 2002). A pharmacokinetic study of regadenoson in healthy volunteers revealed that regadenoson causes a dose (concentration)-dependent sinus tachycardia with minimal or no changes in systolic and diastolic arterial blood pressure (Cannon et al., 2003). This finding is similar to the observations reported in laboratory animals with adenosine (Koos et al., 1993) and more recently in humans using another selective A_{2A} receptor agonist, binodenoson (Barrett et al., 2005). Altogether, these observations suggest that the A_{2A} adenosine receptor mediated sinus tachycardia may not entirely be due to a baroreceptor reflex triggered by a decrease in arterial pressure. Thus, the present study was undertaken to determine whether regadenoson induced tachycardia is solely baroreflex mediated or also due to a direct activation of the SNS.

Material and Methods

Chemicals: Regadenoson (adenosine, 2-[4-(methylamino)carbonyl]-1*H*-pyrazol-1-yl]) was synthesized by the Department of Bio-Organic Chemistry of CV Therapeutics, Inc., and was dissolved in saline. Metoprolol, hexamethonium and sodium nitroprusside were purchased from Sigma (St. Louis, MO) and were dissolved in saline. ZM 241385 (4-(2-[7-Amino-2-(2-furyl)-[1,2,4]-triazolo-[2,3-*a*]-[1,3,5]-triazin-5-ylamino]ethyl)phenol) was purchased from Tocris (Ellisville, MO) and was dissolved in 5% DMSO in deionized water.

Animals: Male Sprague-Dawley rats weighing 300 ± 50 gms with two indwelling cannulas (carotid artery and jugular vein) were purchased from Charles River (Wilmington, MA). Animals were maintained on a 12 hr light and dark cycle and received standard laboratory rat chow and water *ad libitum*. All experimental procedures were performed under the protocol approved by the IACUC (CV Therapeutics, Inc.) and in accordance with the recommendations set forth in the Guide for the Care and Use of Laboratory Animals published by the National Research Council.

Experimental Protocol: All experiments were carried out in unrestrained awake rats. On the day of the experiment (at least one week after the intravascular cannulation), the animal was weighed and placed in a small cage to acclimatize to the environment for at least one hour prior to the commencement of the experiment. Food was withheld during the experiment but water was given *ad libitum*. Baseline measurements were recorded for at least 30 minutes before starting treatment. Rats were given intravenous injections of regadenoson, up to 2 doses each, of

0.3, 1.0, 3, 10, 30, and 50 $\mu\text{g}/\text{kg}$ at 30-minute intervals or longer (at the higher doses) to ensure that the parameters being measured returned to baseline levels before the next dose was given. Sodium nitroprusside (SNP) was given as an iv bolus at 0.1, 3 and 10 $\mu\text{g}/\text{kg}$ doses. In experiments with ZM241385, A_{2A} adenosine receptor antagonist, metoprolol (β_1 -adrenoceptor antagonist) or hexamethonium (ganglionic blocker); these agents were given as an iv bolus to the rats 10 minutes prior to regadenoson. The doses used for these agents were derived from the literature (Poucher et al., 1996) and/or were tested by giving repeated injections of the blocking agents to determine the dose that ensured complete blockade of the response consistent with the action of each agent (data not shown). All test compounds were injected via the jugular vein catheter.

Measurement of Cardiovascular Responses: All measurements were done in unrestrained awake animals. Heart rate and arterial pressure were monitored continuously throughout the experiment. The arterial catheter was connected to a pressure transducer (MLT0698, AD Instruments) and transducer signal conditioner (Model 13-6615-50, Gould Instrument Systems, Inc. Valley View, OH) to monitor arterial pressure. Arterial pressure (diastolic and systolic) and heart rate (HR) were recorded using a Gould PONEMAH's system (Gould Instrument Systems, Inc.). Mean arterial pressure (MAP) was calculated as (mmHg) as: $2/3$ diastolic + $1/3$ systolic blood pressure.

Measurements of Norepinephrine Levels: A separate group of animals were used for measurement of norepinephrine levels. Animals were weighed and placed in metabolic cages with both the arterial and venous catheters attached to infusion sets (Terumo, needles 21G x $3/4$ "

3 ½” tubing, volume 0.15ml). Regadenoson was injected into the venous site and blood samples were drawn from the arterial site. Blood was withdrawn using 1 ml syringe with 25G needle, and placed in a plasma separator tube (on ice) containing EDTA (Microtainer brand). The samples were centrifuged at 8000 rpm (4°C) for 5 minutes, and plasma were stored in a microtube (on ice) containing a 10 µl aliquot of EDTA-Glutathion (Sigma) preservative. Plasma samples were then transferred to -80°C until analyzed. Plasma norepinephrine concentrations were determined by HPLC using the electrochemical detection method as described in detail elsewhere (Boudreau et al., 1993).

Data analysis: Data are presented as percent change \pm SEM from baseline. Baseline values are presented in the results section and/or figure legends. Statistical comparisons among various treatment groups were carried out using two way ANOVA followed by Bonferroni’s post hoc test. Differences between two groups were determined by t-test. Differences among mean values were considered significant when the p value was less than 0.05.

Results

Effect of Regadenoson on Heart Rate and Mean Arterial Pressure: In awake rats the mean baseline value for HR was 345 ± 26 bpm. As illustrated in figure 1 (panel A) regadenoson given as an iv bolus (1, 3, 10, 30, and 50 $\mu\text{g}/\text{kg}$) caused a dose- and time-dependent increase in HR. The increase in HR caused by regadenoson was clearly detectable at 1 minute, although small ($\sim 10\%$, $p < 0.001$), at a dose as low as 1 $\mu\text{g}/\text{kg}$. At higher doses, the increases in HR were in the range of 15-30% above basal ($p < 0.001$). As to the time-course of the changes in HR caused by regadenoson, the increases in HR, independent of the doses, were evident at as early as 1 minute after the iv bolus. At lower doses (1 $\mu\text{g}/\text{kg}$) of regadenoson, the HR returned to baseline within 2.5 minutes whereas at higher doses not only the tachycardia lasted longer (15-30 min) but the magnitude of the effect was also greater (figure 1, panel A).

In awake rats, the mean baseline value for mean arterial pressure (MAP) was 106 ± 4 mmHg. Regadenoson caused dose- and time-dependent changes in MAP (figure 1, panel B). Regadenoson caused a small, not significant, increase in MAP at a dose of 0.3 (figure 2, panel A) and 1 $\mu\text{g}/\text{kg}$ (figure 1, panel B) whereas at higher doses there was a significant ($p < 0.001$) decrease in MAP ($\sim 20\%$ decrease at 50 $\mu\text{g}/\text{kg}$) which was noted as early as 1 minute after the injection. MAP returned to baseline within 5 minutes at lower doses ($< 10 \mu\text{g}/\text{kg}$) of regadenoson whereas at higher doses (30 and 50 $\mu\text{g}/\text{kg}$) MAP remained slightly below baseline up to 30 minutes post-injection. A similar pattern was observed in the effect of regadenoson on systolic and diastolic pressure (data not shown).

Summarized in panel C of figure 1 are the maximal changes (% of control) in MAP and HR caused by various doses of regadenoson. There was no significant change in MAP at lower doses (0.3-3 $\mu\text{g}/\text{kg}$). A significant decrease in MAP was observed only at 10 $\mu\text{g}/\text{kg}$ and higher doses. However, increases in HR were observed at doses of 1 and 3 $\mu\text{g}/\text{kg}$, when there were no appreciable decreases in MAP. The maximal increase in HR was achieved at the 3 $\mu\text{g}/\text{kg}$ dose of regadenoson whereas the maximal decrease in MAP was observed at the highest regadenoson dose studied, 50 $\mu\text{g}/\text{kg}$. It is important to note that the maximal increase in HR was observed at the 3 $\mu\text{g}/\text{kg}$ dose of regadenoson at which there was very small decrease in MAP. Thereafter no further increase in HR was observed with increasing doses of regadenoson even though the peak response in MAP continued to grow.

Temporal Relationship between Changes in Heart Rate and Mean Arterial Pressure: Figure 2 shows the time course of changes in HR and MAP caused by three different doses (0.3, 3 and 50 $\mu\text{g}/\text{kg}$) of regadenoson. At the 0.3 $\mu\text{g}/\text{kg}$ dose (figure 2, panel A) there was a small but significant ($p < 0.05$) increase ($11 \pm 2\%$) in HR at 1 minute while there was no significant change in MAP. HR returned to baseline values within 10 minutes of the regadenoson injection. At 3 $\mu\text{g}/\text{kg}$ dose (figure 2, panel B) there was greater than 20% increase in HR above baseline ($p < 0.01$) while there was no appreciable decrease in MAP. At this dose HR returned to baseline values within 15 minutes after regadenoson injection. At the 50 $\mu\text{g}/\text{kg}$ dose (figure 2, panel C) there was a significant decrease ($18 \pm 2\%$, $p < 0.01$) in MAP however the maximal HR response was not very different from the 3 $\mu\text{g}/\text{kg}$ dose. At the 50 $\mu\text{g}/\text{kg}$ dose of regadenoson HR remained elevated for 30 minutes even though the MAP returned to near normal levels within 5 minutes.

Pharmacological Interventions: In order to determine whether the A_{2A} receptor subtype was responsible for mediating the tachycardia and hypotension caused by regadenoson, the animals were pretreated, 10 minutes prior to administration of regadenoson, with ZM241385 (ZM), an A_{2A} receptor antagonist (figure 3). ZM itself had no significant effect on HR or MAP. Regadenoson (3 $\mu\text{g}/\text{kg}$) caused a $16 \pm 1\%$ ($p < 0.01$) increase in HR and a $5 \pm 3\%$ decrease in MAP. Pretreatment with ZM completely prevented the decrease in MAP and significantly ($p < 0.01$) attenuated the increase in HR (to $6 \pm 1\%$ above baseline) caused by regadenoson.

To investigate the role of the SNS in the tachycardia induced by regadenoson, animals were pretreated with the beta-blocker metoprolol (MET) at a dose of 1 mg/kg (figure 4). Treatment with MET caused a small decrease (5% below baseline) in HR but had no effect on MAP. Regadenoson (3 $\mu\text{g}/\text{kg}$) caused a $16 \pm 1\%$ ($p < 0.01$) increase in HR which was significantly blunted after pretreatment with MET ($-1 \pm 3\%$) (figure 4, panel A). MET treatment had no effect on the hypotension caused by regadenoson (figure 4, panel B)

The ganglionic blocker hexamethonium was used to further establish the role of the SNS in the tachycardia induced by regadenoson (figure 5). Treatment with hexamethonium (HEX) alone caused a significant reduction (40%) in MAP which recovered somewhat within 5 minutes. HEX had no significant effect on HR. Regadenoson (10 $\mu\text{g}/\text{kg}$) caused a significant ($p < 0.01$) increase ($27 \pm 3\%$) in HR which was completely prevented by HEX (figure 5, panel A). Regadenoson caused a significant ($p < 0.01$) decrease ($11 \pm 2\%$) in MAP. Pretreatment with HEX resulted in a greater decrease (49%) in MAP by regadenoson (figure 5, panel B).

Comparison with Sodium Nitroprusside: The effects of regadenoson on HR and MAP were compared with that of the vasodilator sodium nitroprusside (SNP). SNP is a non-selective vasodilator which causes hypotension mediated by nitric oxide that results in baroreflex mediated increases in HR. SNP caused a dose-dependent decrease in MAP which was accompanied by a proportional increase in HR (figure 6, panel A). Similar to SNP, regadenoson decreased MAP in a dose-dependent manner, but unlike SNP the associated increase in HR was not proportional to the magnitude of decrease in MAP (figure 6, panel B). This is further illustrated in panel C of figure 6 which shows the relationship between changes in HR and MAP by SNP and regadenoson. A linear relationship between MAP and HR changes was observed for SNP but not with regadenoson. The increase in HR caused by regadenoson was independent of the decrease in MAP. The maximal increase (99 bpm) in HR was observed at a very small decrease (1.2 mmHg) in MAP. Thereafter, there were no significant increases in HR despite that a greater decrease in MAP occurred.

Effect of Regadenoson on Plasma Catecholamine Levels: Regadenoson given as an iv bolus at a dose of 10 $\mu\text{g}/\text{kg}$ dose caused a significant ($p < 0.05$) increase in norepinephrine (NE) plasma levels (figure 7). The baseline NE levels were 146 ± 11 pg/ml, which increased to a maximum of 269 ± 22 pg/ml. The peak increase in NE concentration effect was observed between 1-5 minutes after the injection of regadenoson, and NE levels returned to baseline by 60 min post injection of regadenoson.

Discussion

The cardiovascular effects of adenosine have been known for over 60 years. However, only when adenosine was given intravenously to conscious humans that it became evident that this nucleoside could produce sympathetic activation (Biaggioni et al., 1987). Interestingly, most animal studies failed to show sympathoexcitation by systemically-administered adenosine. However, studies showed that adenosine could stimulate isolated arterial chemoreceptors (McQueen and Ribeiro, 1981) and studies using selective agonists suggested that this effect was mediated by A_{2A} receptors (McQueen and Ribeiro, 1983; McQueen and Ribeiro, 1986). In animals as well as humans, adenosine and its analogs cause sinus tachycardia and lower systemic blood pressure (Biaggioni et al., 1987; Lappe et al., 1992; Bonizzoni et al., 1995; Alberti et al., 1997; Koos and Chau, 1998; Barrett et al., 2005; Hendel et al., 2005; Schindler et al., 2005). The sinus tachycardia has been attributed to the baroreceptor reflex caused by the decrease in arterial pressure (Webb et al., 1990; Webb et al., 1991; Nekooeian and Tabrizchi, 1996; Alberti et al., 1997). However, there is also evidence that adenosine-mediated tachycardia can occur independent of a decrease in MAP (Lappe et al., 1992; Mathot et al., 1995; Koos and Chau, 1998; Schindler et al., 2005). Thus, the purpose of this study was to establish the role of A_{2A} adenosine receptors and the mechanism underlying the sinus tachycardia caused by the adenosine analog regadenoson. Regadenoson is highly selective for the A_{2A} receptor subtype, has a rapid onset and short duration of action, is readily reversible and causes sinus tachycardia in humans (Gao et al., 2001; Trochu et al., 2003; Hendel et al., 2005). The important and novel finding of the study is that sinus tachycardia mediated by the A_{2A} receptor subtype, is in great part due to a direct activation of the SNS, and not baroreflex mediated.

A_{2A} Receptor Mediated Responses: The effects of regadenoson on MAP and HR described in the present study are consistent with those reported previously using known selective A_{2A} agonists (Webb et al., 1990; Koos et al., 1993; Mathot et al., 1995; Nekooeian and Tabrizchi, 1996; Alberti et al., 1997; Schindler et al., 2005). Hence, decrease in MAP and tachycardia are commonly observed pharmacological effects of A_{2A} agonists. The sinus tachycardia and lowering of MAP caused by regadenoson were completely prevented by pre-treatment with a selective A_{2A} receptor antagonist, ZM241385, confirming that these effects of regadenoson are mediated by the A_{2A} adenosine receptor subtype. Thus, the results of the present study support previous evidence for the role of A_{2A} receptors in mediating adenosine-induced hypotension and tachycardia (Webb, et al., 1991; Koos et al., 1993; Bonizzoni et al., 1995; Nekooeian and Tabrizchi, 1996; Schindler et al., 2005).

Role of Sympathetic Stimulation in Regadenoson-induced Tachycardia: Pretreatment with metoprolol completely abolished the HR response without any change in the effect of regadenoson on MAP indicating that the tachycardia caused by regadenoson is mediated by an increase in sympathetic tone. Similarly, pretreatment with hexamethonium, caused a greater fall in MAP by regadenoson but the increase in HR caused was completely prevented. This finding also suggests that activation of the SNS plays a major role in the tachycardia induced by regadenoson. Furthermore, regadenoson caused almost a 2-fold increase in circulating plasma norepinephrine levels which is indicative of sympathoexcitation. Previously it has been shown that NECA, a non-selective agonist of adenosine receptors, caused increase in plasma norepinephrine levels (Laborit et al., 1990). Similarly, adenosine has been shown to increase

plasma catecholamine levels in sheep (Koos et al., 1993), an effect that was suggested to be mediated by A_{2A} receptors. However, these data do not discriminate between direct and indirect activation of the SNS.

Regadenoson-induced Tachycardia is Independent of Decrease in MAP: Comparison of the dose-response and time-course of the effects of regadenoson on HR and MAP revealed that regadenoson can increase HR independent of decreasing MAP. In support of this conclusion, the 0.3 and 3.0 $\mu\text{g}/\text{kg}$ doses of regadenoson significantly increased HR but caused no decrease in MAP. At the higher doses of regadenoson (30 and 50 $\mu\text{g}/\text{kg}$) which cause significant hypotension, MAP returns to baseline while marked tachycardia was still present. The tachycardia outlasted the lowering of MAP by at least 20-30 minutes. These data indicate that a baroreflex mediated increase in HR may not be the sole mechanism for the tachycardia observed with regadenoson. In fact, this lack of association between changes in HR and MAP has been previously reported for other A_{2A} agonists (Lappe et al., 1992; Koos and Chau, 1998; Schindler et al., 2005). In fetal sheep, CGS 21680 caused a significant increase in HR (61%) without an effect on MAP (Koos and Chau, 1998). Lappe et al., 1992 reported that systemic administration of the A_{2A} agonist DMPA caused sinus tachycardia without a significant change in MAP in awake rats. A recent study shows that peripheral administration of CGS 21860 resulted in both hypotension and tachycardia whereas central administration (lateral ventricle) caused tachycardia without causing hypotension suggesting that the hypotensive effect of this A_{2A} agonist is mediated by peripheral receptors, whereas the tachycardia is mediated by centrally located A_{2A} receptors (Schindler et al., 2005). Altogether these observations suggest that the tachycardia caused by A_{2A} agonists is not solely due to a reflex response caused by their hypotensive effect.

Although the tachycardia and hypotension caused by regadenoson are mediated by the same receptor subtype (A_{2A}), differences in receptor reserve (and coupling efficiency) for these two effects could be responsible for the differential sensitivity and duration of the effect. This however remains to be investigated.

A comparison between cardiovascular responses of regadenoson to that of a pure vasodilator sodium nitroprusside (SNP) provides additional evidence that tachycardia caused by regadenoson is not simply caused by reflex. SNP is a direct vasodilator which causes relaxation of vascular smooth muscle by nitric oxide and cGMP formation (Ignarro and Kadowitz, 1985). SNP caused a dose-dependent decrease in MAP which was accompanied by a proportional increase in HR caused by baroreflex-mediated mechanism to keep arterial pressure from falling. However, the decrease in MAP caused by regadenoson was associated with a much greater response in HR, and compared to SNP appears to be disproportionate to the magnitude of decrease in MAP. This is more evident in figure 6 (panel B) where changes in HR are plotted against changes in MAP for both agents. While a linear relationship exists between MAP and HR changes caused by SNP that was not the case for regadenoson indicating lack of dependence of the increase in HR on MAP changes. This analysis provides evidence that the increase in HR caused by regadenoson may not be solely be due to its vasodilatory effects, as is the case for the pure vasodilator SNP.

Mechanism of Sympathetic Stimulation: The mechanism of direct sympathoexcitation caused by A_{2A} agonists remains to be established. Adenosine reportedly activates a variety of afferent nerve terminals (myocardial, renal and skeletal muscle) leading to sympathetic activation

(Katholi et al., 1984; Cox et al., 1989; Costa et al., 2001). In humans, activation of arterial chemoreceptors is particularly prominent during intravenous administration of adenosine which can lead to increase in ventilation, sympathetic activation with a modest increase in systolic pressure, and increase in HR (Biaggioni et al., 1987; Engelstein et al., 1994). The receptor subtype that mediates adenosine mediated activation of arterial chemoreceptors is suggested to be A_{2A} (McQueen and Ribeiro, 1983; McQueen and Ribeiro, 1986; Koos et al., 1993). In contrast, adenosine induced tachycardia in fetal sheep mediated by A_{2A} receptors was proposed to be due to direct activation of the SNS independent of the peripheral arterial chemoreceptors (Koos et al., 1993). Thus, further studies are needed to elucidate the mechanism of A_{2A} receptor mediated sympathoexcitation.

In conclusion, the results from the present study show that regadenoson causes dose- and time-dependent decreases in MAP and tachycardia in awake rats mediated by A_{2A} adenosine receptors. The dissociation of the effects of regadenoson on HR and MAP by dose, time-course, pharmacological interventions and in comparison with SNP provides evidence that tachycardia the tachycardia observed with regadenoson in this study could not be explained simply by a baroreflex-mediated phenomenon due to the vasodilatory effects of regadenoson. We propose, therefore, that it was due, in part, to a direct stimulation of arterial chemoreceptors, as suggested by previous studies (McQueen and Ribeiro, 1986; Biaggioni et al., 1987; Engelstein et al., 1994). A limitation of the study is that we have not conclusively proven our hypothesis by directly measuring afferent sympathetic nerve traffic arising from arterial chemoreceptors, or studied the effects of regadenoson in chemodenervated animals.

A potential clinical implication of the findings of the present study is that sympathoexcitation caused by A_{2A} agonists should contribute to the diagnostic value of these agents for coronary artery disease in myocardial perfusion imaging studies, as they not only increase coronary vasodilation but also increase heart rate to mimic exercise, a physiological stressor.

References

- Alberti C, Monopoli A, Casati C, Forlani A, Sala C, Nador B, Ongini E, and Morganti A (1997) Mechanism and pressor relevance of the short-term cardiovascular and renin excitatory actions of the selective A_{2A} adenosine receptor agonists. *J Cardiovasc Pharmacol* **30**:320-324.
- Barrett RJ, Lamson MJ, Johnson J, and Smith WB (2005) Pharmacokinetics and safety of binodenoson after intravenous dose escalation in healthy volunteers. *J Nucl Cardiol* **12**:166-171.
- Belardinelli L, Linden J, and Berne RM (1989) The cardiac effects of adenosine. *Prog Cardiovasc Dis* **32**:73-97.
- Belardinelli L, Shryock JC, Snowdy S, Zhang Y, Monopoli A, Lozza G, Ongini E, Olsson RA, and Dennis DM (1998) The A_{2A} adenosine receptor mediates coronary vasodilation. *J Pharmacol Exp Ther* **284**:1066-1073.
- Biaggioni I, Killian TJ, Mosqueda-Garcia R, Robertson RM, and Robertson D (1991) Adenosine increases sympathetic nerve traffic in humans. *Circulation* **83**:1668-1675.
- Biaggioni I, Olafsson B, Robertson RM, Hollister AS, and Robertson D (1987) Cardiovascular and respiratory effects of adenosine in conscious man. Evidence for chemoreceptor activation. *Circ Res* **61**:779-786.

Bonizzoni E, Milani S, Ongini E, Casati C, and Monopoli A (1995) Modeling hemodynamic profiles by telemetry in the rat: A Study with A₁ and A_{2a} adenosine agonists. *Hypertension* **25**:564-569.

Boudreau G, Peronnet F, De Champlain J, and Nadeau R (1993) Presynaptic effects of epinephrine on norepinephrine release from cardiac sympathetic nerves in dogs. *Am J Physiol* **265**:H205-H211.

Cannon RO, Freestone S, Mair SJ, Skettino SL, Belardinelli L, Blackburn BK, and Wolff AA (2003) A randomized, placebo-controlled, crossover, ascending dose tolerance and pharmacokinetic study of CVT-3146: a novel agent for potential use in myocardial perfusion imaging. *Clin Pharmacol Ther* **73**, 31. Ref Type: Abstract

Costa F, Diedrich A, Johnson B, Sular P, Farley G, and Biaggioni I (2001) Adenosine, a metabolic trigger of the exercise pressor reflex in humans. *Hypertension* **37**:917-922.

Cox DA, Vita JA, Treasure CB, Fish RD, Selwyn AP, and Ganz P (1989) Reflex increase in blood pressure during the intracoronary administration of adenosine in man. *J Clin Invest* **84**:592-596.

Dhalla AK, Shryock JC, Shreeniwas R, and Belardinelli L (2003) Pharmacology and therapeutic applications of A₁ adenosine receptor ligands. *Curr Top Med Chem* **3**:369-385.

Engelstein ED, Lerman BB, Somers VK, and Rea RF (1994) Role of arterial chemoreceptors in mediating the effects of endogenous adenosine on sympathetic nerve activity. *Circulation* **90**:2919-2926.

Fredholm BB, IJerman AP, Jacobson KA, Klotz KN, and Linden J (2001) International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev* **53**:527-552.

Gao Z, Li Z, Baker SP, Lasley RD, Meyer S, Elzein E, Palle V, Zablocki JA, Blackburn B, and Belardinelli L (2001) Novel short-acting A_{2A} adenosine receptor agonists for coronary vasodilation: inverse relationship between affinity and duration of action of A_{2A} agonists. *J Pharmacol Exp Ther* **298**:209-218.

Hein TW, Belardinelli L, and Kuo L (1999) Adenosine A_{2A} receptors mediate coronary microvascular dilation to adenosine: role of nitric oxide and ATP-sensitive potassium channels. *J Pharmacol Exp Ther* **291**:655-664.

Hendel RC, Bateman TM, Cerqueira MD, Iskandrian AS, Leppo JA, Blackburn BK, and Mahmarian JJ (2005) Initial clinical experience with regadenoson, a novel selective A_{2A} agonist for pharmacologic stress SPECT myocardial perfusion imaging. *J Am Coll Cardiol*
In press 2005.

Ignarro LJ and Kadowitz PJ (1985) The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. *Annu Rev Pharmacol Toxicol* **25**:171-191.

Iskandrian AS, Verani MS, and Heo J (1994) Pharmacologic stress testing: mechanism of action, hemodynamic responses, and results in detection of coronary artery disease. *J Nucl Cardiol* **1**:94-111.

Johnston DL, Daley JR, Hodge DO, Hopfenspirger MR, and Gibbons RJ (1995) Hemodynamic responses and adverse effects associated with adenosine and dipyridamole pharmacologic stress testing: a comparison in 2,000 patients. *Mayo Clin Proc* **70**:331-336.

Katholi RE, Whitlow PL, Hageman GR, and Woods WT (1984) Intrarenal adenosine produces hypertension by activating the SNS via the renal nerves in the dog. *J Hyperten* **2**:349-359.

Kerensky RA, von Mering GO, Kent KM, Cannon RO, and Belardinelli L (2002) Dose dependent increase in human coronary blood flow velocity following an IV bolus of CVT-3146, a novel A_{2A} adenosine receptor agonist: a potential agent for the use in pharmacological stress testing for myocardial perfusion imaging. *Circulation* 106 (suppl II), 618. Ref Type: Abstract

Koos BJ and Chau A (1998) Fetal cardiovascular and breathing responses to an adenosine A_{2a} receptor agonist in sheep. *Am J Physiol* **274**:R152-R159.

Koos BJ, Mason BA, and Duksay CA (1993) Cardiovascular responses to adenosine in fetal sheep: autonomic blockade. *Am J Physiol* **264**:H526-H532.

Laborit H, Manzo-Fay G, Baron C, and Hasni H (1990) Changes in plasma catecholamine levels after the intraperitoneal and intracerebroventricular administration of adenosine analogues and of clonidine in conscious rats. *Res Commun Chem Pathol Pharmacol* **68**:307-327.

Lappe RW, Sheldon JH, and Cox BF (1992) Selective adenosine-2 agonist produces both direct and reflex tachycardia in normotensive rats. *J Cardiovasc Pharm* **19**:460-463.

Mathot RA, Cleton A, Soudijn W, IJzerman AP, and Danhof M (1995) Pharmacokinetic modelling of the haemodynamic effects of the A_{2a} adenosine receptor agonist CGS 21680C in conscious normotensive rats. *Br J Pharmacol* **114**:761-768.

McQueen DS and Ribeiro JA (1981) Effect of adenosine on carotid chemoreceptor activity in the cat. *Br J Pharmacol* **74**:129-136.

McQueen DS and Ribeiro JA (1983) On the specificity and type of receptor involved in carotid body chemoreceptor activation by adenosine in the cat. *Br J Pharmacol* **80**:347-354.

McQueen DS and Ribeiro JA (1986) Pharmacological characterization of the receptor involved in chemoexcitation induced by adenosine. *Br J Pharmacol* **88**:615-620.

Nekooeian AA and Tabrizchi R (1996) Effects of adenosine A_{2A} receptor agonist, CGS 21680, on blood pressure, cardiac index and arterial conductance in anaesthetized rats. *Eur J Pharmacol* **307**:163-169.

Poucher SM, Keddie JR, Brooks R, Shaw GR, and McKillop D (1996) Pharmacodynamics of ZM 241385, a potent A_{2a} adenosine receptor antagonist, after enteric administration in rat, cat and dog. *J Pharm Pharmacol* **48**:601-606.

Schindler CW, Karcz-Kubicha M, Thorndike EB, Muller CE, Tella SR, Ferre S, and Goldberg SR (2005) Role of central and peripheral adenosine receptors in the cardiovascular responses to intraperitoneal injections of adenosine A₁ and A_{2A} subtype receptor agonists. *Br J Pharmacol* **144**:642-650.

Schindler CW, Karcz-Kubicha M, Thorndike EB, Muller CE, Tella SR, Goldberg SR, and Ferre S (2004) Lack of adenosine A₁ and dopamine D₂ receptor-mediated modulation of the cardiovascular effects of the adenosine A_{2A} receptor agonist CGS 21680. *Eur J Pharmacol* **484**:269-275.

Trochu JN, Zhao G, Post H, Xu X, Belardinelli L, Belloni FL, and Hintze TH (2003) Selective A_{2A} adenosine receptor agonist as a coronary vasodilator in conscious dogs: potential for use in myocardial perfusion imaging. *J Cardiovasc Pharmacol* **41**:132-139.

Udelson JE, Heller GV, Wackers FJ, Chai A, Hinchman D, Coleman PS, Dilsizian V, DiCarli M, Hachamovitch R, Johnson JR, Barrett RJ, and Gibbons RJ (2004) Randomized, controlled dose-ranging study of the selective adenosine A_{2A} receptor agonist binodenoson for pharmacological stress as an adjunct to myocardial perfusion imaging. *Circulation* **109**:457-464.

Verani MS (1991) Pharmacological stress with adenosine for myocardial perfusion imaging. *Semin Nucl Med* **21**:266-272.

Webb RL, Barclay BW, and Graybill SC (1991) Cardiovascular effects of adenosine A₂ agonists in the conscious spontaneously hypertensive rat: a comparative study of three structurally distinct ligands. *J Pharmacol Exp Ther* **259**:1203-1212.

Webb RL, McNeal RB, Barclay BW, and Yasay GD (1990) Hemodynamic effects of adenosine agonists in the conscious spontaneously hypertensive rat. *J Pharmacol Exp Ther* **254**:1090-1099.

Figure Legends

Figure 1: Time course of the effect of increasing doses of regadenoson on (A) mean arterial pressure (MAP) and (B) heart rate (HR) in awake rats. Regadenoson was administered as an iv bolus (1-50 $\mu\text{g}/\text{kg}$). Data are presented as percent change from pre-treatment values. The mean baseline value for HR was 345 ± 26 bpm and 106 ± 4 mmHg for MAP. Panel C shows the maximal (peak effect) change (% of control) in MAP and HR at various doses of regadenoson. Values are mean \pm SE from 4 to 7 rats. *) $p < 0.01$ significantly different from the pretreatment value.

Figure 2: Temporal relationship between the changes (% of control) in MAP and HR caused by regadenoson in awake rats. Regadenoson at doses of 0.3 (A), 3.0 (B) and 50 (C) $\mu\text{g}/\text{kg}$ was administered via a single iv bolus. The mean baseline values for HR was 330 ± 18 , 342 ± 14 and 378 ± 12 bpm and 106 ± 3 , 103 ± 2 and 108 ± 6 mmHg for MAP respectively. Values are mean \pm SEM of single measurements from 5 to 6 rats. *) $p < 0.05$, **) $p < 0.01$ significantly different from the pretreatment value.

Figure 3: Effect of regadenoson (3 $\mu\text{g}/\text{kg}$) on the heart rate (A) and mean arterial pressure (B) of awake rats in the absence and presence of the A_{2A} receptor antagonist ZM241385 (ZM) administered via an iv bolus at the dose of 30 $\mu\text{g}/\text{kg}$. ZM 241385 was given 10 minutes prior to the injection of regadenoson. The mean baseline values for HR in the regadenoson alone and ZM plus regadenoson groups were 377 ± 13 and 369 ± 18 bpm, respectively. The mean baseline values for MAP in the regadenoson alone and ZM plus regadenoson groups were 108 ± 3 and

106 ± 4 mmHg respectively. Values are mean ± SEM of single measurements from 4 to 6 rats.

*) p<0.01 significantly different from the regadenoson alone group.

Figure 4: Effect of regadenoson (3 µg/kg) on the heart rate (A) and mean arterial pressure (B) of awake rats in the absence and presence of a β-adrenergic blocker Metoprolol (MET), at a dose of 1 mg/kg. Metoprolol was given 10 minutes prior to regadenoson. The mean baseline values for HR in the regadenoson alone and MET plus regadenoson groups were 377 ± 13 and 364 ± 9 bpm, respectively. The mean baseline values for MAP in the regadenoson alone and MET plus regadenoson groups were 108 ± 3 and 115 ± 4 mmHg, respectively. Values are mean ± SEM of single measurements from 5 to 6 rats. *) p<0.01 significantly different from the regadenoson alone group.

Figure 5: Effect of regadenoson (10 µg/kg) on the heart rate (A) and mean arterial pressure (B) of awake rats in the absence and presence of the ganglionic blocker hexamethonium (HEX) at a dose of 10 mg/kg. Hexamethonium was given 10 minutes prior to regadenoson. The mean baseline values for HR in the regadenoson alone and HEX plus regadenoson groups were 380 ± 7 and 399 ± 20 bpm, respectively. The mean baseline values for MAP in the regadenoson alone and HEX plus regadenoson groups were 114 ± 5 and 115 ± 4 mmHg, respectively. Values are mean ± SEM of single measurements from 3 to 6 rats. *) p<0.01 indicates values that are significantly different from the regadenoson alone group.

Figure 6: Comparison between the changes in HR and MAP in response to three different doses of the vasodilator (A) sodium nitroprusside (0.3, 10 and 30 $\mu\text{g}/\text{kg}$) and (B) regadenoson (3, 10 and 50 $\mu\text{g}/\text{kg}$) administered via a single iv bolus to awake rats. Values are mean \pm SE from n=3 to 6 in different groups. Panel C shows the relationship between the increases in heart rate caused by the decreases in MAP. Note that there is a linear relationship between changes in HR and MAP in the case of SNP but not for regadenoson.

Figure 7: Effect of regadenoson on plasma norepinephrine levels in awake rats. Regadenoson was given as a single iv bolus at a dose of 10 $\mu\text{g}/\text{kg}$. Values are mean \pm SEM of single measurements from 6 rats in each group. *) $p < 0.05$ significantly different from the saline group.

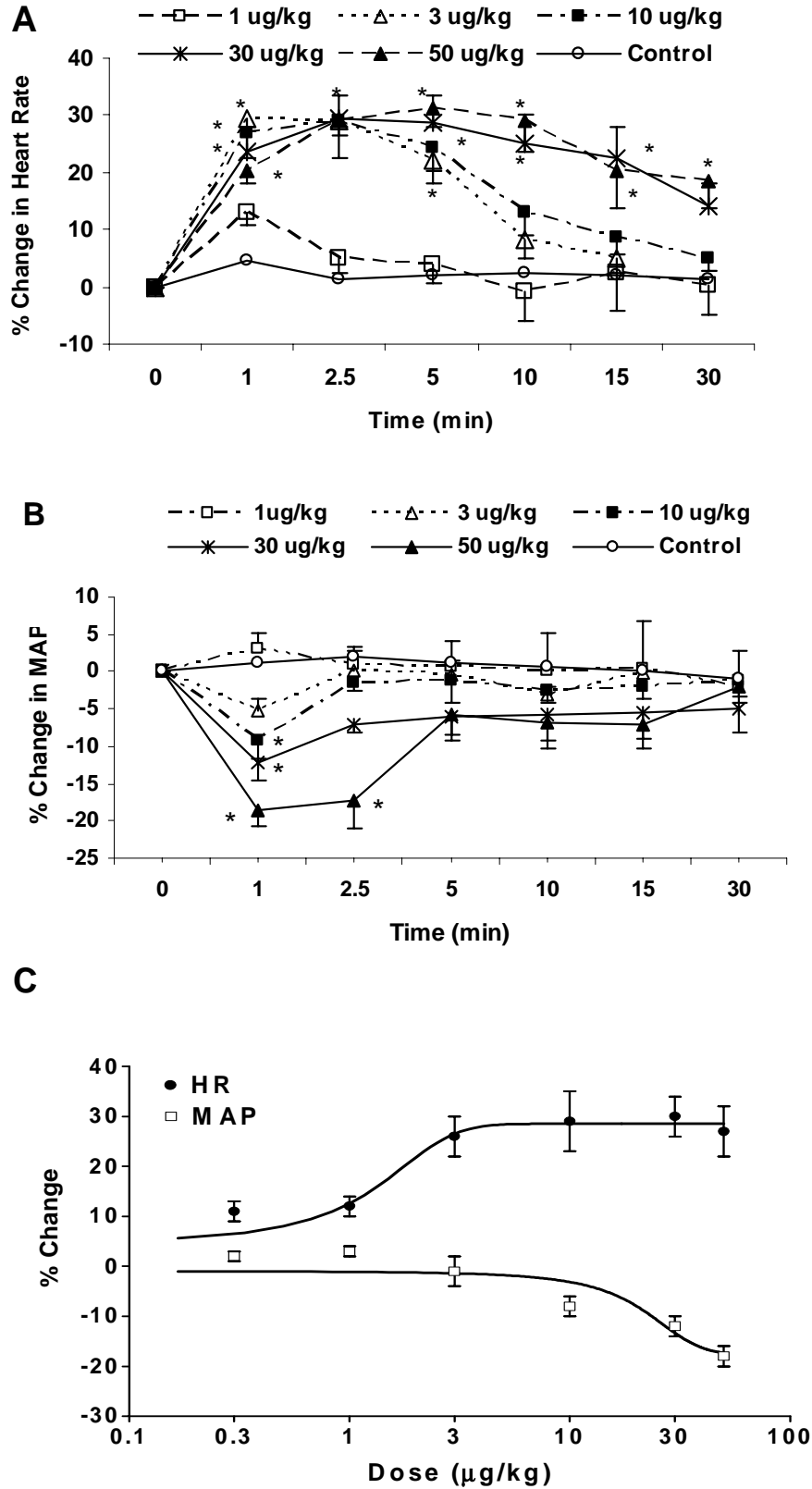


Figure 1

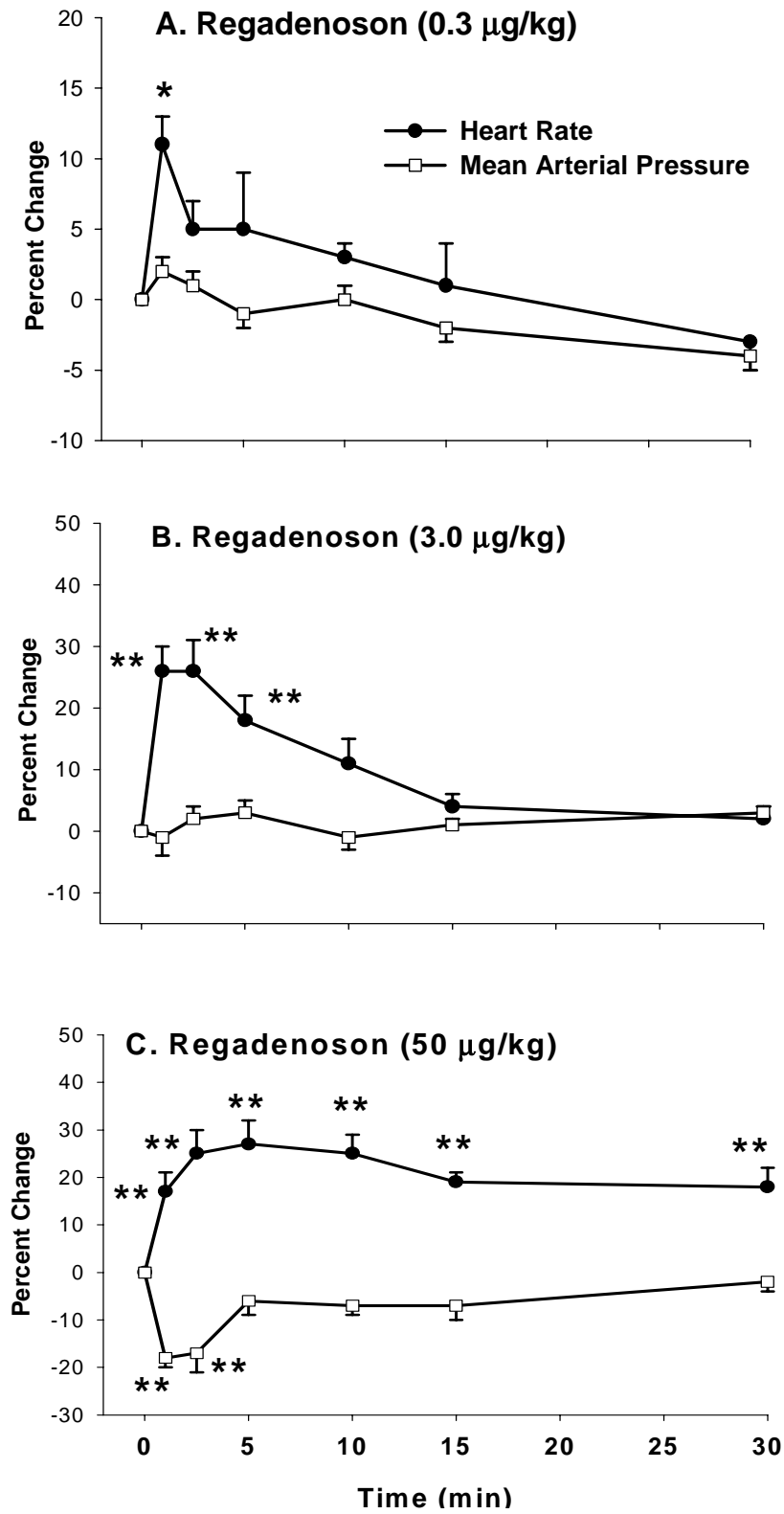


Figure 2

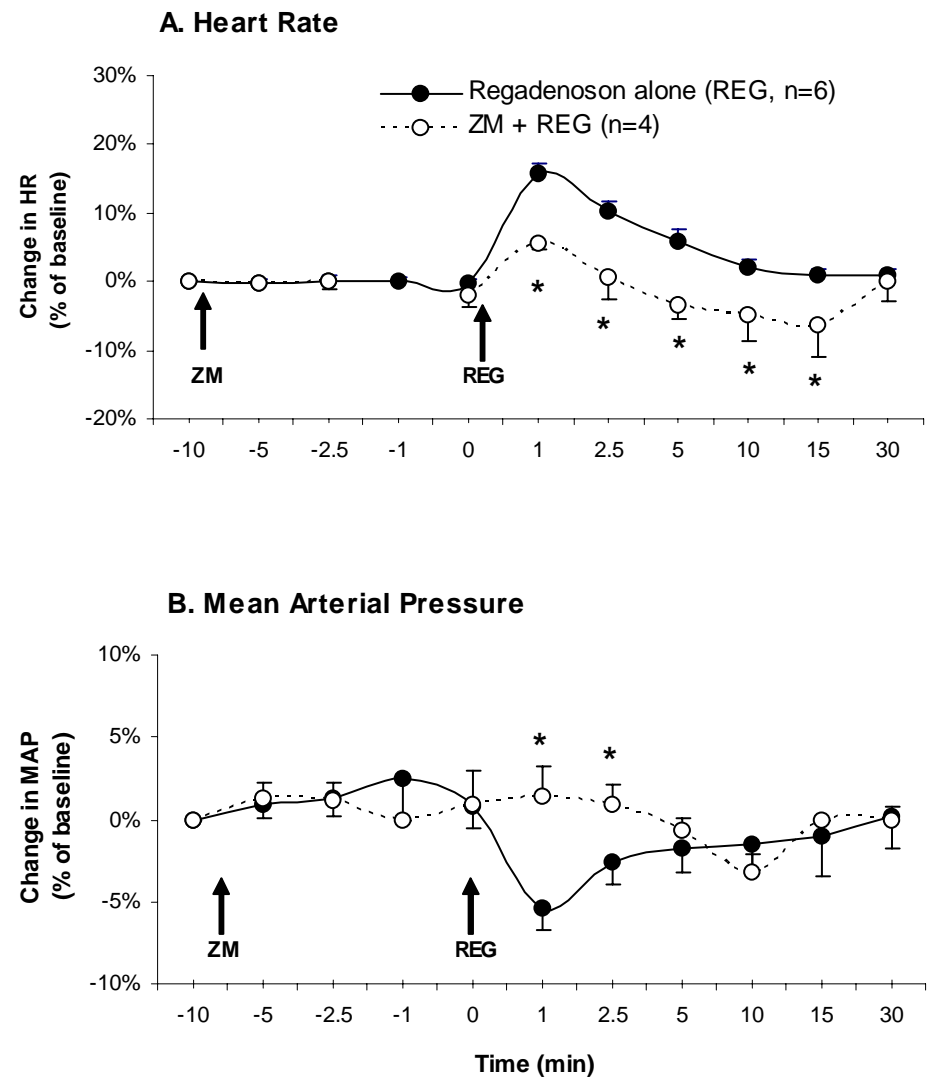


Figure 3

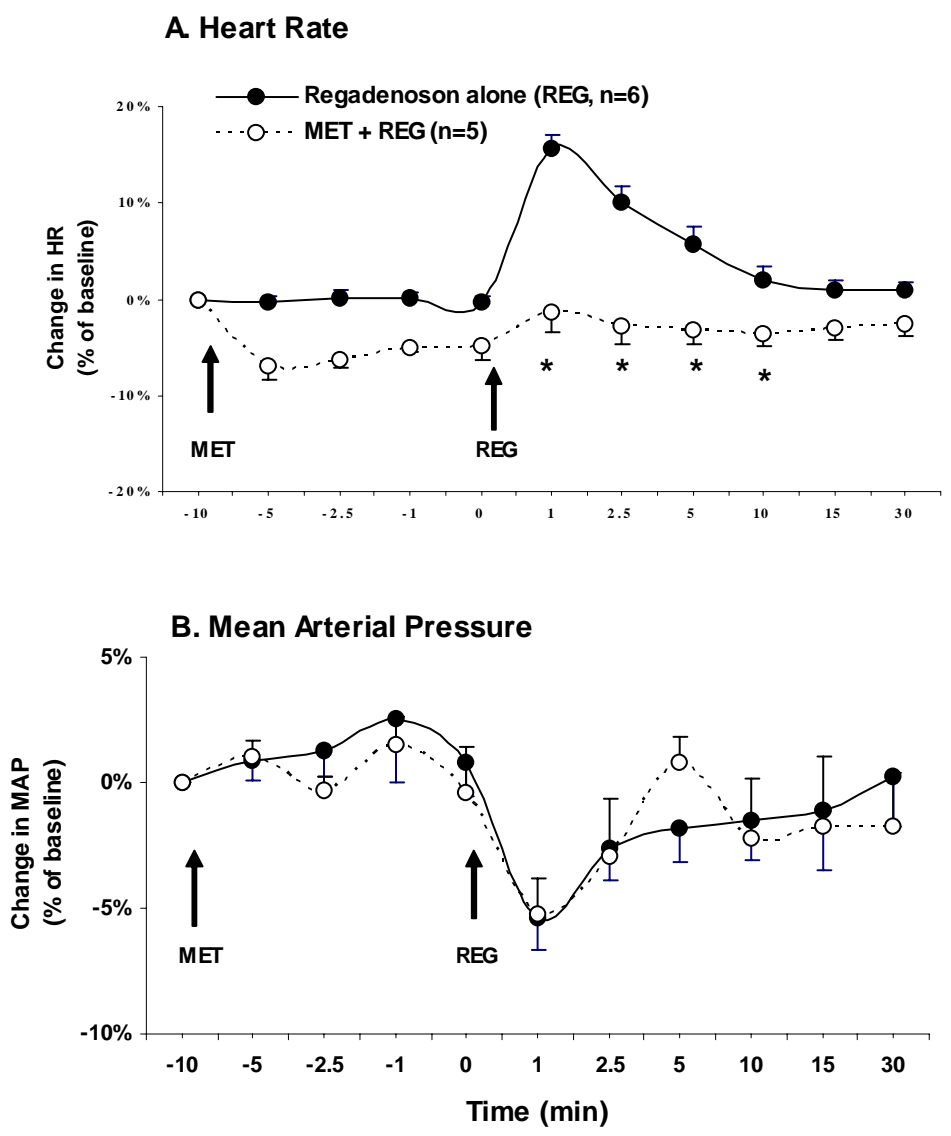


Figure 4

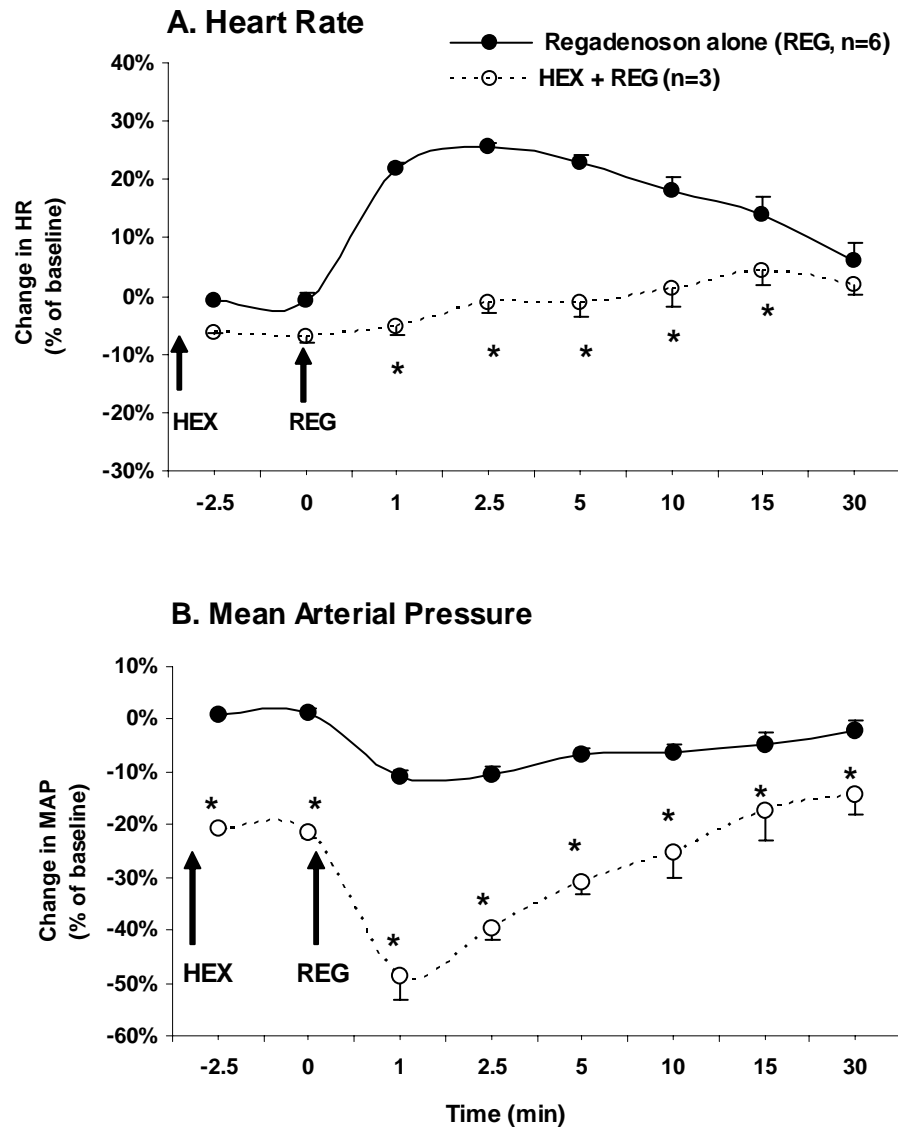


Figure 5

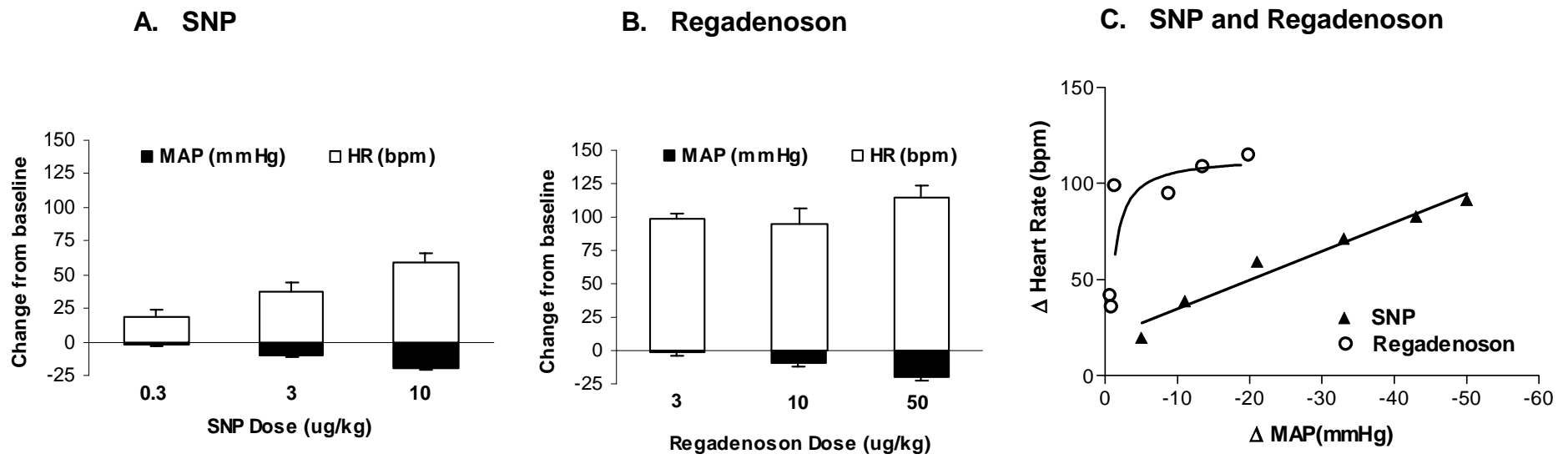


Figure 6

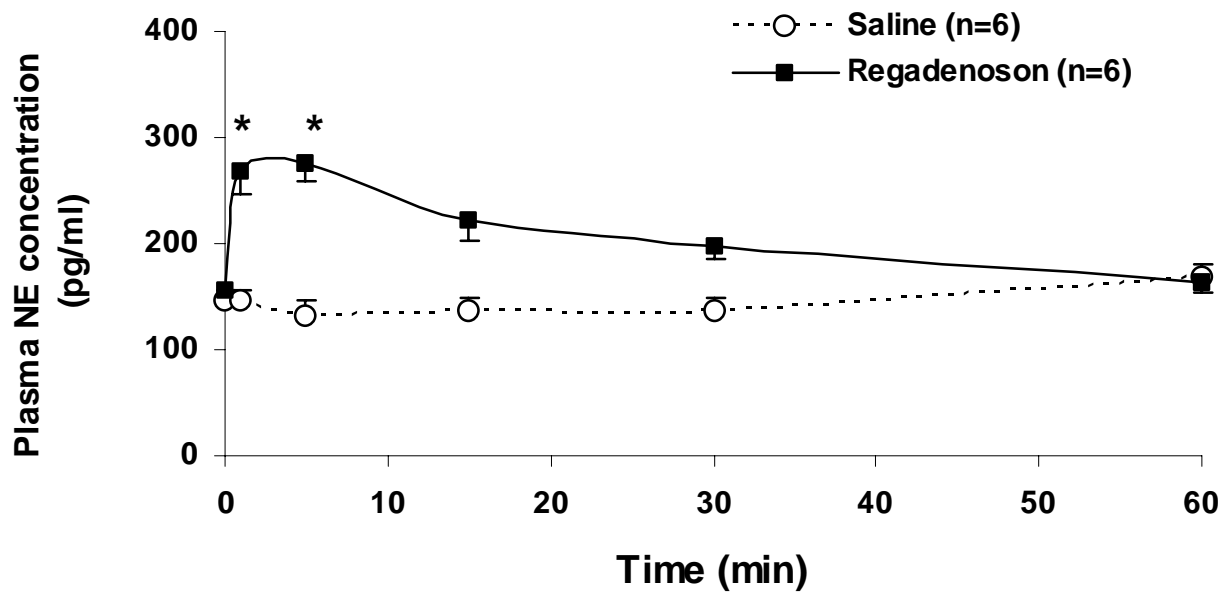


Figure 7