

## PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/25484>

Please be advised that this information was generated on 2017-12-05 and may be subject to change.

# Comparison of hemodynamic and sympathoneural responses to adenosine and lower body negative pressure in man

Gerard A. Rongen, Beverley L. Senn, Shin-ichi Ando, Catherine F. Notarius, James A. Stone, and John S. Floras

**Abstract:** Adenosine increases heart rate and sympathetic nerve activity reflexively in conscious humans through several mechanisms. The purpose of this study was to assess the relative contributions of arterial baroreceptor unloading, carotid chemoreceptor stimulation, and other adenosine-sensitive afferent nerves to these responses. In 12 healthy men, the effect on blood pressure, heart rate (HR), and muscle sympathetic nerve activity (MSNA; peroneal nerve) of lower body negative pressure (LBNP;  $-15$  mmHg (1 mmHg = 133.3 Pa)) was compared with the effect of intravenous adenosine (35, 70, and  $140 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ). In eight subjects, the highest dose was reinfused during 100% oxygen to suppress arterial chemoreceptors. Blood pressure reductions during LBNP and adenosine ( $140 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) were similar. HR did not change significantly during LBNP ( $+2 \pm 2$  beats/min; mean  $\pm$  SE) but increased at the highest adenosine dose ( $+25 \pm 3$  beats/min;  $p < 0.05$ ). MSNA increased significantly ( $p < 0.05$ ) during both interventions ( $+255 \pm 82$  and  $+247 \pm 58$  units/100 beats for adenosine and LBNP, respectively), and there was no difference in the MSNA response to these two stimuli ( $p > 0.1$ ). Oxygen inhibited adenosine-induced increases in HR and MSNA (from  $+305 \pm 99$  to  $+198 \pm 75$  units/100 beats and from  $+26 \pm 4$  to  $+18 \pm 3$  beats/min;  $p < 0.05$  for both comparisons). The MSNA response to these combined stimuli was similar to that observed during LBNP. In contrast, the residual HR response ( $+18 \pm 3$  beats/min) was significantly greater than the response to LBNP ( $+2 \pm 2$  beats/min;  $p < 0.05$ ). These data indicate that arterial baroreceptor unloading cannot account for the marked adenosine-induced increase in HR, but may be sufficient to explain its effect on MSNA. The effect of 100% oxygen confirms that stimulation of carotid chemoreceptors accounts for approximately one-third of the HR and MSNA response to adenosine. However, other mechanisms, such as stimulation of adenosine-sensitive afferent nerves in other vascular beds, are involved in the HR and possibly the MSNA response.

*Key words:* adenosine, human, heart rate, sympathetic nervous system, baroreflex.

**Résumé :** L'adénosine augmente la fréquence cardiaque et l'activité nerveuse sympathique de manière réflexe chez les humains conscients et ce, par l'intermédiaire de divers mécanismes. Le but de la présente étude a été d'évaluer l'influence sur ces réponses d'une décharge des barorécepteurs artériels, d'une stimulation des chémorécepteurs carotidiens, ainsi que d'autres nerfs afférents sensibles à l'adénosine. Chez 12 hommes sains, on a comparé l'effet d'une pression négative des membres inférieurs (PNMI;  $-15$  mmHg (1 mmHg = 133,3 Pa)) avec celui de l'adénosine intraveineuse (35, 70 et  $140 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) sur la pression artérielle, la fréquence cardiaque (FC) et l'activité nerveuse sympathique musculaire (ANSM; nerf péronnier). Chez huit sujets, on a réinjecté la plus forte dose d'adénosine en présence de 100% d'oxygène afin de supprimer les chémorécepteurs artériels. Les réductions de pression artérielle durant la PNMI et l'adénosine ( $140 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) ont été similaires. La fréquence cardiaque n'a pas changé significativement durant la PNMI ( $+2 \pm 2$  battements/min; moyenne  $\pm$  ÉT), mais elle a augmenté à la plus forte dose d'adénosine ( $+25 \pm 3$  battements/min;  $p < 0,05$ ). L'ANSM a augmenté significativement ( $p < 0,05$ ) durant les deux interventions ( $+225 \pm 82$  et  $247 \pm 58$  unités/100 battements pour l'adénosine et la PNMI, respectivement); de plus, il n'y a eu aucune différence dans la réponse de l'ANSM à ces deux stimuli ( $p > 0,1$ ). L'oxygène a inhibé les augmentations de FC et d'ANSM induites par l'adénosine (de  $+305 \pm 99$  à  $198 \pm 75$  unités/100 battements et de  $+26 \pm 4$  à  $+18 \pm 3$  battements/min;  $p < 0,05$  dans les deux cas). La réponse de l'ANSM à ces deux stimuli combinés a été similaire à celle observée durant la PNMI. À l'opposé, la réponse FC résiduelle ( $+18 \pm 3$  battements/min) a été significativement plus forte que la réponse à la PNMI ( $+2 \pm 2$  battements/min;  $p < 0,05$ ). Ces résultats indiquent que la décharge des barorécepteurs artériels ne peut justifier l'augmentation de FC marquée induite par l'adénosine, mais elle pourrait être suffisante pour expliquer son effet sur l'ANSM. L'effet obtenu en présence de 100% d'oxygène confirme que la stimulation des chémorécepteurs carotidiens contribue à environ un tiers de la réponse de l'ANSM et de la FC à l'adénosine. Toutefois, d'autres mécanismes, tels que la stimulation

Received June 25, 1996.

G.A. Rongen, B.L. Senn, S. Ando, C.F. Notarius, J.A. Stone, and J.S. Floras.<sup>1</sup> Divisions of Cardiology, Mount Sinai and Toronto Hospitals, and the Centre for Cardiovascular Research, University of Toronto, Toronto, ON M5G 1X5, Canada.

<sup>1</sup> Author for correspondence at the Division of Cardiology, Mount Sinai Hospital, Suite 1614, 600 University Avenue, Toronto, ON M5G 1X5, Canada.



des nerfs afférents sensibles à l'adénosine dans d'autres lits vasculaires, participent à la réponse de la fréquence cardiaque et possiblement de l'ANSM.

*Mots clés* : adénosine, humain, fréquence cardiaque, système nerveux sympathique, baroréflexe.  
[Traduit par la Rédaction]

## Introduction

The arterial vasodilator actions of adenosine (Smits et al. 1990) have stimulated its use in stress radiotracer imaging for the evaluation of patients presumed to suffer myocardial ischemia (Cerqueira et al. 1994). However, adenosine has, in addition, complex and often opposing effects on the cardiovascular and autonomic nervous systems (Rongen et al. 1997). Adenosine inhibits norepinephrine release from sympathetic nerve endings via a presynaptic mechanism (Kubo and Su 1983; Rongen et al. 1996), and has negative chronotropic, inotropic, and dromotropic effects on the heart (Belardinelli and Berne 1989). These properties have found application in the treatment of supraventricular tachycardias (DiMarco et al. 1983). These direct effects are mediated by adenosine receptors located on the effector cells.

In contrast with these inhibitory direct effects, intravenous adenosine infusion in conscious humans elicits a sympathoexcitation that is accompanied by tachycardia and hyperventilation (Fuller et al. 1987; Clarke et al. 1988; Maxwell et al. 1987; Watt et al. 1989). The mechanism or mechanisms responsible for these changes have not been completely elucidated. The purpose of the present study was to assess the relative contributions of arterial baroreceptor unloading, carotid chemoreceptor stimulation, and other adenosine-sensitive afferent nerves to these responses. Biaggioni et al. (1991a) observed less increase in muscle sympathetic nerve activity (MSNA) in response to intravenous sodium nitroprusside compared with adenosine when infused in doses causing similar reductions in diastolic blood pressure, suggesting that the marked increase in MSNA during adenosine infusion was due to mechanisms other than arterial baroreceptor unloading. However, at the time of these experiments it was not appreciated that sodium nitroprusside, as a nitric oxide donor, could inhibit efferent sympathetic outflow at brainstem or other sites (Sakuma et al. 1992; Bredt et al. 1990). Thus, the relative contribution of the arterial baroreflex to this adenosine-mediated sympathoexcitatory reflex remains uncertain.

Because the excitatory effects of exogenous adenosine can be blunted by breathing 100% oxygen (Biaggioni et al. 1987), activation of chemoreceptors in the carotid body was thought to be the principal stimulus to this excitatory reflex. However, the sympathoexcitatory effects of adenosine uptake inhibition are not abolished completely by 100% oxygen (Engelstein et al. 1994). Other adenosine-sensitive afferent nerves have been recently identified in the human forearm; these may also be present in the heart and kidney (Katholi et al. 1984; Biaggioni et al. 1987; Cox et al. 1989; Watt et al. 1987; Costa and Biaggioni 1993; Thames et al. 1993). When stimulated, these afferents increase efferent sympathetic nerve traffic to muscle, an action that likely contributes to these reflex excitatory responses to intravenous adenosine.

This investigation was performed to elucidate a possible contributory role for arterial baroreflex unloading in the

adenosine-induced activation of the sympathetic nervous system in conscious humans, using a nonpharmacological hypotensive stimulus for comparison. We administered adenosine doses that are used for clinical purposes, e.g., the adenosine-thallium stress test for cardiac ischemia (Cerqueira et al. 1994). The effects of intravenous adenosine were compared with responses to lower body negative pressure (LBNP), a maneuver that initially deactivates cardiopulmonary baroreceptors, and at higher negative pressures aortic and carotid arterial baroreceptors. An LBNP of  $-15$  mmHg ( $1$  mmHg =  $133.3$  Pa) was applied because this stimulus was expected to have effects on blood pressure closely resembling those of adenosine (Floras 1990). (A potential alternative intervention, namely neck pressure, would be unsuitable for the purpose of this experiment, as this maneuver elicits only transient increases in MSNA:  $1-2$  s, despite continuation of this stimulus (Wallin and Eckberg 1982)). The effect of carotid chemoreceptor inhibition, by inhalation of 100% oxygen, on adenosine-induced changes in hemodynamics and MSNA was also examined.

## Methods

### Subjects

After approval of this protocol by our institutional ethics committee, 12 healthy male volunteers (mean age 48 years; range 31–56 years) were recruited for this study. Subjects signed written consent forms before their participation. They had no history of cardiac or pulmonary disease and none were taking medication. Baseline hemodynamic and neural variables are given in Table 1.

### Procedures

All experiments were performed in the morning, after an overnight fast with subjects lying supine. Subjects were instructed to abstain from caffeinated beverages on the morning of the experiment. Caffeine abstinence of this brief duration has no effect on diastolic blood pressure, the proximate stimulus to a subsequent burst of MSNA (Sanders and Ferguson 1989), or on heart rate (G.A. Rongen, S.C. Brooks, S. Ando, B.L. Senn, C.L. Notarius, and J.S. Floras, to be published).

Blood pressure was measured at 1-min intervals by an automatic cuff recorder (model Lifestat 200, Physio-Control, Redmond, Wash.). Heart rate (electrocardiography) was recorded continuously and simultaneously with the sympathetic mean voltage neurogram (model 2800S ink recorder, Gould, Cleveland, Ohio).

Postganglionic multifiber SNA was recorded from a muscle fascicle of the right peroneal nerve posterior to the fibular head, using a microneurographic technique (Delius et al. 1972; Floras et al. 1987). Recordings were made with tungsten microelectrodes with a  $200\text{-}\mu\text{m}$  shaft diameter, tapering to a  $1\text{-}$  to  $5\text{-}\mu\text{m}$  uninsulated tip. A reference electrode was inserted subcutaneously 1 to 3 cm from the recording electrode. Electrodes were connected to a preamplifier with a gain of 1000 and an amplifier with a gain that could be varied from 30 to 90 as required in a subject. Amplification was constant throughout the study in each subject. Neural activity was fed through a bandpass filter with a bandwidth of 700 to 2000 Hz. The filtered neurogram was routed through an amplitude discriminator to a storage oscilloscope



**Table 1.** Baseline hemodynamic and neural characteristics of the healthy subjects.

Variable	Baseline before LBNP	Baseline before adenosine infusion	Baseline before adenosine infusion with 100% oxygen
<i>n</i>	12	12	8
Systolic blood pressure (mmHg)	120.0±2.0	122.7±2.1	122.6±2.7
Diastolic blood pressure (mmHg)	76.0±1.9	76.5±2.0	77.6±2.1
Heart rate (beats/min)	66.0±3.8	63.5±3.4*	58.3±3.3*†
Sympathetic nerve activity			
Bursts/min	41.9±5.1	39.7±4.3	37.5±4.6
Bursts/100 beats	60.9±4.3	60.5±4.3	63.4±5.5†
Arbitrary units	392.2±77.6	356.8±79.5	408.1±132.7
Arbitrary units/100 beats	552.9±87.3	531.2±109.0	668.2±204.2

Note: Values are means ± SE.

\* $p < 0.05$  versus baseline before LBNP.

† $p < 0.05$  versus baseline before adenosine without 100% oxygen (Wilcoxon paired signed rank test).

and a loudspeaker. For recording and analysis, the filtered neurogram was fed through a resistance-capacitance integrating network (time constant, 0.1 s) to obtain a mean voltage neurogram of SNA. There were three criteria for an acceptable recording of muscle SNA. First, weak electrical stimulation (1–10 V, 0.2 ms, 1 Hz) through the electrode in the peroneal nerve elicited involuntary muscle contraction but not paresthesiae. Second, tapping or stretching the muscle or tendons supplied by the impaled fascicle elicited afferent mechanoreceptor discharge, whereas stroking the skin in the distribution of the peroneal nerve did not. Third, the neurogram contained spontaneous, intermittent, pulse-synchronous bursts that increased during held expiration. Evidence from earlier studies that such activity represents efferent postganglionic sympathetic activity includes (i) interruption of activity by local nerve block proximal but not distal to the recording site, (ii) reversible elimination of activity by ganglionic blockade, and (iii) a conduction velocity approximating 1 m/s.

### Protocol

Ten to fifteen minutes after instrumentation, the experiment started with 6-min baseline measurements, immediately followed by a 6-min LBNP at –15 mmHg. Approximately 30 min thereafter, a 4-min baseline period was repeated, immediately followed by 4-min infusions of adenosine (Fujisawa Inc., Markham, Ont.), in 0.9% saline, at sequential doses of 35, 70, and 140  $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ . Fifteen minutes after the last adenosine infusion, 100% oxygen was delivered by a non-rebreathing oxygen mask (Respan Products Inc., Erin, Ont.). After a 4-min baseline period, the highest adenosine dose (140  $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) was reinfused during administration of 100% oxygen.

The intervals of 30 and 15 min between LBNP and adenosine and adenosine with and without inhalation of 100% oxygen, respectively, allowed heart rate and MSNA to return to baseline.

### Statistics

Muscle SNA was expressed as integrated MSNA (units/100 beats): the product of burst incidence (bursts/100 beats) and averaged burst amplitude. Since muscle sympathetic bursts occur pulse synchronously, maximum burst frequency is dictated by heart rate (Delius et al. 1972; Sundlof and Wallin 1977). Adenosine increases heart rate, thereby increasing burst frequency nonspecifically (Fig. 1). To overcome this problem, we used integrated burst incidence (bursts/100 beats) instead of burst frequency to express MSNA. For each variable, all baseline measurements were averaged to obtain a single value. During LBNP and adenosine infusion, mean values for each subsequent 2-min period were derived from all measurements obtained during these time periods. The effects of LBNP or adenosine during each 2-min period were calculated as differences from baseline. Since the response to adenosine reached its maximum after the first 2 min, only

the results of the last 2 min of each adenosine dose are presented. For the comparison of LBNP with adenosine, the three subsequent 2-min values for responses were averaged to one value for each variable. If Friedman nonparametric two-way ANOVA for repeated measures was statistically significant, Wilcoxon paired signed rank tests were performed to determine the particular time interval (LBNP) or adenosine dose at which statistical significance was achieved. Differences between LBNP and adenosine and differences between adenosine with and without concomitant 100% oxygen breathing were analyzed using the Wilcoxon paired signed rank test. Results are expressed as mean ± SE. Values of  $p \leq 0.05$  were considered statistically significant.

## Results

LBNP and adenosine infusions were completed in all 12 subjects. In four volunteers, the last part of the experiment (100% oxygen breathing) was not performed because the MSNA recording site was lost or because of volunteer discomfort (chest pain, flushing, or dyspnea).

Apart from a small difference in heart rate and sympathetic nerve activity when expressed as bursts/100 beats, baseline variables were similar prior to the three procedures (Table 1).

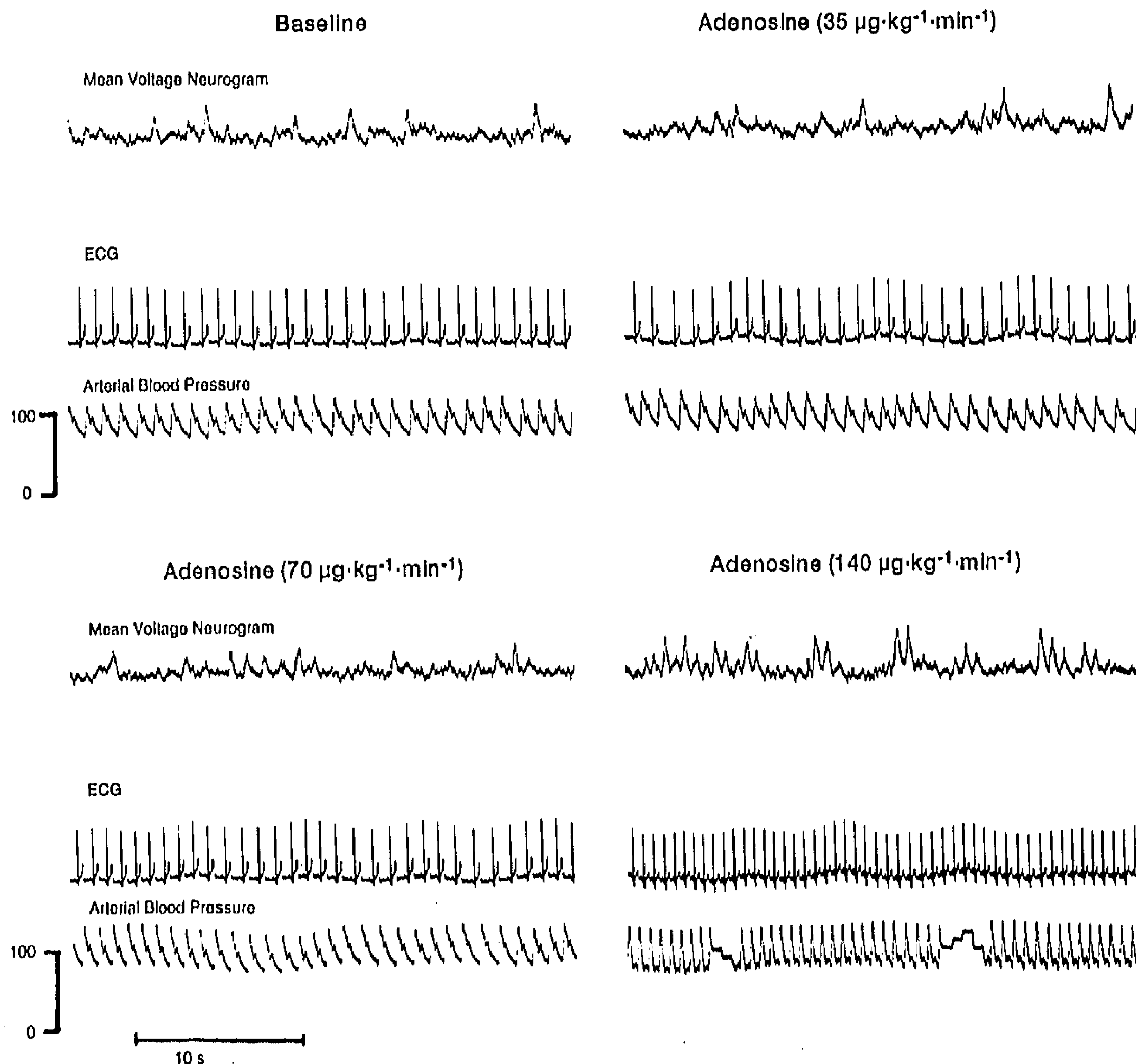
### Hemodynamic and neural effects of LBNP

During LBNP, systolic blood pressure decreased by a maximum of  $5 \pm 1$  mmHg, from  $120 \pm 2$  mmHg at baseline to  $115 \pm 2$  mmHg, 4 min after starting this manoeuvre ( $p < 0.01$ ; Fig. 1). Likewise, diastolic blood pressure decreased by  $3 \pm 1$  mmHg, from  $76 \pm 2$  mmHg at baseline to  $73 \pm 2$  mmHg, at 2 min after the start of the LBNP ( $p < 0.05$ ). There was a modest  $3 \pm 2$  beats/min increase in heart rate from  $66 \pm 4$  beats/min at baseline to  $69 \pm 4$  beats/min 4 min after start of LBNP ( $p = 0.1$ ). MSNA increased significantly, by  $224 \pm 63$  units/100 beats, 2 min after the start of the LBNP ( $p < 0.01$ ; Fig. 2).

### Hemodynamic and neural effects of adenosine

An individual tracing during baseline and adenosine infusion is shown in Fig. 1. Adenosine infusion had nonsignificant effects on systolic blood pressure ( $-3 \pm 2$  mmHg during the highest infusion rate;  $p = \text{ns}$ ; see Fig. 2), but it reduced diastolic blood pressure by  $3 \pm 1$  mmHg during the highest infusion rate ( $p < 0.05$ ). Heart rate increased dose-dependently by a maximum of  $25 \pm 3$  beats/min during the highest infusion

**Fig. 1.** Individual tracing illustrating the response to three infusions of adenosine in a single volunteer. Continuous arterial blood pressure was recorded from the finger in this volunteer for illustrative purposes (Finapres, Ohmeda). The tracing clearly shows the pulse synchronicity of MSNA. Changes in burst frequency and amplitude are preceded by opposite fluctuations in blood pressure, which illustrates the importance of the arterial baroreflex in spontaneous modulations in MSNA. Heart rate and finger systolic blood pressure increased in this particular experiment, which was performed in a subject who voluntarily abstained from caffeine for 24 h before the study.



rate ( $p < 0.01$ ). Likewise, MSNA increased dose-dependently by a maximum of  $255 \pm 82$  units/100 beats during the highest infusion rate ( $p < 0.05$ ; see Fig. 1).

During the highest adenosine infusion rate, the blood pressure reduction and increase in MSNA were not significantly different from the peak responses observed during LBNP (see Fig. 3). In contrast, the heart rate response was significantly higher during adenosine compared with LBNP ( $+25 \pm 3$  versus  $+2 \pm 2$  beats/min for adenosine and LBNP, respectively;  $p < 0.05$ ).

#### Effect of breathing 100% oxygen on the hemodynamic and neural effects of adenosine

In eight subjects, the highest adenosine infusion was repeated during inhalation of 100% oxygen (see Fig. 4). Responses of systolic and diastolic blood pressure to adenosine were not significantly affected by breathing 100% oxygen. The response in heart rate was significantly reduced but not completely abolished:  $+26 \pm 4$  versus  $+18 \pm 3$  beats/min ( $p < 0.05$ ). A comparable result was obtained when heart rate responses were expressed as percent change from baseline. Likewise, breathing oxygen significantly reduced the response in MSNA:  $+305 \pm 99$  versus  $+198 \pm 75$  units/100 beats

( $p < 0.05$ ). The residual MSNA response during 100% oxygen breathing remained significantly different from baseline ( $p = 0.05$ ). Compared with LBNP in these subjects, adenosine with concomitant breathing of 100% oxygen induced similar reductions in blood pressure, a similar increase in MSNA, but a greater increase in heart rate (Fig. 5;  $+17.5 \pm 3.4$  versus  $+1.6 \pm 1.9$  beats/min for adenosine and LBNP, respectively;  $p < 0.05$ ).

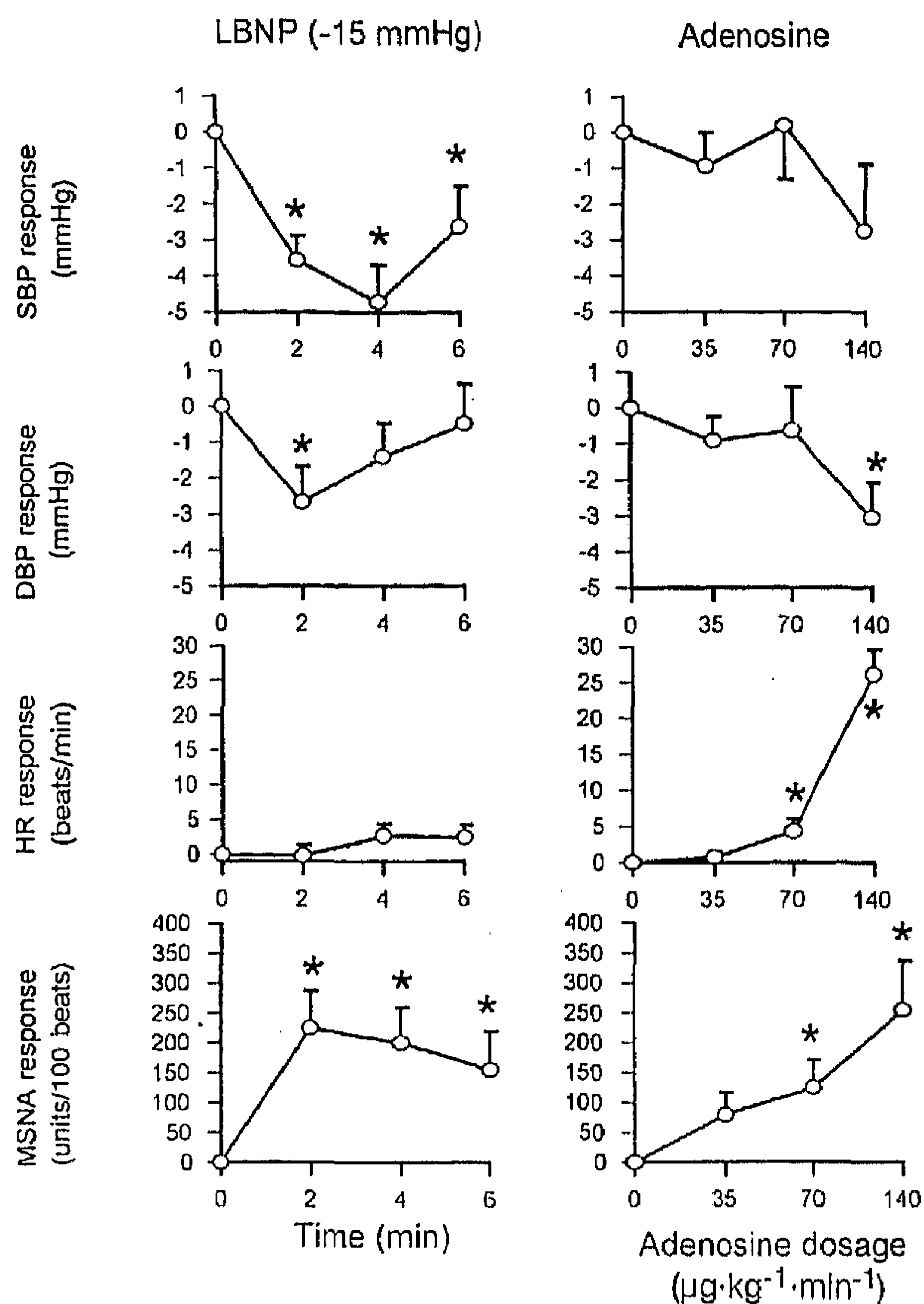
#### Discussion

In this study, adenosine-induced changes in blood pressure, heart rate, and MSNA were compared with the effects of mechanical unloading of baroreceptors. The major new finding of this study is that adenosine but not LBNP induced an increase in heart rate, while both interventions induced similar changes in blood pressure and sympathetic nerve traffic. Thus, while arterial baroreceptors were deactivated to a similar extent during both interventions, the response in heart rate differed markedly, indicating that the adenosine-induced increase in heart rate cannot be explained by unloading of arterial baroreceptors. Inhalation of 100% oxygen significantly inhibited the effect of adenosine on sympathetic nerve traffic and heart rate,

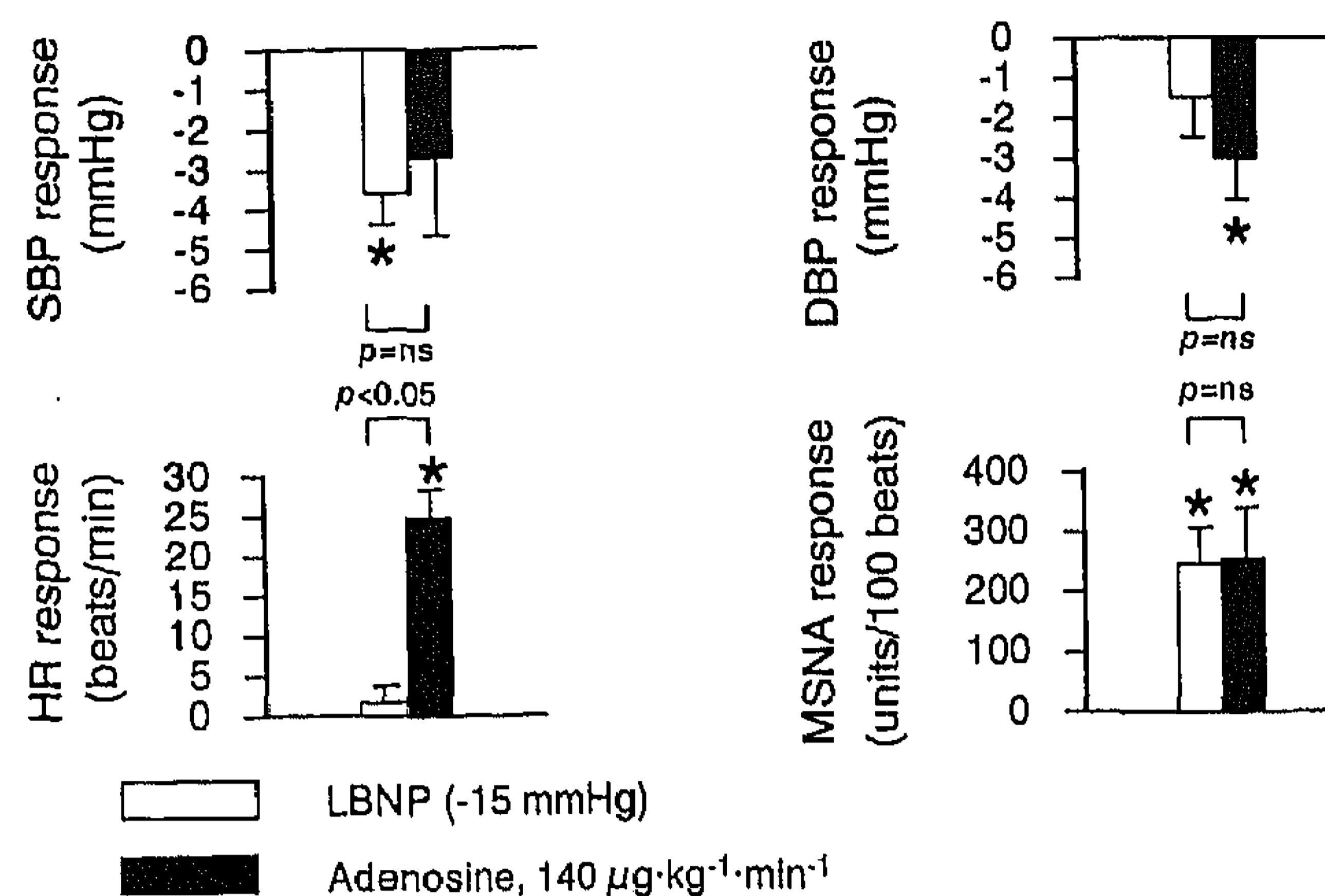


**Fig. 2.** Time course in blood pressure (SBP, systolic blood pressure; DBP, diastolic blood pressure), heart rate (HR), and MSNA responses to LBNP, and dose-response curve for the same variables during graded intravenous infusion of adenosine.

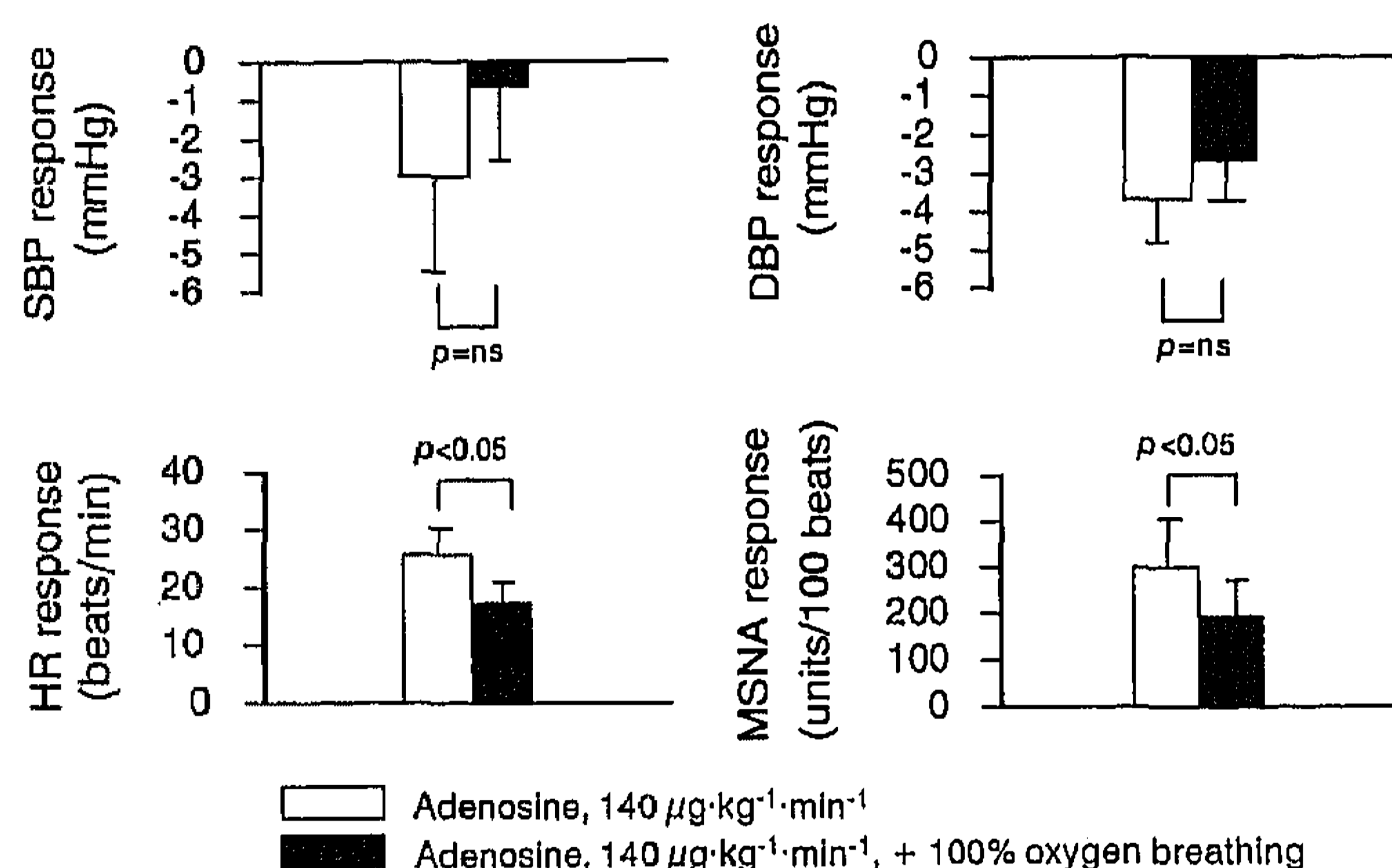
\*Responses statistically significantly different from zero ( $n = 12$ ).



**Fig. 3.** Comparison between LBNP and adenosine ( $140 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ). \*Responses statistically significantly different from baseline. Values of  $p$  indicate level of significance for difference between LBNP and adenosine ( $n = 12$ ).



**Fig. 4.** Effect of 100% oxygen breathing on the effects of adenosine ( $140 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ). Values of  $p$  indicate level of significance for effect of oxygen on responses to adenosine ( $n = 8$ ).



indicating that activation of peripheral chemoreceptors is partially responsible for this response to adenosine.

The hemodynamic response to intravenous adenosine in humans has been studied in considerable detail. Adenosine induces a dose-dependent increase in heart rate and stroke volume and reduces diastolic blood pressure and total peripheral resistance, mainly by vasodilation of skin and splanchnic vascular beds (Biaggioni et al. 1991a; Edlund et al. 1990; Nussbacher et al. 1995; Smits et al. 1987a). The other intervention, LBNP of  $-15 \text{ mmHg}$ , reduces central venous pressure and stroke volume, and to a lesser extent increases peripheral resistance, resulting in modest hypotension (Florás 1990).

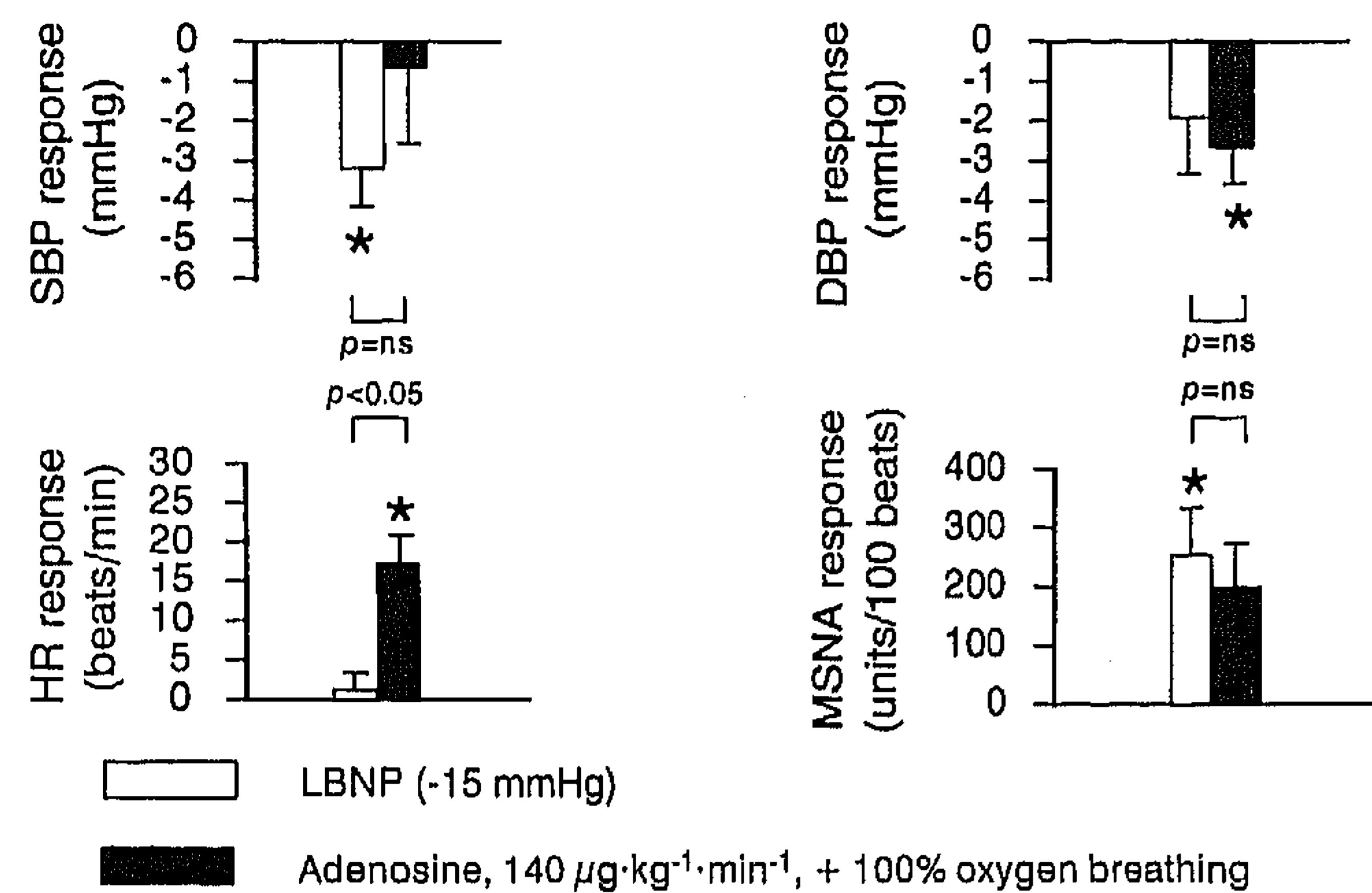
In the present study, the drop in blood pressure was small, but similar during LBNP and adenosine at  $140 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , indicating that arterial baroreceptors were probably deactivated equally. Notwithstanding, heart rate increased during adenosine but not during LBNP, indicating that arterial baroreceptor unloading is probably not involved in the adenosine-induced increase in heart rate. A reduction of parasympathetic tone contributes to this chronotropic response (Conradson et al. 1987a). We cannot exclude an additional effect of adenosine on baroreceptor reflex sensitivity, with subsequent augmentation of the heart rate response to arterial baroreceptor unloading. However, in conscious dogs, adenosine actually reduces the heart rate response to a fall in blood pressure,

probably by its direct negative chronotropic action on the sinoatrial node (Hintze et al. 1985).

Breathing 100% oxygen was used to inhibit peripheral chemoreceptors. This intervention significantly reduced the response of both heart rate and MSNA to adenosine, confirming the involvement of chemoreceptors in these effects. However, a significant residual increase in both variables persisted during oxygen. Arterial oxygen tension of approximately 400 mmHg is associated with complete inhibition of chemoreceptors (Biscoe et al. 1970). We did not sample arterial blood to determine the efficacy of oxygen delivery, but in one volunteer end-expiratory oxygen tension was over 700 mmHg, suggesting that the oxygen administration in these experiments was sufficient to abolish chemoreceptor activation completely. Since the response in MSNA did not differ significantly between LBNP and adenosine, involvement of the baroreflex could still account for the residual increase in MSNA but not for the residual heart rate response, since this was markedly different from the response to LBNP. Adenosine increases tidal volume (Fuller et al. 1987; Watt et al. 1987; Smits et al. 1987b) and thereby stimulates pulmonary stretch receptors. However, the heart rate response to adenosine is not affected



**Fig. 5.** Comparison between LBNP and adenosine ( $140 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) with concomitant breathing of 100% oxygen. \*Responses statistically significantly different from baseline. Values of  $p$  indicate level of significance for difference between LBNP and adenosine ( $n = 8$ ).



by pulmonary denervation in humans, indicating that these receptors are not involved in the heart rate response to adenosine (Morgan-Hughes et al. 1994). Adenosine-sensitive sympathetic efferents in heart, kidney, and (or) skeletal muscle (Katholi et al. 1984; Cox et al. 1989; Costa and Biaggioni 1993; Thames et al. 1993; Edlund et al. 1994) are probably resistant to oxygen administration and could be involved in the adenosine-induced heart rate increase.

Some possible limitations of this study should be discussed. First, we did not measure cardiac filling pressures. Therefore, we cannot exclude the possibility that LBNP and adenosine infusion differentially unloaded cardiopulmonary baroreceptors. Others have shown that adenosine ( $80 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) does not affect central venous pressure of young healthy volunteers (Biaggioni et al. 1991a). In patients who were studied during a diagnostic catheterization, intravenous adenosine ( $140 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) caused a modest (1.6 mmHg) increase in right atrial pressure (Nussbacher et al. 1995). In contrast, previous studies from our laboratory have demonstrated that central venous pressure drops by approximately 5 mmHg in response to an LBNP of  $-15$  mmHg (Floras 1990). Others have shown that application of less negative pressure, which was not accompanied by a reduction in arterial blood pressure and thus probably unloaded cardiopulmonary baroreceptors selectively, increased MSNA by approximately 60–70% (Oren et al. 1993; Victor and Leimbach 1987). Thus, cardiopulmonary baroreceptor unloading probably accounted for most of the LBNP-induced increase in MSNA but none of the effects of adenosine on MSNA. The relatively equivalent effect on MSNA of LBNP and of adenosine, in the presence of 100% oxygen, argues that stimulation of other adenosine-sensitive afferents (kidney, heart, skeletal muscle), not directly related to the baroreflex, causes a sympathoexcitation that is roughly similar to that of cardiopulmonary baroreceptor unloading during LBNP.

A second limitation of the study is related to the relatively short period of caffeine abstinence advised our subjects. Caffeine is an adenosine receptor antagonist in humans in vivo (Smits et al. 1987a; Biaggioni et al. 1991b). In studies in healthy volunteers with less than 12–24 h of caffeine

abstinence, systolic blood pressure does not increase (Watt et al. 1987, 1989; Conradson et al. 1987a, 1987b; Fuller et al. 1987; Clarke et al. 1988; Maxwell et al. 1987; Rongen et al. 1995), whereas in otherwise comparable studies with caffeine abstinence of more than 24 h systolic blood pressure invariably rises (Smits et al. 1987a; Biaggioni et al. 1987, 1991a, 1991b; Edlund et al. 1990; Reid et al. 1987). Since caffeine abstinence was for approximately 12 h in most of our volunteers, the interaction between caffeine and adenosine probably explains why their systolic blood pressure fell. This probably also explains why the increase in MSNA in our present study was less than in a previous report by others who studied the effect of adenosine after at least 24 h of caffeine abstinence (Biaggioni et al. 1991a). A 12- to 24-h caffeine abstinence period is consistent with the current clinical practice in nuclear medicine where adenosine infusion ( $140 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) is used for the evaluation of cardiac ischemia. Our study shows that adenosine-induced sympathoexcitation can occur in the clinical setting of adenosine-thallium scanning. These increases in sympathetic nerve traffic to the heart and periphery may have adverse implications for patients with ischemic heart disease.

In conclusion, the adenosine-evoked increase in heart rate was not observed during a LBNP stimulus that reduced arterial blood pressure to a similar extent. MSNA was equally increased during LBNP and adenosine infusion. These observations indicate that arterial baroreceptor unloading is probably not involved in the heart rate response to adenosine. Oxygen partially inhibited the increase in heart rate and MSNA, confirming the involvement of chemoreceptors in this response to adenosine. Other well-documented adenosine-sensitive sympathetic afferents may be involved in the residual heart rate response.

## Acknowledgements

This work was supported by a grant-in-aid from the Heart and Stroke Foundation of Ontario. Dr. Rongen is the recipient of a research fellowship from the Department of Medicine Research Fund of the Mount Sinai Hospital. Dr. Ando is the recipient of a Canadian Hypertension Society/Merck Frosst fellowship. Dr. Notarius is supported by research fellowships from the Department of Medicine and Centre for Cardiovascular Research of the University of Toronto. Dr. Floras is a Career Investigator of the Heart and Stroke Foundation of Ontario.

## References

- Belardinelli, L., and Berne, R.M. 1989. The cardiac effects of adenosine. *Prog. Cardiovasc. Dis.* **32**: 73–97.
- Biaggioni, I., Olafsson, B., Robertson, R.M., Hollister, A.S., and Robertson, D. 1987. Cardiovascular and respiratory effects of adenosine in conscious man. Evidence for chemoreceptor activation. *Circ. Res.* **61**: 779–786.
- Biaggioni, I., Killian, T.J., Mosqueda Garcia, R., Robertson, R.M., and Robertson, D. 1991a. Adenosine increases sympathetic nerve traffic in humans. *Circulation*, **83**: 1668–1675.
- Biaggioni, I., Paul, S., Puckett, A., and Arzubiaga, C. 1991b. Caffeine and theophylline as adenosine receptor antagonists in humans. *J. Pharmacol. Exp. Ther.* **258**: 588–593.
- Biscoe, T.J., Purves, M.J., and Sampson, S.R. 1970. The frequency of nerve impulses in single carotid body chemoreceptor afferent fibres recorded in vivo with intact circulation. *J. Physiol. (London)*, **208**: 121–131.



- Bredt, D.S., Hwang, P.M., and Snyder, S.H. 1990. Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature (London)*, **347**: 768–770.
- Cerqueira, M.D., Verani, M.S., Schwaiger, M., Heo, J., Iskandrian, A.S., and The Investigators of the Multicenter Adenoscan Trial. 1994. Safety profile of adenosine stress perfusion imaging: results from the Adenosine Multicenter Trial Registry. *J. Am. Coll. Cardiol.* **23**: 384–389.
- Clarke, B., Conradson, T.B., Dixon, C.M.S., and Barnes, P.J. 1988. Reproducibility of heart rate changes following adenosine infusion in man. *Eur. J. Clin. Pharmacol.* **35**: 309–311.
- Conradson, T.B., Clarke, B., Dixon, C.M., Dalton, R.N., and Barnes, P.J. 1987a. Effects of adenosine on autonomic control of heart rate in man. *Acta Physiol. Scand.* **131**: 525–531.
- Conradson, T.B.G., Dixon, C.M.S., Clarke, B., and Barnes, P.J. 1987b. Cardiovascular effects of infused adenosine in man: potentiation by dipyridamole. *Acta Physiol. Scand.* **129**: 387–391.
- Costa, F., and Biaggioni, I. 1993. Adenosine activates afferent fibers in the forearm, producing sympathetic stimulation in humans. *J. Pharmacol. Exp. Ther.* **267**: 1369–1374.
- Cox, D.A., Vita, A., Treasure, C.B., Fish, R.D., Selwyn, A.P., and Ganz, P. 1989. Reflex increase in blood pressure during intracoronary administration of adenosine in man. *J. Clin. Invest.* **84**: 592–596.
- Delius, W., Hagbarth, K.E., Hongell, A., and Wallin, B.G. 1972. General characteristics of sympathetic activity in human muscle nerves. *Acta Physiol. Scand.* **84**: 65–81.
- DiMarco, J.P., Sellers, T.D., Berne, R.M., West, G.A., and Belardinelli, L. 1983. Adenosine: electrophysiologic effects and therapeutic use for terminating paroxysmal supraventricular tachycardia. *Circulation*, **68**: 1254–1263.
- Edlund, A., Sollevi, A., and Linde, B. 1990. Haemodynamic and metabolic effects of infused adenosine in man. *Clin. Sci.* **79**: 131–138.
- Edlund, A., Ohlson, H., and Sollevi, A. 1994. Renal effects of local infusion of adenosine in man. *Clin. Sci.* **87**: 143–149.
- Engelstein, E.D., Lerman, B.B., Somers, V.K., and Rea, R.F. 1994. Role of arterial chemoreceptors in mediating the effects of endogenous adenosine on sympathetic nerve activity. *Circulation*, **90**: 2919–2926.
- Floras, J.S. 1990. Sympathoinhibitory effects of atrial natriuretic factor in normal humans. *Circulation*, **81**: 1860–1873.
- Floras, J.S., Aylward, P.E., Abboud, F.M., and Mark, A.L. 1987. Inhibition of muscle sympathetic nerve activity in humans by arginine vasopressin. *Hypertension (Dallas)*, **10**: 409–416.
- Fuller, R.W., Maxwell, D.L., Conradson, T.B., Dixon, C.M., and Barnes, P.J. 1987. Circulatory and respiratory effects of infused adenosine in conscious man. *Br. J. Clin. Pharmacol.* **24**: 306–317.
- Hintze, T.H., Belloni, F.L., Harrison, J.E., and Shapiro, G.C. 1985. Apparent reduction in baroreflex sensitivity to adenosine in conscious dogs. *Am. J. Physiol.* **249**: H554–H559.
- Katholi, R.E., Whitlow, P.L., Hageman, G.R., and Woods, W.T. 1984. Intrarenal adenosine produces hypertension by activating the sympathetic nervous system via the renal nerves in the dog. *J. Hypertens.* **2**: 349–359.
- Kubo, T., and Su, C. 1983. Effects of adenosine on [<sup>3</sup>H]norepinephrine release from perfused mesenteric arteries of SHR and renal hypertensive rats. *Eur. J. Pharmacol.* **87**: 349–352.
- Maxwell, D.L., Fuller, R.W., Conradson, T.B., Dixon, C.M., Aber, V., Hughes, J.M., and Barnes, P.J. 1987. Contrasting effects of two xanthines, theophylline and enprofylline, on the cardiorespiratory stimulation of infused adenosine in man. *Acta Physiol. Scand.* **131**: 459–465.
- Morgan-Hughes, N.J., Corris, P.A., Healey, M.D., Dark, J.H., and McComb, J.M. 1994. Cardiovascular and respiratory effects of adenosine in humans after pulmonary denervation. *J. Appl. Physiol.* **76**: 756–759.
- Nussbacher, A., Arie, S., Kalil, R., Horta, P., Feldman, M.D., Bellotti, G., Pileggi, F., Ellis, M., Johnson, W.H., Camarano, G.B., and Kass, D.A. 1995. Mechanism of adenosine-induced elevation of pulmonary capillary wedge pressure in humans. *Circulation*, **92**: 371–379.
- Olsson, R.A., and Pearson, J.D. 1990. Cardiovascular purinoceptors. *Physiol. Rev.* **70**: 761–845.
- Oren, R.M., Schobel, H.P., Weiss, R.M., Stanford, W., and Ferguson, D.W. 1993. Importance of left atrial baroreceptors in the cardiopulmonary baroreflex of normal humans. *J. Appl. Physiol.* **74**: 2672–2680.
- Reid, P.G., Watt, A.H., Routledge, P.A., and Smith, A.P. 1987. Intravenous infusion of adenosine but not inosine stimulates respiration in man. *Br. J. Clin. Pharmacol.* **23**: 331–338.
- Rongen, G.A., Smits, P., Ver Donck, K., Willemsen, J.J., de Abreu, R.A., Van Belle, H., and Thien, T. 1995. Hemodynamic and neurohumoral effects of various grades of selective adenosine transport inhibition in humans. Implications for its future role in cardioprotection. *J. Clin. Invest.* **95**: 658–668.
- Rongen, G.A., Lenders, J.W.M., Lambrou, G., Willemsen, J.J., Van Belle, H., Thien, T., and Smits, P. 1996. Presynaptic inhibition of norepinephrine release from sympathetic nerve endings by endogenous adenosine. *Hypertension (Dallas)*, **27**: 933–938.
- Rongen, G.A., Floras, J.S., Lenders, J.W.M., Thien, T., and Smits, P. 1997. Cardiovascular pharmacology of purines. *Clin. Sci.* **92**: 13–24.
- Sakuma, I., Togashi, H., Yoshioka, M., Saito, H., Yanagida, M., Tamura, M., Kobayashi, T., Yasuda, H., Gross, S.S., and Levi, R. 1992. N<sup>G</sup>-Methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo: a role for nitric oxide in the central regulation of sympathetic tone? *Circ. Res.* **70**: 607–611.
- Sanders, J.S., and Ferguson, D.W. 1989. Diastolic pressure determines autonomic responses to pressure perturbation in humans. *J. Appl. Physiol.* **66**: 800–807.
- Smits, P., Boekema, P., Thien, T., de Abreu, R., and van't Laar, A. 1987a. Evidence for an antagonism between caffeine and adenosine in the cardiovascular system in man. *J. Cardiovasc. Pharmacol.* **10**: 136–143.
- Smits, P., Schouten, J., and Thien, T. 1987b. Respiratory stimulant effects of adenosine in man after caffeine and enprofylline. *Br. J. Clin. Pharmacol.* **24**: 816–819.
- Smits, P., Lenders, J.W., and Thien, T. 1990. Caffeine and theophylline attenuate adenosine-induced vasodilation in humans. *Clin. Pharmacol. Ther.* **48**: 410–418.
- Sollevi, A., Lagerkranser, M., Irestedt, L., Gordon, E., and Lindquist, C. 1984. Controlled hypotension with adenosine in cerebral aneurysm surgery. *Anesthesiology*, **61**: 400–405.
- Sundlof, G., and Wallin, B.G. 1977. The variability of muscle nerve sympathetic activity in resting recumbent man. *J. Physiol. (London)*, **272**: 383–397.
- Thames, M.D., Kinugawa, T., and Diberner Dunlap, M.E. 1993. Reflex sympathoexcitation by cardiac sympathetic afferents during myocardial ischemia. Role of adenosine. *Circulation*, **87**: 1698–1704.
- Victor, R.G., and Leimbach, W.N., Jr. 1987. Effects of lower body negative pressure on sympathetic discharge to leg muscles in humans. *J. Appl. Physiol.* **63**: 2558–2562.
- Wallin, B.G., and Eckberg, D.L. 1982. Sympathetic transients caused by abrupt alterations of carotid baroreceptor activity in humans. *Am. J. Physiol.* **242**: H185–H190.
- Watt, A.H., Reid, P.G., Stephens, M.R., and Routledge, P.A. 1987. Adenosine-induced respiratory stimulation in man depends on site of infusion. Evidence for an action on the carotid body? *Br. J. Clin. Pharmacol.* **23**: 486–490.
- Watt, A.H., Bayer, A., Routledge, P.A., and Swift, C.G. 1989. Adenosine-induced respiratory and heart rate changes in young and elderly adults. *Br. J. Clin. Pharmacol.* **27**: 265–267.