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 μg·kg⁻¹·min⁻¹). In eight subjects, the highest dose was reinjected during 100% oxygen to suppress arterial chemoreceptors. Blood pressure reductions during LBNP and adenosine (140 μg·kg⁻¹·min⁻¹) were similar. HR did not change significantly during LBNP (+2 ± 2 beats/min; mean ± SE) but increased at the highest adenosine dose (+25 ± 3 beats/min; p < 0.05). MSNA increased significantly (p < 0.05) during both interventions (+255 ± 82 and +247 ± 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 ± 99 to +198 ± 75 units/100 beats and from +26 ± 4 to +18 ± 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 ± 3 beats/min) was significantly greater than the response to LBNP (+2 ± 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 artériaux, 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 μg·kg⁻¹·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 artériaux. Les réductions de pression artérielle durant la PNMI et l’adénosine (140 μg·kg⁻¹·min⁻¹) ont été similaires. La fréquence cardiaque n’a pas changé significativement durant la PNMI (+2 ± 2 battements/min; moyenne ± ÉT), mais elle a augmenté à la plus forte dose d’adénosine (+25 ± 3 battements/min; p < 0.05). L’ANSM a augmenté significativement (p < 0.05) durant les deux interventions (+225 ± 82 et 247 ± 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 ± 99 à +198 ± 75 unités/100 battements et de +26 ± 4 à +18 ± 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 ± 3 battements/min) a été significativement plus forte que la réponse à la PNMI (+2 ± 2 battements/min; p < 0,05). Ces résultats indiquent que la décharge des barorécepteurs artériaux 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...
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 microneuromographic technique (Delius et al. 1972; Floras et al. 1987). Recordings were made with tungsten microelectrodes with a 200-μm shaft diameter, tapering to a 1- to 5-μm 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.

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

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).

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 ± 1 mmHg, from 120 ± 2 mmHg at baseline to 115 ± 2 mmHg, 4 min after starting this manoeuvre (p < 0.01; Fig. 1). Likewise, diastolic blood pressure decreased by 3 ± 1 mmHg, from 76 ± 2 mmHg at baseline to 73 ± 2 mmHg, 2 min after the start of the LBNP (p < 0.05). There was a modest 3 ± 2 beats/min increase in heart rate from 66 ± 4 beats/min at baseline to 69 ± 4 beats/min 4 min after start of LBNP (p = 0.1). MSNA increased significantly, by 224 ± 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. Arousal infusion had nonsignificant effects on systolic blood pressure (−3 ± 2 mmHg during the highest infusion rate; p = ns; see Fig. 2), but it reduced diastolic blood pressure by 3 ± 1 mmHg during the highest infusion rate (p < 0.05). Heart rate increased dose-dependently by a maximum of 25 ± 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.

Baseline

Mean Voltage Neurogram

ECG

Arterial Blood Pressure

0
100

Adenosine (35 μg·kg⁻¹·min⁻¹)

Mean Voltage Neurogram

ECG

Arterial Blood Pressure

0
100

Adenosine (70 μg·kg⁻¹·min⁻¹)

Adenosine (140 μg·kg⁻¹·min⁻¹)

rate (p < 0.01). Likewise, MSNA increased dose-dependently by a maximum of 255 ± 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 ± 3 versus +2 ± 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 ± 4 versus +18 ± 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 ± 99 versus +198 ± 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 ± 3.4 versus +1.6 ± 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.
Adenosine dosage
Responses statistically significantly different from zero (n = 12).

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 µg·kg\(^{-1}\)·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 µg·kg\(^{-1}\)·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 mmHg, reduces central venous pressure and stroke volume, and to a lesser extent increases peripheral resistance, resulting in modest hypotension (Floras 1990).

In the present study, the drop in blood pressure was small, but similar during LBNP and adenosine at 140 µg·kg\(^{-1}\)·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 (Hinze 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.
Values of Fig. 5. Comparison between LBNP and adenosine (140 Jig-kg\(^{-1}\)-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 \(\mu\)g-kg\(^{-1}\)-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 \(\mu\)g-kg\(^{-1}\)-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 \(\mu\)g-kg\(^{-1}\)-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.

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