In vivo evidence against a role for adenosine in the exercise pressor reflex in humans

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Riksen, Niels P., Egidia E. M. van Ginneken, Petra H. H. van den Broek, Paul Smits, and Gerard A. Rongen. In vivo evidence against a role for adenosine in the exercise pressor reflex in humans. J Appl Physiol 99: 522–527, 2005. First published April 7, 2005; doi:10.1152/japplphysiol.00108.2005.—The pressor response to exercise is of great importance in both physiology and pathophysiology. Whether endogenous adenosine is a trigger for this reflex remains controversial. Muscle interstitial adenosine concentration can be determined by microdialysis. However, there are indications that local muscle cell damage by the microdialysis probe confounds these measurements in exercising muscle. Therefore, we used the nucleoside uptake inhibitor dipyridamole as pharmacological tool to bypass this confounding. We used microdialysis probes to measure endogenous adenosine in forearm skeletal muscle of healthy volunteers during two cycles of 15 min of intermittent isometric handgripping. During the second contraction, dipyridamole (12 μg·min⁻¹·dl forearm⁻¹) was administered into the brachial artery. Dipyridamole potentiated the exercise-induced increase in dialysate adenosine from 0.30 ± 0.08 to 0.48 ± 0.10 μmol/l (n = 9, P < 0.05), but it did not potentiate the exercise-induced increase in blood pressure. A time-control study without dipyridamole revealed no difference in exercise-induced increase in adenosine between both contractions (n = 8). To exclude the possibility that the dipyridamole-induced increase in dialysate adenosine originates from extravasation of increased circulating adenosine, we simultaneously measured adenosine with microdialysis probes in forearm muscle and antecubital vein. In a separate group of nine volunteers, simultaneous intrabrachial infusion of 100 μg·min⁻¹·dl⁻¹ dipyridamole and 5 μg·min⁻¹·dl⁻¹ adenosine increased dialysate adenosine from the intravenous but not the interstitial probe, indicating preserved endothelial barrier function for adenosine. We conclude that dipyridamole significantly inhibits uptake of interstitial adenosine without affecting the pressor response to exercise, suggesting that interstitial adenosine is not involved in the pressor response to rhythmic isometric exercise.

blood pressure; microdialysis; purine nucleoside

THE INCREASE IN ARTERIAL BLOOD pressure resulting from strenuous exercise of skeletal muscle is known as the exercise pressor reflex (1, 16). The exercising muscle releases several metabolites, which stimulate metabosensitive afferents that trigger this reflex (16). Several studies have provided evidence that the endogenous nucleoside adenosine contributes to this metabosensitive afferent activation (5–7, 21). On the contrary, other studies challenge the role of adenosine in triggering the exercise pressor reflex, and thus the role of adenosine remains controversial (12, 13, 19, 20, 25, 29). The present study aims to explain this controversy in terms of methodological shortcomings. Because of the very short half-life of adenosine (22) and the strong endothelial barrier for adenosine (23), it has long been very difficult to measure the concentration of endogenous adenosine in the muscle interstitial compartment, where the thin-fiber afferents responsible for initiating the pressor reflex are mainly located. However, with the recently introduced microdialysis method, it is possible to accurately measure endogenous interstitial adenosine in resting muscle in humans in vivo (8, 14). Several investigators also used microdialysis in exercising muscle and showed that exercise increases interstitial endogenous adenosine (7, 8, 14, 17, 18). The demonstration of an association between exercise intensity, muscle interstitial adenosine concentration, and increase in muscle sympathetic nerve activity (MSNA) strongly favors a role for adenosine in triggering the pressor reflex. However, there are indications that measurements of adenosine with microdialysis in exercising muscle are confounded by mechanical damage of muscle cells located in the vicinity of the probe (24). Consequently, the increased dialysate adenosine concentration during exercise does not necessarily reflect a generalized increase in muscle interstitial adenosine but rather a local increase by probe-related muscle cell injury.

We performed the present study to directly study the role for adenosine as trigger of the exercise pressor reflex with the microdialysis method, but we bypassed the possible confounding of local muscle cell damage by using the nucleoside uptake inhibitor dipyridamole as pharmacological tool to increase the interstitial adenosine concentration. We found that dipyridamole potentiates the exercise-induced increase in dialysate adenosine, indicating significant interstitial adenosine uptake inhibition, but not the exercise-induced pressor response. To exclude the possibility that the dipyridamole-induced increase in dialysate adenosine originates from extravasation of increased circulating adenosine, we performed an additional series of experiments with simultaneous measurements of interstitial and circulating adenosine.

METHODS

The study was approved by the Institutional Review Board of the Radboud University Nijmegen Medical Centre, and each subject gave written, informed consent before participation.

Subjects. The demographic data of the 26 participants of the three different study protocols are shown in Table 1. The subjects were normotensive nonsmokers and were not taking any medications ex-
except for oral contraceptives. The subjects underwent a physical examination, including electrocardiography, before entering the study. Subjects with a history of pulmonary or cardiovascular disease were excluded. All experiments were performed after at least 24 h of caffeine abstinence and at least 2 h of food abstinence.

**Experimental protocol.** The experiments started in the morning in a temperature-controlled laboratory (23°C), with the subjects in supine position. The brachial artery of the nondominant arm was cannulated for drug infusion and blood pressure recording, as previously described (28). In the first protocol, blood pressure was measured at 2-min intervals on the dominant arm, using an automated device (Dinamap).

After local anesthesia, a microdialysis probe (CMA70 brain microdialysis catheter, CMA, Stockholm, Sweden) was inserted into the anterior forearm muscle compartment of the nondominant arm, guided by a 14-gauge Venflon cannula. The probe had a dialysis tubing of 0.9% NaCl with a microdialysis pump and tetrabutylammonium hydrogen sulfate (10 mM) as the ion-pair reagent set at 254 nm. A binary low-pressure gradient elution was used with eluent A, consisting of dipotassium hydrogen phosphate (0.1 M) and tetrabutylammonium hydrogen sulfate (10 mM) as the ion-pair forming agent. The pH was adjusted to 6.5 with HCl. Solvent B contained, in addition, 40% (vol/vol) methanol. In addition, in the dialysate samples (10 μl) from the time-control study of protocol 1, the concentration of creatine was determined spectrophotometrically (kit no. 12320, Merck) without use of creatininase. Also, for each sample, creatine was measured after enzymatic conversion of phosphocreatine by creatine kinase. The concentration of phosphocreatine was calculated by taking the difference between these two creatine concentrations.

**Analytical procedures.** Dialysate samples (15 μl) were analyzed for concentrations of adenosine using HPLC, equipped with a UV detector set at 254 nm. A binary low-pressure gradient elution was used with eluent A, consisting of dipotassium hydrogen phosphate (0.1 M) and tetrabutylammonium hydrogen sulfate (10 mM) as the ion-pair forming agent. The pH was adjusted to 6.5 with HCl. Solvent B contained, in addition, 40% (vol/vol) methanol. In addition, in the dialysate samples (10 μl) from the time-control study of protocol 1, the concentration of creatine was determined spectrophotometrically (kit no. 12320, Merck) without use of creatininase. Also, for each sample, creatine was measured after enzymatic conversion of phosphocreatine by creatine kinase. The concentration of phosphocreatine was calculated by taking the difference between these two creatine concentrations.

**Dialysate dipyridamole concentration** was determined using HPLC with fluorescence detection set at 286/470 nm.

**Drugs and solutions.** Adenosine (Adencor, Sanofi-Synthelabo) and dipyridamole (Persantin, Boehringer Ingelheim) were diluted in normal saline to obtain the different concentrations.

**Data analysis and statistics.** In the first protocol, all blood pressure values during the 15-min contraction periods were averaged to one value. This value was compared with the mean value of a baseline period of 15 min immediately before contraction. All results are expressed as means ± SE, unless indicated otherwise.

The effect of dipyridamole on exercise-induced increases in adenosine and blood pressure was tested with a paired Student’s t-tests. The effect of dipyridamole on interstitial and intravascular adenosine concentrations was assessed with repeated-measures ANOVA. P < 0.05 was considered statistically significant.

**RESULTS**

**Protocol 1:** the effect of dipyridamole on muscle interstitial adenosine concentration and pressor response during exercise. Infusion of dipyridamole into the brachial artery during the second period of contraction significantly potentiated the exercise-induced increase in dialysate adenosine from 0.30 ± 0.08 μmol/l during the first bout of exercise to 0.48 ± 0.10 μmol/l (P < 0.05 for effect of dipyridamole). In contrast,
dipyridamole did not potentiate the exercise-induced increase in blood pressure (systolic blood pressure [SBP]/diastolic blood pressure [DBP]): 9.6 ± 2.4/4.5 ± 2.0 mmHg and 10.4 ± 2.2/7.0 ± 1.5 mmHg in the absence and presence of dipyridamole, respectively (P > 0.1).

In the time-control study, dialysate adenosine concentration increased by 0.25 ± 0.10 and 0.15 ± 0.07 μmol/l during the successive exercise periods (P > 0.1 for the comparison between the first and the second period of contractions; n = 8, Fig. 1). The exercise-induced rise in blood pressure (SBP/DBP) was 15.1 ± 2.8/9.8 ± 2 mmHg for the first contraction period and 17.3 ± 3.7/14.3 ± 1.6 mmHg for the second contraction period (P > 0.1 for SBP, P < 0.05 for DBP for comparison between first and second period).

In the time-control group, dialysate (phospho)creatine was high immediately after insertion because of mechanical injury of muscle cells (330 ± 82 μmol/l, n = 7). After 90 min, (phospho)creatine had stabilized (65 ± 11 μmol/l). During subsequent exercise, dialysate (phospho)creatine increased by 151 ± 58 μmol/l. Before the second bout of exercise, (phospho)creatine had returned to baseline and increased by 45 ± 19 μmol/l during the second period of exercise. The ratio of dialysate (phospho)creatine to adenosine just after insertion did not significantly differ from the ratio during the exercise periods (1.291 ± 477 vs. 1.033 ± 434 and 805 ± 181, P > 0.1).

In an additional study, antecubital venous plasma (phospho)creatine concentrations were determined before and immediately after 15 min of rhythmic exercise. (Phospho)creatine concentration was 50 ± 28 and 47 ± 22 μmol/l before and after exercise, respectively (n = 8, P > 0.1).

Protocol 2: simultaneous measurement of interstitial and circulating adenosine during intrabrachial infusion of adenosine with and without dipyridamole. Intra-arterial infusion of only adenosine or dipyridamole did not significantly affect dialysate adenosine concentrations from either the intravascular or intramuscular probe (Fig. 2). However, confusion of adenosine with dipyridamole increased adenosine in dialysate from the intravenous probe from 0.11 ± 0.02 μmol/l during baseline to 0.39 ± 0.14 μmol/l (P < 0.05; n = 9), without affecting adenosine in the dialysate from the intramuscular probe (0.05 ± 0.01 μmol/l at baseline vs. 0.08 ± 0.02 μmol/l during administration of dipyridamole and adenosine; P > 0.1, n = 9).

The in vitro adenosine recovery from the intramuscular and intravenous probes averaged 42 ± 4 vs. 49 ± 6%, respectively (P > 0.1).

During intrabrachial administration, dipyridamole effectively diffused into the interstitial compartment: the dialysate concentration of dipyridamole from the intravenous probe was 3.18 ± 1.6 μmol/l and from the interstitial probe was 1.59 ± 1.0 μmol/l. Absolute concentration cannot be calculated because we did not determine recovery for dipyridamole.

DISCUSSION

In the present study, we showed that local infusion of the nucleoside uptake inhibitor dipyridamole into the brachial artery potentiates the handgrip exercise-induced increase in
ADENOSINE AND THE EXERCISE PRESSOR REFLEX

muscle interstitial adenosine but not the exercise-induced pressor response. This observation argues against a role for adenosine as a trigger for the exercise pressor reflex to intermittent static handgripping in healthy young volunteers.

It has long been known that exercise elicits sympathetic activation and a rise in blood pressure (1), which are induced both by central command and by activation of a reflex originating in the exercising skeletal muscle (16). The afferent limb of this reflex is composed of type III and IV muscle afferents, which can be activated by mechanical as well as chemical stimuli (16). Much research has been devoted to elucidate the role of the endogenous nucleoside adenosine as one of these chemical triggers of the exercise pressor reflex, but still no consensus has been reached. Adenosine is an attractive candidate for triggering this reflex because it is known to excite a variety of afferent fibers. Activation of metabosensitive afferents by adenosine has been demonstrated in the kidney (15), carotid and aortic chemoreceptors (3), forearm (6), and heart (9), although not all studies confirm this afferent fiber activation in the heart (26).

The concentration of endogenous adenosine in the muscle interstitial compartment, where the metabosensitive afferents that trigger the exercise pressor reflex are mainly located, can only be determined by microdialysis. In resting muscle, microdialysis provides an accurate method to determine interstitial adenosine (8, 14). However, using microdialysis to measure muscle interstitial adenosine during muscle contraction is more complicated. It has been suggested that in this situation the microdialysis probe injures muscle cells in the vicinity of the probe (24). Consequently, an exercise-induced increase in dialysate adenosine might not reflect a genuine increase in interstitial adenosine but rather local mechanical cellular disruption. To circumvent this possible source of confounding, we used dipyridamole as pharmacological tool to inhibit adenosine uptake during exercise, to further increase the extracellular interstitial adenosine concentration without a concomitant increase in mechanical damage. We showed that infusion of dipyridamole into the brachial artery significantly potentiates the exercise-induced increase in dialysate adenosine, whereas dipyridamole does not potentiate the pressor response.

On the basis of these observations, we concluded that dipyridamole effectively inhibits adenosine uptake in the interstitial compartment. This conclusion is not confounded by any local mechanical cellular injury because the source of the increased adenosine is not relevant. However, this conclusion could critically be confounded by disruption of the endothelial barrier for adenosine by either dipyridamole (11) or mechanically by insertion of the microdialysis probe. In that case, extravasation of increased circulating adenosine could account for the observed increase in dialysate adenosine. Therefore, in an additional series of experiments, we tested whether the endothelial barrier for adenosine is still intact during interstitial microdialysis. We simultaneously measured interstitial adenosine with a microdialysis probe inserted into the flexor muscle of the forearm and circulating adenosine with a microdialysis probe inserted retrogradely into the medial cubital vein during concomitant infusion of dipyridamole and adenosine into the brachial artery. Infusion of adenosine and dipyridamole significantly increases dialysate adenosine from the intravascular probe but not from the interstitial probe. This clearly confirms that after insertion of a microdialysis probe in forearm muscle, the endothelial barrier for adenosine is still intact. Therefore, the potentiation of the exercise-induced increase in dialysate adenosine by dipyridamole indeed represents a genuine increase of adenosine in the interstitial compartment.

Up to now, three types of experiments have been performed to study the role of adenosine in triggering the pressor reflex, and each has yielded inconsistent results. First, exogenous adenosine has been injected locally into the arterial supply of limbs. Indeed, injection of adenosine into the brachial artery in humans increases blood pressure (5–7, 21). Moreover, this is not caused by venous spillover of adenosine, because adenosine was administered during venous occlusion (5–7, 21), intravenous administration of the same dose did not affect blood pressure (5), and the blood pressure response was blunted by axillary ganglionic blockade (7). In contrast, in anesthetized cats and rabbits, intra-arterial injection of adenosine or the stable adenosine analog 2-chloroadenosine does not elicit pressor responses (13, 20, 29). In particular, MacLean et al. (20) administered adenosine into the arterial supply of cat triceps surae muscle in vivo in a sufficient dose to significantly increase muscle interstitial adenosine, but they still did not observe any hemodynamic changes. This discrepancy is attributed to the effects of anesthesia and substantial interspecies differences in the actions of adenosine (2). Although the evidence that exogenous adenosine stimulates muscle afferents in the forearm is very robust (5–7), no conclusion can be derived considering the role of endogenous adenosine during exercise. The endothelium acts as a very strong metabolic barrier for adenosine between the intravascular and interstitial compartment (23). In the cat hindlimb, <3% of the intraluminally administered adenosine dose diffused into the interstitial compartment, where the metabosensitive afferent fibers reside (20). Consequently, intravenously administered exogenous adenosine probably does not directly stimulate the metabosensitive afferents, which are involved in the pressor response to exercise.

Second, the role of adenosine as trigger of the pressor reflex has been studied by the use of adenosine receptor antagonists during exercise. Costa and Biaggioni (6) demonstrated that intra-arterially administered theophylline attenuates the pressor response to sustained isometric handgrip, whereas Notarius et al. (25) showed that systemic infusion of caffeine does not affect the pressor response to isometric handgrip and posthandgrip ischemia in healthy subjects but does so only in patients with cardiac failure during posthandgrip ischemia.

Third, studies directly measuring endogenous interstitial adenosine have contributed to increasing knowledge on the role of adenosine in the exercise pressor reflex. It has long been impossible to actually measure endogenous adenosine in vicinity of the muscle afferents in the interstitium, because of the very short half-life of adenosine (22). Moreover, circulating adenosine does not reflect interstitial adenosine because of the strong endothelial barrier for adenosine (23). With microdialysis, direct measurement of interstitial adenosine is possible. Using microdialysis probes inserted into exercising muscle, several groups demonstrated that dialysate adenosine increases during exercise (14, 17, 18). Moreover, a role for adenosine as a trigger in the pressor reflex was suggested by showing that the dialysate adenosine concentration correlates to exercise intensity on the one hand and to the increase in MSNA on the other hand (7).
Recently, Nordsborg et al. (24) provided evidence that, when using microdialysis in exercising muscle, local mechanical disruption of cellular membranes of muscle cells in the vicinity of the probe occurs. They showed that during exercise, high concentrations of carnosine were found in the dialysate. Biaggioni (6) in which it was demonstrated that theophylline (4).

Because carnosine is found in high concentrations in muscle cells but not in plasma, this finding indicates disruption of cellular membranes. Because the intracellular and extracellular concentration of purine nucleotides and nucleosides also importantly differ, membrane rupture might significantly confound measurements of interstitial adenosine. To determine whether local muscle cell injury also occurred in our experiments, we measured the dialysate concentrations of (phospho)creatine. (Phospho)creatine is found in high intracellular concentrations in muscle cells, but it cannot pass the cellular membrane (30). We assumed that an increase of these substances in the dialysate implicates local mechanical disruption of the membranes of cells located in the vicinity of the microdialysis probe. Immediately after insertion of the microdialysis probe, dialysate levels of adenosine and (phospho)creatine are elevated, reflecting pure mechanical injury of muscle fibers (Fig. 1). During exercise, a similar increase in (phospho)creatine and adenosine occurs, and the ratio of (phospho)creatine to adenosine does not significantly differ between samples obtained during exercise and those obtained immediately after insertion of the probe, although there is a trend toward a reduction of this ratio. This observation suggests that local mechanical injury of muscle cells significantly contributes to the increase of adenosine in the dialysate during exercise. To exclude the possibility that exercising muscle cells release creatine without mechanical damage, we measured venous (phospho)creatine in a separate group of subjects before and immediately after 15 min of intermittent isometric forearm exercise. This exercise protocol was not accompanied by an increase in forearm venous (phospho)creatine, which suggests that exercise itself does not trigger the release of this substance by viable cells.

In general, our findings suggest that microdialysis is not well suited for measurement of interstitial purines in exercising muscle, which is very important in the interpretation of previously published studies on this topic.

On the basis of the above-mentioned observations, we conclude that adenosine does not importantly trigger the pressor response to intermittent handgrip in these healthy young volunteers. It is important to realize that the isometric exercise used in the present study, as in previous studies with microdialysis, was performed rhythmically without superimposed ischemia to allow volunteers to sustain the exercise for a sufficient period of time to complete microdialysis sampling. Therefore, skeletal muscle perfusion was restored during each 5-s interval of relaxation, thus potentially allowing washout of accumulated metabolites. In contrast, in the study by Costa and Biaggioni (6) in which it was demonstrated that theophylline inhibits the exercise-induced sympathetic activation and pressor response, sustained isometric handgrip was used. The intermittent restoration of muscle perfusion in our study prevents accumulation of metabolites such as adenosine and could explain why, in our experimental setup, adenosine is not importantly involved in triggering the pressor reflex. This phenomenon could also well explain the previous observation that the adenosine receptor antagonist caffeine inhibits the pressor response to exercise in patients with heart failure, who have compromised skeletal muscle perfusion, but not in healthy controls (25). The intermittent, nonisometric exercise performed in the present study is highly relevant to daily life. However, with use of this particular stimulus, the observed pressor response is resulting from stimulation of chemosensitive afferents as well as from central command. Nevertheless, if adenosine were involved as one of the stimuli of metabosensitive afferents, a significant increase in interstitial adenosine resulting from dipyridamole infusion should have potentiated the pressor response, even when central command and mechanoreceptor stimulation also contribute to sympathetic activation.

Because caffeine is a potent adenosine receptor antagonist, it is important to consider the duration of caffeine abstinence before experimentation. In the present study, subjects abstained from caffeine-containing beverages for at least 24 h, whereas in some previous studies on this topic, 72 h of abstinence were used (6). Rongen et al. (27) studied the effect of the duration of caffeine abstinence (6, 30, 78, 150, and 318 h) on the hemodynamic effects of acute systemic administration of adenosine. They showed that this duration did not influence heart rate and DBP responses. The adenosine-induced increase in SBP was significantly higher only after 1 wk of abstinence. Consequently, studies using caffeine abstinence for 1 or 3 days can be compared without confounding by this variable.

Finally, it needs to be realized that dipyridamole is proposed to have alternative mechanisms of actions besides inhibition of nucleoside transport (10). However, our group has shown previously that dipyridamole-induced vasodilation in the concentration range used in the present study is indeed due to adenosine uptake inhibition, because it could be inhibited by the adenosine receptor antagonist theophylline (4).

In conclusion, in the present study, we showed that dipyridamole effectively inhibits interstitial adenosine uptake, but does not potentiate the pressor response to intermittent isometric handgrip, which argues against a role for adenosine as a metabolic trigger of the exercise pressor response.

GRANTS

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