Adenosine, a Metabolic Trigger of the Exercise Pressor Reflex in Humans

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Abstract—There is substantial evidence that adenosine activates muscle afferent nerve fibers leading to sympathetic stimulation, but the issue remains controversial. To further test this hypothesis, we used local injections of adenosine into the brachial artery while monitoring systemic muscle sympathetic nerve activity (MSNA) with peroneal microneurography. The increase in MSNA induced by 3 mg intrabrachial adenosine (106±32%) was abolished if forearm afferent traffic was interrupted by axillary ganglionic blockade (21±9%, n=5, P<0.05). Furthermore, the increase in MSNA induced by intravenous adenosine was 3.7-fold lower and later (onset latency 20.9±4.8 seconds versus 8.5±1 seconds) than intrabrachial adenosine. Finally, we used forearm exercise (dynamic handgrip at 50% and 15% maximal voluntary contraction, MVC), with or without superimposed ischemia, to modulate interstitial levels of adenosine (estimated with microdialysis) while monitoring MSNA. Fifteen minutes of intense (50% MVC) and moderate (15% MVC) exercise increased adenosine dialysate concentrations from 0.31±0.1 to 1.24±0.4 μmol/L (528±292%) and from 0.1±0.02 to 0.419±0.16 μmol/L (303±99%), respectively (n=7, P<0.01). MSNA increased 88±25% and 38±28%, respectively. Five minutes of moderate exercise increased adenosine from 0.095±0.02 to 0.25±0.12 μmol/L, and from 0.095±0.02 to 0.48±0.19 μmol/L when ischemia was superimposed on exercise (n=7, P=0.01). The percent increase in MSNA induced by the various interventions correlated with the percent increase in dialysate adenosine levels (r=0.96). We conclude that adenosine activates muscle afferent nerves, triggering reflex sympathetic activation. (Hypertension. 2001;37:917-922.)

Key Words: exercise ■ microdialysis ■ muscles ■ sympathetic nervous system

Adenosine acts as an endogenous physiological modulator, particularly during ischemic events, when interstitial adenosine levels increase as oxygen demands exceed blood supply.1,2 Its actions are aimed to protect the tissue against ischemia by inducing local vasodilation and inhibiting platelet aggregation, neutrophil activation, and norepinephrine release. In contrast, adenosine also stimulates afferent nerve fibers (eg, myocardial and renal afferents and arterial chemoreceptors), triggering reflex sympathetic activation.3–6 This apparent paradoxical neuroexcitatory effect may actually contribute to the protective effect of adenosine; by inducing reflex sympathetic activation, adenosine will improve perfusion pressure to the ischemic tissue while protecting it from sympathologically mediated vasoconstriction through its local sympatholytic effects.

Given the excitatory effects of adenosine on other afferent fibers, it has been suggested that adenosine also activates muscle afferents involved in the exercise pressor reflex. This reflex arises in part from afferent fibers located in the skeletal muscle that are activated by metabolites generated by intense exercise, triggering a reflex that results in sympathetic activation and increased blood pressure (BP).7,8

We previously suggested that endogenous adenosine contributes to the activation of muscle afferents involved in the exercise pressor reflex in humans,9 in part based on the observation that intra-arterially administered adenosine produces reflex sympathetic activation by stimulating forearm afferents.6 Some investigators have provided independent confirmation of these findings,10,11 but others have expressed concern that the sympathetic activation may be explained by a spillover of adenosine into the systemic circulation with the activation of peripheral arterial chemoreceptors.12,13 Controversy also has arisen about the ability of adenosine to activate myocardial afferents.14,15

In the present study, we used complementary approaches to test the hypothesis that adenosine activates forearm afferent fibers. First, to discriminate whether the effects of intrabrachial adenosine are due to the activation of forearm muscle afferents or to spillover into the systemic circulation with activation of peripheral receptors, we used axillary ganglionic blockade to determine whether the interruption of forearm afferent nerve traffic suppresses the sympathetic activation induced by intrabrachial adenosine. We also compared the magnitude and onset latency of the sympathetic activation...
induced by intravenous and intrabrachial injections of adenosine. Finally, we measured interstitial concentrations of adenosine in the forearm muscle while monitoring muscle sympathetic nerve activity (MSNA) to determine the relationship between these variables to the intensity of exercise and the presence of ischemia. We used a microdialysis probe, which allows the passage of only low-molecular-weight molecules, to estimate interstitial adenosine levels.

**Methods**

**Subjects**

We studied a total of 18 normal volunteers, aged 21 to 42 years (mean age 29.2 ± 2.3 years). Subjects were nonsmokers, were not taking medications, and had abstained from methyloxanthines for ≥72 hours before the study. The protocol was approved by the Vanderbilt University Institutional Review Board. Volunteers were informed of the characteristics of the study and gave written consent.

**Instrumentation**

For each study session, subjects were fasted and in the supine position. Heart rate was monitored with surface ECG coupled to a rate computer. A catheter was inserted into the right antecubital vein for drug administration. When indicated, an indwelling catheter was placed into the brachial artery for intra-arterial drug administration and connected through 3-way valves to a pressure transducer. BP was measured continuously from the brachial artery or through finger plethysmography (Finapres2300; Ohmeda). Cardiovascular signals were modulated on signal conditioners and displayed on a thermal array recorder (model TA6000; Gould Inc) or digitized with a Windaq system (DA-220; DATAQ Instruments).

**Axillary Ganglionic Blockade**

The left brachial plexus was blocked using an axillary perversual approach. With the left humerus abducted 90° and in full external rotation, the axillary artery was palpated in the arm, and a 1% lidocaine skin wheal was placed over the artery under sterile conditions. A 22-gauge, 1.5-inch needle was advanced toward the axillary artery pulsation until the hub of the needle was observed to exhibit triphasic pulsations. The needle was then advanced proximally 0.5 cm parallel to the axillary artery while the triphasic pulsations were maintained. Tubing was then connected to the needle hub, and 40 mL of 1% lidocaine was intermittently injected with frequent, careful aspiration and monitoring of vital signs. The needle was then removed, and a sterile dressing was applied. The subject was questioned regarding the onset of paresthesias and observed for the onset of vasodilatation, cutaneous warmth, and loss of proprioception.

**Transcutaneous Muscle Microdialysis**

A microdialysis probe, CMA/20 (CMA), was introduced into the flexor digitorium superficialis muscle of the left forearm, as previously described in detail. The probe had a dialysis tubing (10×0.5 mm with a 20 000 molecular mass cutoff) and was perfused continuously with saline at a rate of 2 μL/min ("perfusion") with a microinjection pump (CMA/102 Microdialysis Pump). The effluent ("dialysate") was recovered with a fraction collector. The in vitro recovery of adenosine from microdialysis probes averaged 36±6% in these studies.

**Microneurography**

MSNA was recorded from the right peroneal nerve, as previously described, and digitized with a Windaq system (DA-220; DATAQ Instruments). Previously published criteria for an adequate MSNA recording were followed.

**Protocol 1: To Determine Whether Axillary Ganglionic Blockade, Which Interrupts Forearm Afferent Traffic, Suppresses Sympathetic Activation Induced by Intrabrachial Adenosine**

In 5 subjects, an intrabrachial catheter was placed for drug administration, and microneurography was performed as described earlier.

The subjects rested for 20 minutes after instrumentation. BP and MSNA were monitored continuously. Intrabrachial boluses of either saline or adenosine (1, 2, and 3 mg) were then administered in a randomized and single-blinded fashion, with venous occlusion as described earlier. Time was allowed between doses for MSNA and BP to return to baseline levels. To test the effectiveness of ganglionic blockade, cold pressor test was performed by placing the hand in ice water for 1 minute. BP and MSNA were recorded. We performed axillary ganglionic blockade as described, and the preceding protocol (intrabrachial injections of adenosine and the cold pressor test) was then repeated.

**Protocol 2: To Determine Differences in Magnitude and Latency of Onset of Sympathetic Activation Between Intrabrachial and Intravenous Adenosine Administration**

Volunteers were instrumented so we could monitor MSNA, BP, and heart rate, as described. Bolus injections of either 3 mg adenosine or saline were administered either intravenously, intrabrachially, intrabrachially during venous occlusion, or intrabrachially during arterial occlusion. The injections were single-blinded, and the order was randomized. Venous occlusion was induced by inflating a proximal pneumatic cuff to 50 mm Hg immediately before injection and maintaining inflation for 2 minutes while data were collected. Arterial occlusion was induced immediately before intrabrachial injections by inflating a proximal pneumatic cuff to 50 mm Hg above systolic BP and maintaining inflation for 2 minutes.

**Protocol 3: To Examine the Effect of Varying Exercise Intensity and Superimposed Ischemia on Muscle Interstitial Levels of Adenosine and Reflex Sympathetic Activation**

In 8 subjects, a microdialysis probe was inserted in the forearm, and microneurography was set up, as previously described. After a 1-hour equilibration period, 2 consecutive 15-minute dialysate samples were collected to determine resting adenosine levels. Subjects were then asked to perform intense dynamic handgrip at 50% of maximal voluntary contraction (MVC) for 15 minutes (5-second contractions at 10-second intervals). One 15-minute dialysate sample was collected during handgrip. Immediately after the release of handgrip, a proximal pneumatic cuff on the upper arm was inflated to 50 mm Hg above systolic BP and maintained for 2 minutes (posthandgrip coronary arrest). Two consecutive 15-minute recovery dialysate samples were collected immediately after exercise. MSNA was recorded continuously.

Subjects returned 3 weeks later for a second study day with a similar setup and protocol. Three interventions were performed in random order, 1 hour apart: (1) dynamic handgrip at 15% MVC for 15 minutes followed by posthandgrip coronary arrest, (2) dynamic handgrip at 15% MVC for 5 minutes, followed by posthandgrip coronary arrest, and (3) dynamic handgrip at 15% MVC for 5 minutes while ischemia was superimposed with a proximal pneumatic cuff inflated to 50 mm Hg above systolic BP, followed by posthandgrip coronary arrest. Dialysate samples were collected during each exercise period (15 or 5 minutes) and at 15-minute intervals during all resting periods. MSNA was continuously recorded. The 3 interventions included in this protocol were randomized.

**Drugs**

Adenosine was purchased from Sigma Chemical Co and prepared for human use by our investigational pharmacy as a 6 mg/mL solution. Lidocaine HCl 2% (Xylocaine) was purchased from Astra USA, Inc and dissolved in saline.

**Analytical Methods and Statistical Analysis**

Adenosine samples were analyzed using a microbore HPLC system (Isco microLC system; Isco Inc) according to the method of Jackson et al.

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Costa et al  Adenosine, Trigger of the Pressor Reflex  919

For protocol 1, MSNA was determined from the original tracings of the mean voltage neurograms using a digitizer tablet coupled to Sigma Scan software (Jandel Scientific). The amplitude of each “burst” was measured in millimeters, and total activity was defined as the sum of “burst” amplitude over 60-second periods and was expressed in arbitrary units. For protocol 2, data were analyzed with software written in our laboratory using PV-WAVE (Visual Numerics Inc). Bursts were detected with an automated detection algorithm with artifact elimination, dynamic noise level detection, and signal-to-noise estimation. Bursts were accepted if the signal-to-noise ratio was >2:1 and synchronization to the previous cardiac event was \(\pm 1.6\) seconds. To determine the latency of onset of sympathetic activation, values for MSNA were determined at 1-second intervals, and a 10-second sliding window average was used to generate each data point. Time 0 is the average of data from 5 seconds before to 5 seconds after injection. The effect of a given intervention on MSNA was expressed as percent change from the preceding resting period. A 3-minute period before injection was used as baseline, and the average and standard deviation values of all 1-second measurements were calculated. For each adenosine injection, we determined the time when MSNA exceeded a value equivalent to 2 SDs around this average. The latency of onset was defined as the time in seconds immediately before this threshold was reached. Individual latency of onset times from individual intra-arterial or intravenous adenosine injections were then averaged.

Statistical analysis was performed using \(t\) test for single comparisons and ANOVA for multiple comparisons. Post hoc tests (Duncan’s) were used to determine individual differences only if significant group differences were found with ANOVA. Values of \(P<0.05\) were considered significant. Results are expressed as mean±SEM.

Results

Effect of Interruption of Afferent Nerve Traffic on the Sympathetic Activation Induced by Intrabrachial Adenosine

Axillary ganglionic blockade inhibited sympathetic activation induced by 1, 2, and 3 mg intrabrachial adenosine from 57±19%, 95±30%, and 106±32%, respectively, to 6±8%, 24±9%, and 21±19% (n=5, \(P<0.05\), Figure 1A). The increase in mean BP after adenosine 1, 2, and 3 mg was also inhibited, from 7±2, 9±1, and 9±1 mm Hg, respectively, to 3±2, 4±2, and 5±3 mm Hg. These values were the MSNA and BP averaged over the first minute immediately after the injections.

Axillary ganglionic blockade inhibited the increase in MSNA produced by cold exposure (from 163±46% to 4±14%) and the increase in mean BP (from 19±5 to 4±2 mm Hg), demonstrating the effectiveness of ganglionic blockade (n=5, \(P<0.05\), Figure 1B).

Effect of the Route of Administration of Adenosine on the Magnitude and Latency of Onset of Sympathetic Activation

IV injections of 3 mg adenosine increased MSNA by 67±28%, with a latency of onset of 17±3 seconds (n=14, 95% CI 12 to 23 seconds, Figure 2A). Intrabrachial injections of adenosine produced a greater (248±49%) and earlier (7.6±1 seconds, 95% CI 6 to 10 seconds) increase in MSNA (n=13, Figure 2A). There was a significant difference in latency of onset of sympathetic activation produced by intrabrachial and intravenous adenosine injections as determined by 2-tailed unpaired \(t\) test (\(P=0.0031\)) or nonparametric (Mann-Whitney) test (\(P=0.0015\). Intrabrachial injections of adenosine produced a mild increase in mean BP (5.5±2.1 mm Hg at 27 seconds). IV adenosine did not produce significant changes in BP. Neither IV nor intrabrachial injections of saline (placebo) affected MSNA or BP (not shown). Likewise, there was no consistent effect of adenosine or saline on heart rate.

The increase in MSNA produced by intrabrachial injections of adenosine during venous occlusion was not as pronounced but sustained, compared with that produced by intrabrachial adenosine (Figure 2B). The onset latency was similar. Intrabrachial adenosine during arterial occlusion produced a moderate and delayed increase in MSNA.
(115±32% at 64 seconds, n=8, Figure 2B) and a more gradual and delayed increase in mean BP (5.6±1.5 mm Hg at 75 seconds). Representative neurograms taken after intrabronchial and intravenous injections of adenosine are shown in Figure 2C.

**Figure 3.** Effect of exercise intensity on adenosine levels and MSNA. A, Adenosine dialysate concentrations at rest (BASE) and during 15 minutes of moderate dynamic handgrip (15% maximal voluntary contraction, MVC, 5-second duration at 10-second intervals, EXER 15) and intense exercise (50% MVC, EXER 50). Bars represent 15-minute sample collections. B, Simultaneous recording of MSNA during moderate (○) and intense (●) exercise, during the last 2 minutes of exercise (EXER 14’, EXER 15’) and the 2 minutes of posthandgrip circulatory arrest (PHCA 1’, PHCA 2’). MSNA is expressed as percent change (%Δ) from the preceding resting period (n=7). Probability values are for t test (A) and ANOVA (B).

**Effects of Varying Exercise Intensity on Muscle Interstitial Adenosine and MSNA**

Adenosine muscle dialysate levels were higher during intense (50% MVC) handgrip (from 0.307±0.07 to 1.237±0.42 μmol/L, 528±292% increase from baseline) compared with moderate (15% MVC) handgrip (from 0.104±0.02 to 0.419±0.16 μmol/L, 303±99% increase, n=7, P<0.01, Figure 3A). The concentrations of adenosine returned to rest levels within 30 minutes after exercise.

We found a greater increase in MSNA (88±25%) during intense handgrip compared with moderate handgrip (38±28%, n=7, P=0.01, Figure 3B). MSNA remained elevated during posthandgrip circulatory arrest (58±21% and 28±31% above resting levels after intense and moderate handgrip, respectively).

**Effect of Combining Ischemia and Exercise on Muscle Interstitial Adenosine and MSNA**

Adenosine dialysate concentrations increased from 0.095±0.02 to 0.249±0.12 μmol/L during moderate dynamic exercise and from 0.095±0.02 to 0.476±0.19 μmol/L when ischemia was superimposed on moderate exercise (487±181% and 132±77% increase, respectively; n=7, P=0.01, Figure 4A). Similarly, a greater increase in MSNA was produced when ischemia was superimposed on handgrip (74±13% versus 40±9%, n=6, P<0.01, Figure 4B). MSNA remained elevated during posthandgrip circulatory arrest (47±17% after handgrip with superimposed ischemia and 16±9% after handgrip without ischemia).

There was a significant correlation between the percent increases in adenosine levels and MSNA produced by these interventions (r=0.96, P<0.01 by linear regression, Figure 5).

**Figure 4.** Effect of combining ischemia and exercise on muscle adenosine levels and MSNA. A, Adenosine dialysate concentrations at rest (BASE, 15-minute collections), during 5-minute moderate dynamic exercise handgrip (15% MVC, EXER), and ischemia superimposed on this level of exercise (EXER + ISC). B, Simultaneous recording of MSNA during exercise alone (○) or with superimposed ischemia (●) during the last 2 minutes of exercise (EXER 4’, EXER 5’) and the 2 minutes of posthandgrip circulatory arrest (PHCA 1’, PHCA 2’). MSNA is expressed as percent change (%Δ) from the preceding resting period (n=7). Probability values are for t test (A) and ANOVA (B).

**Figure 5.** Relationship between the increase in dialysate adenosine (Ado) and sympathetic activity produced by exercise and ischemia. X axis shows percent increase in dialysate adenosine from the corresponding resting period produced by moderate and intense exercise (data derived from Figure 3) and moderate exercise with or without superimposed ischemia (data derived from Figure 4), respectively. Y axis shows the percent increase in MSNA during the corresponding posthandgrip circulatory arrest periods.
5). For this analysis, we used the increase in MSNA observed during the posthandgrip circulatory arrest period because this sympathetic activity during this period is not confounded by central command or muscle mechanoreceptor activation and more closely reflects chemoreceptor activation.

Discussion

The main conclusions derived from this study are that, first, interruption of forearm muscle afferent nerve traffic with axillary blockade suppresses the increase in MSNA and BP induced by intrabradial adenosine, providing evidence that intrabradial adenosine triggers sympathetic activation through activation of neural afferents in the forearm; Second, this hypothesis is further supported by the observation that exogenous adenosine produces earlier and greater increases in MSNA and BP when administered intrabradially compared with intravenously. Third, exercise intensity and the presence of ischemia directly determine interstitial levels of adenosine in the forearm muscle, and these levels correlate with the magnitude of reflex sympathetic activation induced by these maneuvers.

Adenosine concentrations in the muscle are reported to increase during ischemia in animal models and in the human myocardium and pericardium. Previous attempts to measure adenosine concentrations from total tissue or from venous samples have resulted in inconsistent results. Because most adenosine contained in the muscle is of intracellular origin muscle sampling has not been helpful in estimating the amount of adenosine released into the extracellular space during exercise. In addition, the detection of any increase in interstitial adenosine is limited by cellular uptake and metabolism, mechanisms that are particularly efficient in humans. Consequently, the very short half-life of adenosine limits the possibility of obtaining reliable values from venous samples. Also, the endothelium constitutes an important barrier for adenosine, so interstitial adenosine may not completely reach the intravascular compartment; furthermore, it is likely that endothelial cells generate adenosine into the intravascular compartment. Adenosine determinations from venous samples therefore may not reflect muscle interstitium adenosine levels. The microdialysis technique has the advantage that the semipermeable microdialysis membrane excludes enzymes such as adenosine deaminase (molecular weight 41 kDa) that metabolize adenosine and cells that take up adenosine. Our results with this technique suggest that the release of adenosine during exercise is related to exercise intensity, because dialysate adenosine levels are higher during intense exercise (dynamic handgrip at 50% MVC) compared with during moderate exercise (15% MVC), and to the presence of ischemia, because dialysate concentrations of adenosine increased further when ischemia was superimposed on moderate exercise.

In addition, we found that the magnitude of sympathetic activation induced by these interventions correlated with the increase in interstitial adenosine. Parallel increases in adenosine and MSNA do not prove that one is the consequence of the other, but we have previously shown that the blockade of forearm adenosine receptors with intrabradial theophylline blunts the exercise pressor reflex induced by sustained handgrip. It should be noted that forearm ischemia imposed in a resting forearm is not sufficient to induce sympathetic activation or to increase muscle interstitial adenosine. This suggests that both ischemia and increased metabolic demands are required to induce the release of sufficient adenosine (and perhaps other metabolites) to stimulate muscle afferents and trigger sympathetic activation.

MacLean et al recently proposed that the sympathetic activation induced by injections of adenosine into the femoral artery is not due to stimulation of leg muscle afferents but rather to spillover of adenosine into the systemic circulation with subsequent activation of peripheral arterial chemoreceptors. This is at variance with our conclusions. We believe the explanation of this discrepancy lies in differences in the experimental design and doses used. Their protocol called for increasing the doses of adenosine injected into the femoral artery until an “unquestionable increase in MSNA above baseline” was obtained. This resulted in the use of larger doses in their study (range 2.5 to 9 mg) compared with ours (1 to 3 mg). We agree with their conclusion that these larger doses will result in spillover into the systemic circulation and activation of peripheral arterial chemoreceptors and sympathetic activation. We do not believe that the same applies to our studies. They observed an onset latency for the increase in MSNA of 15.8±0.8 seconds from the time of injection, whereas our onset latency was 8.5±1 seconds. We also found that during a 2-minute occlusion of the forearm circulation, used in this study to mimic MacLean et al’s protocol, the effects of intrabradial adenosine on MSNA and BP were significantly inhibited. We speculate that when the local circulation is blocked, adenosine injected intra-arterially does not reach the muscle afferents because it remains at the site of injection and is not mobilized or distributed within the limb until the circulation is reestablished. Furthermore, our comparisons between the effects of intra-arterial and intravenous adenosine injections clearly demonstrate that under the careful conditions of our study, intrabradial adenosine selectively activated muscle afferents in the forearm. Finally, the hypothesis that adenosine activates forearm afferents is strengthened by the fact that axillary ganglionic blockade blunted sympathetic activation induced by intrabradial adenosine.

From the results of the present study, we conclude that adenosine is released by the skeletal muscle during dynamic handgrip and that interstitial adenosine concentrations and sympathetic activation are directly related to exercise intensity and the presence of ischemia. Furthermore, both ischemia and increased metabolic demands are required for adenosine to be released and for the triggering of sympathetic activation. These results provide additional and conclusive evidence that adenosine activates muscle afferents in humans, a finding that is in agreement with its proposed role as a metabolic trigger of the exercise pressor reflex.

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References


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