Local adenosine receptor blockade accentuates the sympathetic responses to fatiguing exercise

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Cui J, Leuenberger UA, Blaha C, Yoder J, Gao Z, Sinoway LI. Local adenosine receptor blockade accentuates the sympathetic responses to fatiguing exercise. Am J Physiol Heart Circ Physiol 298: H2130–H2137, 2010. First published April 16, 2010; doi:10.1152/ajpheart.00083.2010.—The role adenosine plays in evoking the exercise pressor reflex in humans remains controversial. We hypothesized that localized forearm adenosine receptor blockade would attenuate muscle sympathetic nerve activity (MSNA) responses to fatiguing handgrip exercise in humans. Blood pressure (Finometer), heart rate, and MSNA from the peroneal nerve were assessed in 11 healthy young volunteers during fatiguing isometric handgrip, postexercise circulatory occlusion (PECO), and passive muscle stretch during PECO. The protocol was performed before and after adenosine receptor blockade by local infusion of 40 mg aminophylline in saline via forearm Bier block (regional intravenous anesthesia). In the second experiment, the same amount of saline was infused via the Bier block. After aminophylline, the MSNA and blood pressure responses to fatiguing handgrip, PECO, and passive stretch (all P < 0.05) were significantly greater than during the control condition. Saline Bier block had no similar effects on the MSNA and blood pressure responses. These data suggest that adenosine receptor antagonism in the exercising muscles may accentuate sympathetic activation during fatiguing exercise.

EXERCISE ELICITS INCREASES in muscle sympathetic nerve activity (MSNA), peripheral vasoconstriction, heart rate, cardiac output, and blood pressure (1, 21, 31). In addition to central mechanisms (i.e., central command) (39), inputs from mechanically and chemically sensitive afferents in the exercising muscle are important in evoking sympathetic activation (21, 24, 27). Group III and IV afferent fibers in muscles are suggested to be involved in this reflex (24, 40).

A number of substances are potential muscle afferent stimulants (16). However, the identity of the ischemic metabolites that engage the muscle metaboreceptors and activate the sympathetic nervous system is controversial (17). For example, interstitial adenosine concentration increases during exercise, and the adenosine level depends on the exercise strength (6, 7, 14). However, it is unclear if the released adenosine actually stimulates muscle afferents and evokes sympathetic excitation (15). An early study in cats suggested that adenosine had only trivial activating effects on group III and IV afferents in resting muscles (30). However, Costa and colleagues found that bolus infusion of adenosine in a brachial artery evoked an increase in MSNA in healthy humans (5, 6), whereas adenosine receptor antagonism with intrabrachial theophylline blocked the increase in MSNA during exercise (5). Thus these investigators concluded that adenosine stimulates the muscle afferents and evokes sympathetic activation. On the other hand, MacLean et al. (20) found that intra-arterial adenosine administered in the femoral artery did not evoke MSNA increase when the leg circulation was arrested. It should be noted that in these studies (5, 6, 20), which employed systemic drug infusions, nonspecific effects on other pools of afferents could not be excluded (20). Moreover, varied approaches and study designs (e.g., administration of adenosine or adenosine antagonists) in these different studies may have contributed to the different cardiovascular effects noted (5, 6, 20).

Middlekauff and colleagues reported that inhibition of adenosine receptors with aminophylline had no effect on the MSNA activation during low-level rhythmic forearm exercise in healthy individuals (25) and heart failure patients (26). They concluded that muscle mechanoreceptors are not sensitized by the adenosine produced during low-level rhythmic handgrip exercise. However, it is unknown whether the adenosine produced during intense exercise (e.g., fatiguing exercise) contributes to the sensitization of muscle mechanoreceptors, since adenosine concentration will depend on the strength of the exercise stimulus (6, 7, 14). In previous studies (9, 11), our data demonstrate that passive muscle stretch evokes a further increase in the already elevated MSNA levels seen during postexercise circulatory occlusion (PECO) that follows fatiguing handgrip. This suggested that muscle metabolites (9), including prostaglandins (11), sensitize the muscle mechanoreceptors. This approach has not been used to examine the role of adenosine in sensitization of muscle mechanoreceptors during high-intensity exercise.

Based on the previous observations, we speculate that adenosine in muscles may play a role in enhancing sympathetic responses during fatiguing exercise, probably via directly stimulating the muscle afferents and sensitizing the muscle mechanoreceptors. Thus we hypothesize that local adenosine receptor antagonism attenuates the MSNA responses to fatiguing exercise, PECO, and the passive stretch during PECO. The adenosine receptor antagonism in the exercising forearm was accomplished by local infusion of aminophylline via the regional intravenous anesthesia technique (Bier block) (3, 10, 11).

METHODS

Subjects. Eleven subjects [6 male, 5 female; age: 25 ± 1 (SE) yr; height: 170 ± 2 cm, weight: 69 ± 2 kg] participated in the study. All subjects were normotensive (supine blood pressures <140/90 mmHg), were not taking any medication, and were in good health. Subjects refrained from caffeine, alcohol, and exercise 24 h before the study. The experimental protocol was approved by the Institutional Review Board of the Milton S. Hershey Medical Center and conformed with the Declaration of Helsinki. Each subject had the pur-

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poses and risks of the protocol explained to them before written informed consent was obtained.

**Measurements.** Blood pressure was recorded on a beat-by-beat basis from a finger with a Finapres device (Finapres Medical System, Amsterdam, The Netherlands). Resting blood pressures obtained from the Finapres were verified by an automated sphygmomanometer (Dinamap; Critikon, Tampa, FL). A standard electrocardiogram was used to monitor heart rate. Respiratory excursions were monitored with pneumography. Multifiber recordings of MSNA were obtained with a tungsten microelectrode inserted in the peroneal nerve under the fibula head. A reference electrode was placed subcutaneously 2–3 cm from the recording electrode. The recording electrode was adjusted until a site was found in which muscle sympathetic bursts were clearly identified using previously established criteria (38). The nerve signal was amplified, band-pass filtered with a bandwidth of 500–5000 Hz, and integrated with a time constant of 0.1 s (Iowa Biomeengineering, Iowa City, IA). The nerve signal was also routed to a loudspeaker and a computer for monitoring throughout the study. Heart rate, blood pressure, MSNA, and respiratory excursions were recorded throughout the studies. Passive stretch and handgrip forces were measured with transducers. Brachial artery limb blood flow was determined by combined pulsed and echo Doppler ultrasound (ATL 5000; Philips Medical System, Bothell, WA). The Doppler shift signal was demodulated to provide instantaneous mean blood velocity data in real time. Blood flow was determined from both the blood velocity and the vessel cross-sectional area. To minimize the influences of arm position on the blood flow measurement, the position and “posture” of the arm were fixed with supports during rest and static handgrip exercise. The blood flow recording site was marked on the arm. Thus, although the arm moved during the Bier block procedure, it was placed in the same position and posture after Bier block as before. In other words, the relative position of the Doppler probe was the same before and after the Bier block.

**Experimental design.** All subjects were tested in the supine position. An intravenous catheter was inserted in the antecubital fossa of the nondominant arm. The maximal voluntary contraction (MVC) of the nondominant hand was tested during each visit. To ensure the strength of the passive stretch was as vigorous as possible without evoking pain, the stretch strength for each subject was tested before the study. A specifically designed brace with a joint at the wrist was used to support subjects’ forearm and hand. The hand was flexed in the dorsal direction [the extension of wrist (EOW)] as the force was measured with a digital force gauge (IMADA, DPS-220, Northbrook, IL). During EOW, the position of the forearm and wrist remained fixed. The maximal force used to stretch the muscles without inducing pain was obtained during the first visit and was used for all stretch protocols performed on the two study days. No subjects complained of pain with EOW on day 2.

**Preaminophylline trial (control trial).** After instrumentation, 6-min baseline measures of heart rate, blood pressure, MSNA, and respiratory excursion were collected with the subject in the resting condition. Rest brachial artery blood flows of the two arms were assessed alternately. Each subject then performed static isometric handgrip at 30% MVC to fatigue followed by 4 min of PECO. A visual force indicator was used so that subjects could maintain the force necessary to maintain 30% MVC. During grip, subjects were asked to report their perceived level of effort using the Borg Scale of 0–20 (2). The determination of fatigue rested with: 1) the inability of the volunteer to maintain the desired force production; and 2) the assessment of the volunteers that the work was “extremely hard.” When a Borg scale of ∼19 (extremely hard) was reported, a cuff on the upper arm was inflated to 250 mmHg before the subject stopped grip. The PECO was performed for 1.5 min, which started at 1 min (5 subjects) or 2.5 min (6 subjects) from the onset of cuff inflation. Passive stretch was employed to determine whether adenosine produced during contraction contributes to the sensitization of mechanoreceptors. The presented PECO data were obtained in a 1.5-min window, which started at 2.5 or 1 min from the onset of cuff inflation (see Fig. 1). The first minute data of PECO were not included. Subjects did not complain of any pain caused by the EOW during PECO. During the handgrip, brachial arterial blood flow of the exercising arm was measured.

**Bier block.** After 10–15 min of recovery from the control trial, a modified Bier block procedure was used to regionally administer aminophylline in the forearm. Aminophylline is a nonspecific adenosine receptor antagonist that is clinically used in therapy for respiratory diseases such as asthma. To drain the forearm vasculature, the arm was elevated. The arm was fitted with occlusion cuffs arranged in a continuous fashion from the wrist to the elbow (3–4 cuffs). A final cuff was placed on the upper arm. From the wrist to the upper arm, the cuffs were inflated to 250 mmHg in sequence. Next, the cuffs on the forearm were deflated and removed, and the upper arm cuff remained inflated. Thereafter, 40 mg aminophylline (Aminophylline Injection; American Regent, Shirley, NY) in 40 ml of saline were infused in the occluded arm via the catheter. This allows the drug to distribute in the previously emptied vascular system and to diffuse into the forearm tissue. The dose of aminophylline was determined in pilot studies, and the effect of adenosine receptor blockade was verified during a separate visit (see below). After 20 min, the cuff was deflated and the subjects rested for an additional 15–20 min.

**Aminophylline trial.** Another 6 min of baseline data were collected, and the blood flows of both arms were measured at rest. The handgrip exercise at the same intensities as those employed in the preaminophylline trial followed by 4 min PECO was repeated. The passive stretch was performed at the same strength and the same time window during PECO as those in the preaminophylline trial. During the handgrip, brachial arterial blood flow of the exercising arm was measured.

To separate the effects of aminophylline from the Bier block procedure itself, a control study (saline Bier block) was performed in all subjects on another visit to the laboratory. The saline Bier block study and the aminophylline study were performed in a random sequence. Two studies were separated by 1 mo. Hemodynamic variables and MSNA were recorded in the same fashion during the two visits. The forearm blood flows were measured in 6 subjects during the saline Bier block study and in all 11 subjects during the aminophylline study. The presaline trial (control trial) in the control study was the same as the preaminophylline trial. During the Bier block procedure, only 40 ml saline (no aminophylline) were infused in the arm. The handgrip exercise and PECO protocol were repeated for the saline Bier block trial.

The effect of the adenosine receptor blockade was verified in three subjects on another visit to the laboratory. MSNA was not recorded while other variables were measured during this visit. The blood flows of both arms were measured simultaneously with echo Doppler ultrasound (ATL 5000; Philips Medical System). A saline Bier block was performed. After 15 min, 3 mg of adenosine were infused through the venous catheter while the blood flow of both brachial arteries was measured simultaneously. After over 40 min rest, the Bier block with aminophylline, as described above, was performed. After 15 min rest, 3 mg of adenosine were infused through the venous catheter while the blood flow velocity was measured in both arms simultaneously.

**Data analysis.** Data were sampled at 200 Hz via a data acquisition system (MacLab; AD Instruments, Castle Hill, Australia). MSNA bursts were first identified in real time by visual inspection of the data, coupled with the burst sound from the audio amplifier. These bursts were further evaluated by a computer software program that identified bursts based on fixed criteria, including an appropriate latency following the R-wave of the electrocardiogram (8, 10). Integrated MSNA was normalized by assigning a value of 100 to the mean amplitude of the largest 10% of the bursts during the 6-min baseline period. Normalization of the MSNA signal was performed to reduce

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variability between subjects attributed to factors including needle placement and signal amplification. Total MSNA was identified from burst area of the integrated neurogram (8, 10). Mean arterial pressure (MAP) was calculated from the Finometer waveform during handgrip exercise and PECO, whereas the baseline MAP was verified by an automated sphygmomanometer from an upper arm. Forearm vascular conductance (FVC) was calculated from the MAP and forearm blood flow. MSNA, MAP, heart rate, and FVC during the last minute of handgrip were used for the fatiguing exercise.

Statistics. Differences in the absolute values of hemodynamic parameters among the baselines before the four exercise trials were evaluated via repeated-measures one-way ANOVA. Differences in the absolute values of the hemodynamic parameters between respective baseline, the last minute of fatiguing exercise, PECO, and passive stretch during PECO (PECO + EOW) were evaluated for each trial via Tukey’s post hoc analysis after repeated-measures one-way ANOVA. Changes in hemodynamic parameters from the prior exercise baseline to the last minute of fatiguing exercise (i.e., ΔMAP, Δheart rate, and ΔMSNA) were used to examine two main effects: 1) the effect of the Bier block procedure (factor 1: before vs. after); and 2) the effects of aminophylline (factor 2: aminophylline vs. saline) via repeated measures two-way ANOVA. When appropriate, Tukey’s post hoc analyses were employed. In a similar manner, changes from the prior exercise baseline to PECO and the changes from the prior exercise baseline to passive stretch during PECO were compared “across” Bier block and the drugs, respectively. The difference in MSNA response to EOW (changes from PECO only to PECO + EOW) before and after aminophylline was evaluated using a Student’s t-test. The differences between the absolute values of baseline FVC of each arm before and after the Bier block were evaluated using a Student’s t-test for the paired data. The effects of exercise and Bier block on “absolute” levels of FVC were analyzed using a two-way ANOVA. All values are reported as means ± SE. P values of <0.05 were considered statistically significant.

RESULTS

Baseline values for MSNA, blood pressure, and heart rate obtained before the four trials did not differ (Table 1). Recordings of heart rate, integrated MSNA, and blood pressure during the handgrip, PECO, and PECO + EOW in a representative subject are shown in Fig. 1. Isometric fatiguing handgrip evoked increases in MSNA, heart rate, and MAP in the four trials (P < 0.001). After the aminophylline Bier block, MSNA and MAP responses (i.e., changes from the rest baseline) during the last minute of handgrip before fatigue were significantly greater than that in the control trial (preaminophylline trial) (Fig. 2 and Table 2). In contrast, the saline Bier block had no similar effect on the MSNA response to handgrip. Thus, after the aminophylline Bier block, the MSNA responses to exercise were significantly greater than those after saline Bier block (Fig. 2). There were no significant differences in the heart rate responses during exercise in the trials.

MSNA and MAP during PECO in all of the four trials were significantly greater than the prior exercise baseline (all P < 0.001). When expressed as change from prior exercise baseline, MSNA and MAP during PECO (without passive stretch) after aminophylline were significantly greater than the control trial (Fig. 3 and Table 2). The saline Bier block had no similar effect on the MSNA response to the PECO. There was no significant difference in heart rate responses to PECO between the trials. The MSNA and MAP changes from the baselines during the passive stretch under PECO conditions (PECO + EOW) after aminophylline were significantly greater than the control trial (Fig. 4 and Table 2). The passive stretch caused increases in MSNA from the PECO only condition in both control and after aminophylline trials. However, there was no significant difference in MSNA responses (changes from PECO only to PECO + EOW) by the passive stretch between the trials before and after aminophylline (116 ± 34 to 147 ± 76 units/min, P = 0.59).

There was no significant difference in the Borg scale between the four trials (before aminophylline: 18.8 ± 0.2; after aminophylline: 18.6 ± 0.2; before saline: 18.7 ± 0.1; after saline: 18.8 ± 0.2; 2-way ANOVA; P = 0.68 for Bier block; P = 0.80 for the drugs; P = 0.19 for the interaction), which were reported by subjects at the end of the exercise. However, exercise duration decreased significantly after the Bier block (2-way ANOVA; P = 0.03 for Bier block; P = 0.92 for the interaction). After aminophylline Bier block, the exercise duration was significantly shorter than that before aminophylline (239 ± 24 to 194 ± 19 s, P = 0.04). After the saline Bier block, the exercise duration tended to be shorter than that before saline (235 ± 22 to 193 ± 16 s, P = 0.06). There was no significant difference in the exercise duration between the trial after aminophylline and the trial after saline (P = 0.97).

Administration of aminophylline with Bier block significantly increased resting FVC in the treated arm (45.3 ± 9.1 to 65.6 ± 16.3 ml·min⁻¹·100 mmHg⁻¹, P < 0.03, N = 11) but did not affect resting FVC of the untreated arm (43.4 ± 7.8 to 47.0 ± 10.8 ml·min⁻¹·100 mmHg⁻¹, P = 0.75, N = 11). At the end of fatiguing exercise, FVC in the exercising arm increased significantly from the rest baselines in both the control and the aminophylline trials (Fig. 5). However, there was no significant difference in the absolute FVC during the last minute of exercise between the control and the aminophylline trials (P = 0.32). Thus, the increase in FVC evoked by exercise (i.e., the change from the baseline) after aminophylline tended to be less than that before aminophylline (from 103 ± 20 to 67 ± 15 ml·min⁻¹·100 mmHg⁻¹, P = 0.09). The saline Bier block did not significantly affect the resting FVC in either the treated (51.2 ± 10.0 to 52.5 ± 12.5 ml·min⁻¹·100 mmHg⁻¹, N = 6) or untreated (47.4 ± 7.7 to 43.6 ± 8.5 ml·min⁻¹·100 mmHg⁻¹, N = 6) arm.

After aminophylline Bier block, bolus intravenous infusion of 3 mg adenosine induced a transient decrease in blood

Table 1. Prior exercise baselines before and after administration of aminophylline or saline

<table>
<thead>
<tr>
<th></th>
<th>Before Aminophylline</th>
<th>After Aminophylline</th>
<th>Before Saline</th>
<th>After Saline</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mmHg</td>
<td>127 ± 3</td>
<td>129 ± 4</td>
<td>123 ± 4</td>
<td>126 ± 4</td>
<td>0.39</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>64 ± 2</td>
<td>67 ± 2</td>
<td>65 ± 2</td>
<td>65 ± 2</td>
<td>0.38</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>85 ± 2</td>
<td>87 ± 2</td>
<td>83 ± 2</td>
<td>84 ± 2</td>
<td>0.16</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>65 ± 2</td>
<td>65 ± 2</td>
<td>66 ± 2</td>
<td>63 ± 2</td>
<td>0.34</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>14 ± 2</td>
<td>13 ± 2</td>
<td>12 ± 2</td>
<td>12 ± 2</td>
<td>0.65</td>
</tr>
<tr>
<td>MSNA, units/min</td>
<td>177 ± 22</td>
<td>176 ± 26</td>
<td>189 ± 28</td>
<td>165 ± 28</td>
<td>0.66</td>
</tr>
<tr>
<td>Respiration, cycles/min</td>
<td>16 ± 1</td>
<td>18 ± 1</td>
<td>18 ± 1</td>
<td>17 ± 1</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Values are means ± SE. SBP, DBP, and MAP: systolic, diastolic, and mean arterial blood pressures, which were measured by an automated sphygmomanometer from an upper arm; MSNA: muscle sympathetic nerve activity. There is no significant difference in the measurements among the trials. P: P value from the 1-way repeated ANOVA.
pressure (9.8 ± 2.4 mmHg) and a transient increase in heart rate (21.8 ± 12.8 beats/min). After aminophylline Bier block, bolus intravenous infusion of adenosine induced a transient but clear increase in FVC in the control arm (65.1 ± 16.9% changes from the prior adenosine baseline), but not in the treated arm (5.3 ± 8.4%). The bolus intravenous infusion of adenosine after saline Bier block also induced a transient decrease in blood pressure (12.6 ± 3.5 mmHg) and a transient increase in heart rate (15.2 ± 8.4 beats/min). After saline Bier block, bolus intravenous infusion of adenosine induced clear FVC increases in both the control arm (95.3 ± 15.2% change from the prior adenosine baseline) and the treated arm (67.1 ± 20.5%).

**DISCUSSION**

The main findings of this study are that local administration of aminophylline in the exercising forearm accentuates the MSNA responses to fatiguing exercise and the postexercise ischemia. The local administration of aminophylline does not affect the MSNA response to passive stretch performed during the period of ischemia that follows fatiguing exercise. These results rejected our hypothesis. Thus adenosine receptor antagonism in the exercising muscles increases sympathetic activation during fatiguing exercise.

Adenosine, a metabolic by-product of ATP utilization, has potent vasodilator effects in cardiac and skeletal muscle (4). Previous studies have demonstrated that adenosine is present in the human muscle interstitium during exercise (6, 7, 14). Adenosine has been proposed to be involved in the regulation of many processes of importance in human skeletal muscle during exercise, such as blood flow (13) and by inducing local vasodilation in response to ischemia (12). Aminophylline (a combination of theophylline and the pharmacologically inactive solvent ethylendiamine), a nonspecific adenosine receptor antagonist, has been used to block adenosine receptors in previous studies (5, 18, 25, 26). In these previous studies,
aminophylline was administered in a brachial artery at varied doses/rates. In the present study, 40 mg of aminophylline were administered with the Bier block method. The following evidence demonstrates that the adenosine receptors were only blocked in the treated arm by aminophylline: 1) the intravenous bolus of adenosine did not cause vasodilation in the aminophylline Bier block forearm but did cause vasodilation in the untreated arm and in the postsaline Bier block situation; and 2) there were no differences in baseline MSNA, heart rate, and blocked in the treated arm by aminophylline: 1) the intravenous bolus of adenosine did not cause vasodilation in the aminophylline Bier block forearm but did cause vasodilation in the untreated arm and in the postsaline Bier block situation; and 2) there were no differences in baseline MSNA, heart rate, and

Table 2. Mean blood pressure and heart rate response during fatiguing handgrip exercise, postexercise circulatory occlusion, and passive muscle stretch before and after aminophylline or saline

<table>
<thead>
<tr>
<th></th>
<th>Before Aminophylline</th>
<th>After Aminophylline</th>
<th>Before Saline</th>
<th>After Saline</th>
<th>P for Bier Block</th>
<th>P for Drugs</th>
<th>P for Interaction</th>
</tr>
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<tbody>
<tr>
<td>ΔMAP, mmHg</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>HG</td>
<td>18 ± 2</td>
<td>27 ± 2*</td>
<td>22 ± 2</td>
<td>23 ± 2</td>
<td>0.02</td>
<td>0.68</td>
<td>0.04</td>
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<tr>
<td>PECO</td>
<td>14 ± 2</td>
<td>22 ± 2*</td>
<td>19 ± 3</td>
<td>19 ± 3</td>
<td>0.17</td>
<td>0.97</td>
<td>0.001</td>
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<tr>
<td>PECO + EOW</td>
<td>18 ± 2</td>
<td>26 ± 2*</td>
<td>22 ± 3</td>
<td>23 ± 4</td>
<td>0.03</td>
<td>0.97</td>
<td>0.04</td>
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<tr>
<td>ΔHeart rate, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HG</td>
<td>21 ± 2</td>
<td>20 ± 2</td>
<td>23 ± 4</td>
<td>27 ± 4</td>
<td>0.16</td>
<td>0.17</td>
<td>0.06</td>
</tr>
<tr>
<td>PECO</td>
<td>6 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>7 ± 2</td>
<td>0.86</td>
<td>0.70</td>
<td>0.05</td>
</tr>
<tr>
<td>PECO + EOW</td>
<td>8 ± 2</td>
<td>7 ± 3</td>
<td>5 ± 2</td>
<td>6 ± 2</td>
<td>0.89</td>
<td>0.27</td>
<td>0.13</td>
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</table>

The values are changes (Δ) from the prior exercise baseline. HG, the last minute of fatiguing exercise; PECO, postexercise circulatory occlusion (without stretch); PECO + EOW, passive muscle stretch by flexing the in the dorsal direction under PECO condition. Mean arterial blood pressure (MAP) was measured by Finometer. *P < 0.02 vs. before aminophylline. These differences were evaluated via Tukey’s post hoc analysis after repeated-measures 2-way ANOVA across Bier block (before vs. after) and the drugs (aminophylline vs. saline).
blood pressure values seen during the four trials, which suggests that the infused drug had a limited systemic effect. Thus local infusion of aminophylline via the Bier block method evoked local adenosine receptor blockade with no measureable systemic effect.

Previous studies had shown that intra-arterial aminophylline increases baseline FVC (18). A possible explanation for this baseline shift is that aminophylline is a phosphodiesterase inhibitor, as well as an aminophylline receptor antagonist, which likely resulted in increased baseline cAMP levels in the vascular smooth muscle, and a consequent increase in FVC (18). In the present study, the baseline FVC of the treated arm was elevated after aminophylline, whereas the FVC of the untreated arm was not affected. These data also suggest that the effects of the aminophylline were mainly localized to the treated arm. It is interesting to note that there were no significant differences in the maximal FVC (absolute value) seen during fatiguing exercise in the aminophylline and the control trials, although the baseline FVC in the aminophylline trial was higher and likely due to the direct dilator effects of aminophylline. Interestingly, when this higher baseline is taken into consideration, the vasodilatation evoked by exercise tended to be attenuated after the blockade of adenosine receptors. This effect has been suggested in previous studies (22, 23).

The exercise duration after Bier block was shorter than that before the Bier block. A previous study (35) demonstrated that previous fatiguing contraction shortens the exercise duration of the subsequent fatiguing contraction. Thus the accentuated MSNA responses after aminophylline are not likely due to an increase in the relative intensity of handgrip, since the exercise duration after aminophylline was not different from that after saline. There was no difference in the Borg scales (i.e., perceived exertion) between the trials, which was reported by the

![Fig. 4. MSNA response during passive stretch in PECO condition before and after aminophylline or saline Bier block. The passive stretch started at 2.5 or 1 min from the onset of cuff inflation (see Fig. 1). The values are changes from the prior exercise baseline. The changes were analyzed across the Bier block procedure (before vs. after) and the drugs (aminophylline vs. saline) with repeated-measures 2-way ANOVA. For MSNA burst rate change (top), P = 0.005 for Bier block, P = 0.01 for the drugs, and P = 0.03 for the interaction. For MSNA total activity change (bottom), P = 0.01 for Bier block, P = 0.01 for the drugs, and P = 0.22 for the interaction. *P ≤ 0.005 vs. respective control trial. #P ≤ 0.003 vs. aminophylline Bier block trial.

![Fig. 5. Absolute values of forearm vascular conductance (FVC) in the exercising (treated) arm during the fatiguing handgrip exercise before and after aminophylline or saline Bier block. Data were analyzed across the exercise (baseline vs. last minute of the fatiguing exercise) and the Bier block procedure (before vs. after) via a 2-way ANOVA. For the aminophylline trial (top), P < 0.001 for the drugs, P = 0.01 for the exercise, and P = 0.21 for the interaction. For the saline trial (bottom), P = 0.02 for the exercise, P = 0.39 for Bier block, and P = 0.25 for the interaction. Administration of aminophylline significantly increased prior exercise baseline (resting) FVC in the treated arm; however, there was no significant difference in the absolute FVC during the last minute of fatiguing exercise between the control and the aminophylline trials (top). Subject number: N = 11 for before and after aminophylline trials; N = 6 for before and after saline trials. Units are ml·min⁻¹·100 mmHg⁻¹. *P < 0.05 vs. prior exercise baseline. #P < 0.03 vs. baseline FVC before aminophylline.
subjects at the end of handgrip. Moreover, there was no difference in the heart rate responses among the trials, which suggests that central command input was similar (41). Thus we speculate that adenosine in exercising muscles may play a role in inhibition/attenuation of activity of muscle afferents activated by exercise.

This result is contrasted with the conclusion from the studies of Costa and colleagues (5–7). Costa et al. (5–7) showed that intra-arterial bolus infusion of adenosine (up to 4 mg) induced MSNA activation. These authors thus concluded that adenosine might activate muscle afferent nerves, triggering reflex sympathetic activation. However, in anesthetized animals, injection of adenosine in the arterial supply of hindlimb skeletal muscle did not evoke a pressor reflex (37). Moreover, 2-chloroadenosine, an adenosine analog, had only trivial effects on the discharge of group III and IV muscle afferents in resting cats (30). In humans, MacLean et al. (20) demonstrated that the inflation of a leg cuff to 220 mmHg abolished MSNA activation by the intra-arterial bolus infusion of adenosine, which was injected distal to the leg cuff. Thus their data suggested that the MSNA response to the adenosine injection could be evoked via some other chemically sensitive pool of afferents in the system, but not directly via stimulating the thin fiber muscle afferents in the leg (20).

In the study by Costa and Biaggioni (5), the MSNA and blood pressure responses to 3 min handgrip exercise at 30% MVC and postexercise ischemia were abolished after intra-arterial infusion of aminophylline. The present data showed that MSNA and blood pressure responses to fatiguing exercise and PECO were accentuated after aminophylline was administered via Bier block. The differences in the drug administration methods might contribute to the differences in the results. It should be noted that, in the studies of Middlekauff and colleagues (25, 26), the administering method and dose for aminophylline were similar to those employed in the study of Costa et al. (5), whereas the MSNA response to low levels of rhythmic handgrip exercise (25, 26) was not abolished.

We previously observed that passive muscle stretch during PECO caused a further MSNA increase (9), probably because of stimulation of sensitized mechanoreceptors. In the present study, the passive muscle stretch during PECO evoked further MSNA increases in both control and aminophylline trials. However, there was no significant difference in the responses to the stretch (i.e., the change from PECO only to PECO + EOW) before and after the aminophylline. These data suggest that adenosine may not be involved in sensitizing mechanoreceptors during fatiguing exercise. This result is consistent with the observations of Middlekauff and colleagues (25, 26). In those studies, the MSNA response to a 3-min light rhythmic handgrip exercise paradigm was not affected by intra-arterial aminophylline in either healthy subjects (25) or those with congestive heart failure (26). Thus, their data suggest that adenosine produced during light rhythmic exercise does not sensitize the mechanoreceptors. Similarly, our data suggest that adenosine produced during intense exercise does not sensitize the mechanoreceptors.

We can only speculate on the mechanism(s) responsible for accentuated MSNA responses seen after aminophylline. One possibility is that adenosine may directly inhibit muscle afferent activity. Adenosine and adenosine analogs inhibit capsaicin-mediated vanilloid receptor 1 activation (29), and this type of receptor may be involved in evoking the muscle pressor reflex (19, 36). It should be noted that adenosine exerts complex effects on peripheral sensory nerves. For example, it can inhibit or augment pain by actions on nociceptive afferents via adenosine A1 and A2A receptors, and this results from decreases and increases in cAMP, respectively (33, 34). However, the population of specific receptor subtypes activated during exercise is not clear. The subtype of the adenosine receptors, which may predominately play a role in inhibition/attenuation of muscle afferent activity during exercise, should be examined in further studies. It is also possible that adenosine in exercising muscle may indirectly cause attenuation of sympathetic responses. It is known that adenosine contributes to the vasodilation in exercising muscles (22, 23, 28, 32). Adenosine receptor blockade reduces the vasodilation during exercise (23), which is also suggested in the present study. Thus it is possible that interstitial metabolite accumulation in the exercising muscles was increased by adenosine receptor blockade, which in turn would cause a greater MSNA response. In other words, adenosine in exercising muscle induces vasodilation, which decreases the concentration of metabolites, and in turn the muscle “metaboreceptor” activity decreases. This can be another possible mechanism for the role of adenosine in attenuating the sympathetic activation during exercise. To verify this speculation, the concentration of metabolites and flow should be examined in future studies.

Study limitations. Because the half-life of aminophylline is over 2 h, the aminophylline trial was always performed after the control trial. Thus, we cannot exclude some order effects. To separate these factors, the saline Bier block trial was performed. As in the previous studies (5, 25, 26), only aminophylline was employed as an adenosine receptor blockade in the present study. It is unclear whether aminophylline itself can affect the production of metabolites, and/or alter the sensitivity of afferent fibers. Thus, it will be necessary to employ other adenosine antagonists to verify our observations. Aminophylline is a nonspecific adenosine antagonist. Thus, the subtype of the adenosine receptors engaged cannot be determined. However, there is an obvious limitation to the drugs that can be employed safely in human studies. Therefore, animal studies with more specific drugs will be necessary to verify whether adenosine inhibits the muscle afferent activity during exercise, and to examine the subtype of the receptors engaged.

In conclusion, the present results show that MSNA responses to fatiguing exercise and the postexercise ischemia are increased after local administration of aminophylline. These observations suggest that adenosine receptor antagonism in muscles accentuates sympathetic activation during fatiguing exercise.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

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