Sympathetic cholinergic nerve contributes to increased muscle blood flow at the onset of voluntary static exercise in conscious cats

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Am J Physiol Regul Integr Comp Physiol 295: R1251–R1262, 2008. First published August 13, 2008; doi:10.1152/ajpregu.00076.2008.—We examined whether a sympathetic cholinergic mechanism contributed to increased blood flow of the exercising muscle at the onset of voluntary static exercise in conscious cats. After six cats were operantly conditioned to perform static bar press exercise with a forelimb while maintaining a sitting posture, a Transonic or pulsed Doppler flow probe was implanted on the brachial artery of the exercising forelimb, and catheters were inserted into the left carotid artery and jugular vein. After the baseline brachial blood flow and vascular conductance decreased and became stable in progress of postoperative recovery, the static exercise experiments were started. Brachial blood flow and vascular conductance began to increase simultaneously with the onset of exercise. Their initial increases reached 52 ± 8% and 40 ± 6% at 3 s from the exercise onset, respectively. Both a sympathetic ganglionic blocker (hexamethonium bromide) and atropine sulfate or methyl nitrate blunted the increase in brachial vascular conductance at the onset of static exercise, whereas an inhibitor of nitric oxide synthesis (N\(^\text{\textsuperscript{-}}\)-nitro-L-arginine methyl ester) did not alter the increase in brachial vascular resistance. Brachial blood flow and vascular conductance increased during natural grooming behavior with the forelimb in which the flow probe was implanted, whereas they decreased during grooming with the opposite forelimb and during eating behavior. Thus it is likely that the sympathetic cholinergic mechanism is capable of evoking muscle vasodilatation at the onset of voluntary static exercise in conscious cats.

exercise hyperemia; acetylcholine and nitric oxide; autonomic blockade; central command; voluntary behavior

SYMPATHETIC CHOLINERGIC NERVE fibers innervate blood vessels of skeletal muscle in several species, especially cat, dog, sheep, fox, mongoose, and jackal (9, 12). Activation of sympathetic cholinergic nerve elicited by stimulation of the hypothalamic or midbrain defense area increases skeletal muscle blood flow in anesthetized or decerebrate cats (1, 2, 18, 26, 31). Recently, we have found, using an X-ray angiography in anesthetized cats, that stimulation of the hypothalamic defense area evokes remarkable increases in the internal diameter and cross-sectional area of small arteries in the hindlimb triceps surae muscle, which are abolished by atropine (32, 33). Such vasodilatation in skeletal muscle is observed in the conscious cat during the immobile alerting stage of the defense reaction or during fighting behavior produced by electrical stimulation of the hypothalamus or by auditory, visual, and cutaneous stimuli (2–4). Since the muscle vasodilatation is attenuated by atropine, the sympathetic cholinergic system plays a role in eliciting a preparatory increase in muscle blood flow in association with an emotional state of the defense reaction or fighting (2, 19). Furthermore, an existence of the sympathetic cholinergic mechanism in humans has been suggested, because emotional stress increases forearm blood flow, and the increase in forearm blood flow is blocked by either atropine or sympathetectomy (7, 8, 37). Thus, if centrally induced activation of the sympathetic cholinergic nerve to skeletal muscle may happen during voluntary exercise, a profound vasodilatation of small arteries will be expected to increase upstream muscle blood flow.

Whether or not the sympathetic cholinergic system is functionally operating during voluntary exercise in conscious animals or humans is still controversial. Ellison and Zanchetti (19) reported an interesting finding using conscious cats that atropine-sensitive vasodilatation in skeletal muscle occurred at the time of striking movements during fighting behavior and at the time of hindlimb flexion movement evoked by a conditioned auditory stimulus preceding an unconditioned electrical shock to a hind paw. The evidence implied a possibility that central activation of the sympathetic cholinergic nerve to skeletal muscle may contribute to vasodilatation, not only during fighting behavior or a classical conditioning task, but also during voluntary exercise. Sanders et al. (40) observed using humans that an increase in muscle blood flow in the nonexercising forearm at the beginning of static handgrip exercise was blocked by atropine, but not by propranolol. Other studies, however, reported no significant influences of surgical sympathectomy or ganglionic or muscarinic blockade on the increase in muscle blood flow in the exercising skeletal muscle during treadmill exercise in dogs (11, 15) and during dynamic or static forearm contraction in humans (10, 13, 24). The absence of sympathetic cholinergic vasodilatation in the exercising limb is surprising, because central stimulation of the hypothalamic or midbrain defense area is capable of causing a substantial increase in muscle blood flow via activation of the sympathetic cholinergic nerve (26, 31–33). As a reason responsible for the discrepancy, it should be taken into account that the sympathetic cholinergic vasodilatation might be masked by other mechanisms, such as vasodilatation due to metabolites released in contracting muscle, flow-mediated vasodilatation, and/or muscle pump in association with rhythmical contraction (28, 38, 42).

We hypothesized that the centrally induced sympathetic cholinergic vasodilatation is functionally operating during a
more voluntary type of exercise with a smaller muscle mass, instead of treadmill exercise with the whole body mass. To test this hypothesis, we examined whether sympathetic ganglionic and muscarinic blockers would blunt the responses in brachial blood flow and vascular conductance of the exercising forelimb during voluntary static exercise in conscious cats. In addition, we examined the effect of an inhibition of nitric oxide synthesis on the responses in brachial blood flow and vascular conductance to test a role of nitric oxide-mediated vasodilatation in exercise hyperemia.

**METHODS**

The present study was conducted using seven cats weighing 3.3 ± 1.2 kg. Six cats were used for voluntary static exercise protocol, and three cats for electrically evoked muscle contraction protocol. Two cats were used for both protocols. The experimental protocols were approved by the Committee of Research Facilities for Laboratory Animal Science, Natural Science Center for Basic Research and Development, Hiroshima University. All experiments were performed in accordance with the “Guiding Principles for the Care and Use of Animals in the Fields of Physiological Sciences” approved by the Physiological Society of Japan.

*Exercise training.* The six cats were operantly conditioned to sit quietly in a transparent plastic box (width 35 × height 40 × depth 50 cm) with a small window (width 5 × height 7 cm), extend the dominant forelimb through the window, and press a bar while maintaining a sitting posture, as previously described in detail (14, 21, 27, 29). As long as the cats produced an appropriate force to press the bar, a sound of a buzzer was emitted as an audio feedback. If the animal completed static exercise for a certain period, food was given as reward. The training for static exercise was continued over a period of 2–4 mo (5 days/wk). When the force applied to the bar during exercise was measured in four cats, the peak force during exercise was 317–375 g in the absence of any drugs, which corresponded to 11% of their body weight (Table 1).

*Implantation surgery.* After finishing the training procedure, sterile surgery was conducted to implant catheters and a Doppler flow probe. After an overnight fast, atropine sulfate (0.1–0.2 mg/kg im) was given as a preanesthetic medication to reduce salivation and bronchial secretion. Anesthesia was introduced by inhalation of a mixture of 4% halothane (Fluothane, Takeda Chemical Industries, Osaka, Japan), N₂O (0.5 l/min), and O₂ (1.0 l/min), and an endotracheal tube was inserted. Subsequently, the cats inhaled the halothane-N₂O-O₂ mixture through the endotracheal tube. Electrocardiogram, heart rate (HR), rectal temperature, and respiration were continuously monitored. To maintain an appropriate level of surgical anesthesia, the concentration of halothane was usually preset at 1–1.5% and increased up to 2–2.5%, if an increase in HR and/or respiration and/or withdrawal of the limb in response to noxious pinch of the paw and/or a surgical procedure was observed. Rectal temperature was maintained at 37–38°C with a heating pad. Polyurethane catheters were inserted into the left external jugular vein for administering drugs and into the left carotid artery for measuring arterial blood pressure (AP). We implanted a Transonic Doppler flow probe (1.5R or 2R, Transonic System) on the right brachial artery in four cats, or a pulsed Doppler flow probe (lumen size, 2.0 mm) in two cats. A cable of the flow probe and the arterial and venous catheters were subcutaneously tunnelled and were brought to the exterior in the interscapular region. After finishing implantation surgery, antibiotics (benzylpenicillin potassium, 20,000 U/kg im) were injected, and the cats were housed in their cages. Antibiotics (benzylpenicillin benzathine, Bicillin tablets, 100,000 units, Banyu Pharmaceutical, Tokyo, Japan) were orally given for 5–7 postoperative days.

*Recording of data.* Brachial blood volume flow was measured with the flow probe connected to a Transonic Doppler blood flowmeter (T206, Transonic System). Brachial blood flow velocity was measured with the Doppler flow probe connected to a pulsed Doppler flowmeter (model 200, Triton Technology). Mean AP (MAP) was measured through the carotid artery catheter connected to a pressure transducer (DPTIII, Baxter, Tokyo, Japan). HR was derived from the arterial pressure pulse by a tachometer (model 1321, GE Marquette Medical Systems, Tokyo, Japan). The onset and end of static exercise were manually marked with an electric switch. The force that the cats applied to the bar was measured with strain gauges (KFG-N2-120, Kyowa Electronic Instruments, Tokyo, Japan) affixed on the bar. Brachial blood volume flow or flow velocity, HR, AP, and the timing signal for the onset and end of exercise were simultaneously recorded on an eight-channel pen-writing recorder (8M14, GE Marquette Medical System, Tokyo, Japan) and were also stored in a computer via an analog-to-digital converter (MP100, BIOPACK systems, Santa Barbara, CA) at a sampling frequency of 400 Hz. The mean values of brachial blood volume flow or flow velocity, HR, and AP were calculated every cardiac cycle, and then their corresponding average values over 1 s were stored on a hard disk using a customized software program (Cordit II, Data Integrated Scientific Systems, Pinckney, MI) for offline analysis.

*Experimental protocols.* We started the static exercise experiments a few days after implantation surgery when the cats were in good condition and were able to perform static exercise voluntarily. Each cat was put into the transparent plastic box. A period of >30 min was allowed to establish that the animal was quiescent and the cardiovascular variables became stable. When sitting quietly, the cat voluntarily extended the dominant forelimb through the window and pressed the bar while maintaining the sitting posture. The baseline HR, MAP, and brachial blood flow and vascular conductance and their responses during static exercise were recorded for 6 postoperative days after surgery.

The baseline levels of HR, MAP, and brachial blood flow and vascular conductance were high immediately after surgery and decreased in the course of the postoperative recovery (see Fig. 3). To avoid any influences of the changes in the baseline hemodynamics on

### Table 1. The baseline cardiovascular values before and after administration of hexamethonium, atropine, and L-NAME

<table>
<thead>
<tr>
<th></th>
<th>Hexamethonium</th>
<th>Atropine</th>
<th>L-NAME</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>158±10</td>
<td>175±10</td>
<td>165±5</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>101±7</td>
<td>79±3*</td>
<td>111±2</td>
</tr>
<tr>
<td>Brachial blood flow, ml/min</td>
<td>10.2±1.7</td>
<td>14.4±2.4*</td>
<td>8.2±1.2</td>
</tr>
<tr>
<td>Brachial vascular conductance, ml/min (\times 10^{-4})</td>
<td>0.11±0.02</td>
<td>0.19±0.03*</td>
<td>0.07±0.01</td>
</tr>
<tr>
<td>Exercise duration, s</td>
<td>28.5±2.4</td>
<td>21.5±3.9</td>
<td>28.2±1.7</td>
</tr>
<tr>
<td>Peak force development, g</td>
<td>317±80</td>
<td>193±54</td>
<td>370±44</td>
</tr>
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</table>

Values are means ± SE; \(n = 4\) cats. L-NAME, \(N^\text{\textsuperscript{\text{-}}}\text{nitro}-L\)-arginine methyl ester; HR, heart rate; MAP, mean arterial blood pressure. *Significant difference \((P < 0.05)\) from the control value before drug administration.

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the exercise responses, the following four kinds of pharmacological interventions were carried out at least 1 wk after the implantation surgery, except for one cat. First, to examine whether sympathetic postganglionic nerve contributed to increased muscle blood flow of the exercising forelimb, the brachial blood flow response to exercise was compared before and at 2–18 min and at 18–65 min (recovery phase) after intravenous injection of hexamethonium bromide (2–3 mg/kg) in five cats; a total of 20–44 exercise trials were performed before and after hexamethonium. The dose of hexamethonium was sufficient to cause complete sympathetic ganglionic blockade in conscious cats, because the same dose of hexamethonium abolished renal and cardiac sympathetic nerve activity (30, 35). Second, the brachial blood flow and vascular conductance responses during exercise were compared before and after intravenous injection of a muscarinic receptor blocker, atropine sulfate (0.06–0.1 mg/kg), in two cats or atropine methyl nitrate (0.05 mg/kg) in three cats; a total of 45–52 exercise trials were performed before and after atropine. Atropine methyl-nitrate is less likely to penetrate the blood-brain barrier than atropine sulfate, while atropine sulfate has fewer side effects on the intestinal system than atropine methyl-nitrate. To identify an influence of atropine sulfate on the central nervous system, we compared the effects of muscarinic blockade on the cardiovascular and brachial blood flow responses to static exercise between atropine sulfate and methyl-nitrate. Since the effects of muscarinic blockade on the cardiovascular and brachial blood flow responses were not different between the two drugs, these data were pooled together for further analysis. Third, the brachial blood flow and vascular conductance responses to exercise were compared before and after intravenous injection of a nitric oxide synthesis inhibitor \[N^\circ\text{-nitro-L-arginine methyl ester (L-NAME), 5–10 mg/kg}\] in four cats; a total of 34–51 trials were performed before and after L-NAME. A 5 mg/kg dose of L-NAME, which was sufficient to significantly decrease baseline brachial blood flow and vascular conductance, did not change the relative response in brachial vascular conductance during static exercise. The insignificant effect of L-NAME was confirmed by administering L-NAME with a higher dose of 10 mg/kg in one cat. Finally, the effect of a combined administration of atropine and L-NAME on the cardiovascular and brachial blood flow responses to exercise was examined in four cats; a total of 27–36 trials were performed before and after the combined administration.

The right brachial blood flow response during naturally occurring behavior, i.e., grooming (n = 23 trials in 6 cats) and eating (n = 19 trials in 6 cats), was examined. During grooming behavior, the cats performed rhythmic scratching exercise with one forelimb. The brachial blood flow response in the right forelimb was recorded during grooming with either the right forelimb (n = 14 trials) or the left forelimb (n = 9 trials). During eating behavior, the cats ate canned tuna food in the sitting or standing posture without using a forelimb.

To examine the direct effects of evoked contraction on muscle blood flow and vascular conductance, brachial blood flow, HR, and AP were recorded during electrical stimulation of the triceps brachialis muscle in three cats anesthetized with pentobarbital (25–40 mg/kg iv); two of the three cats were instrumented with arterial and venous catheters and a Doppler flow probe and used in the conscious experiments. Rectal temperature was maintained at 37–38°C with a heating pad. Electrocardiogram, AP, HR, rectal temperature, and respiration were continuously monitored throughout the experiments. The animals were placed in the lateral position, and the joint angles of the right forelimb were fixed within a normal range. The triceps brachialis muscle was intermittently stimulated at 1 s on and 1 s off for 30 s using an electrical stimulator (SEN-7103, Nihon Kohden, Tokyo, Japan). In each stimulation period, electrical pulses (0.1–0.2 ms in duration and 3–8 V in amplitude) were delivered at 20–50 Hz.

Data and statistical analyses. Brachial blood flow was measured with two types of flow probes (pulsed and Transonic Doppler probes). Transonic Doppler flowmetry provides blood volume flow data, whereas pulsed Doppler flowmetry provides blood flow velocity data. Brachial vascular conductance was estimated from a ratio of brachial blood volume flow and MAP. Assuming that the cross-sectional area of the brachial artery remained constant throughout the experiments, brachial vascular conductance was also estimated from a ratio of brachial blood flow velocity, recorded by the pulsed Doppler flow probe, and MAP. The average brachial blood flow and vascular conductance during the preexercise period for 5 s were defined as the 100% baseline control values, and their relative percent changes during and after exercise were sequentially calculated. The relative changes in the blood flow and vascular conductance during static exercise and the effects of the autonomic blockers and L-NAME on their changes were the same between the two types of flow probes. Thus the data taken with the two types of flow probes were pooled for all exercise trials, but not for one trial from each cat.

![Fig. 1. A: responses in heart rate (HR), arterial blood pressure (AP), and brachial blood flow during voluntary static exercise in a conscious cat. As soon as static exercise started, brachial blood flow abruptly increased, as well as HR and AP, and then remained elevated throughout the exercise. Stars indicate the cardiovascular and brachial blood flow responses in association with eating behavior. It was noted that, when eating behavior was started following exercise, brachial blood flow was decreased, despite the second increases in HR and AP. B: responses in HR, AP, and brachial blood flow during passive extension of the forelimb, so as to mimic limb movement during static exercise, were examined in the same cat. When the forelimb was passively extended, brachial blood flow showed little change, as well as HR and AP.](http://ajpregu.physiology.org/)

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The effects of a given drug on the baseline hemodynamics were examined by a paired t-test. The effects of a given drug on the cardiovascular and brachial blood flow responses during static exercise were statistically analyzed by a two-way ANOVA without repeated measures, whose main effects were time and drug (before, during, and recovery). Subsequently, the time course data of the changes in the cardiovascular and brachial blood flow responses were statistically analyzed by a one-way ANOVA without repeated measures. If a significant F value in the main effect of time was present, a Dunnett post hoc test was performed to detect a significant difference in mean values at a given time from the baseline control. Finally, the relationship between the baseline value and the exercise response of a given cardiovascular parameter, obtained in the course of 6 postoperative days, was analyzed with a linear regression model and Pearson correlation coefficient (R). A level of statistical significance was defined at \( P < 0.05 \) in all cases. The data in the text, Table 1, and Figs. 2, 6, and 8 are expressed as means ± SE.

RESULTS

Effects of autonomic blockades and L-NAME on baseline brachial blood flow. Table 1 summarizes the effects of autonomic blockades and L-NAME on the baseline hemodynamics in four conscious cats, in which a Transonic Doppler blood flow probe was implanted. Hexamethonium significantly \( (P < 0.05) \) increased the baseline HR and brachial blood flow but decreased MAP. Accordingly, calculated brachial vascular conductance was increased 33% by hexamethonium, suggesting vasodilatation of the brachial vascular bed by sympathetic ganglionic blockade and an existence of the ongoing sympathetic vasoconstrictor activity. On the other hand, L-NAME decreased \( (P < 0.05) \) brachial blood flow and vascular conductance 26–27%, suggesting vasoconstriction of the brachial...
vascular bed by L-NAME and a vasodilator mechanism due to endogenous release of nitric oxide. Although atropine largely increased HR, it did not significantly ($P > 0.05$) alter MAP and brachial blood flow and vascular conductance. The similar effects of autonomic blockades and L-NAME on the baseline hemodynamics were observed in the remaining two cats, in which a pulsed Doppler blood flow probe was implanted.

Response in brachial blood flow during voluntary static exercise. An example of the brachial blood flow response in the exercising forelimb during voluntary static exercise is represented in Fig. 1. As soon as the cat started static exercise, brachial blood flow increased, as well as HR and AP. Brachial blood flow increased throughout the exercise and remained elevated for a while after exercise. It was interesting that, when eating behavior followed static exercise, brachial blood flow was decreased, despite the similar increases in HR and AP (Fig. 1A). When the forelimb was passively extended so as to mimic the forelimb movement during static exercise, brachial blood flow showed little change (Fig. 1B), suggesting that the increase in brachial blood flow during voluntary static exercise was not an artifact in association with limb movement.

The effects of hexamethonium on the changes in HR, MAP, and brachial blood flow and vascular conductance during voluntary static exercise are summarized in Fig. 2. In the absence of hexamethonium, brachial blood flow and vascular conductance, HR, and MAP began to increase significantly ($P < 0.05$) as soon as static exercise started. The increases in brachial blood flow and vascular conductance reached the initial peaks of $52 \pm 8\%$ and $40 \pm 6\%$ at 3 s from the exercise onset; the initial increases in HR and MAP were $24 \pm 4$ beats/min and $10 \pm 2$ mmHg, respectively. Thereafter, the increases in brachial blood flow and vascular conductance continued to $63 \pm 11\%$ and $45 \pm 9\%$ until the end of exercise and the rise in MAP was sustained throughout the exercise, whereas HR returned gradually toward the preexercise level.

Hexamethonium had a significant ($P < 0.05$) main effect and interaction for all responses of the cardiovascular and blood flow variables at the onset of static exercise (Fig. 2).

Fig. 3. The responses in HR (A) and brachial vascular conductance (B) during voluntary static exercise over postoperative 2–6 days in the same cat. The responses in HR or brachial vascular conductance are superimposed over 7–8 trials on a given day (represented by different traces). In progress of postoperative recovery, the baseline levels of HR and brachial vascular conductance were reduced, and their baseline variation became much less. Interestingly, the average increase in brachial vascular conductance during static exercise (as indicated by thick lines) was constant, independent of the changes in the preexercise baseline level, whereas the average increase in HR was inversely related to the baseline level, i.e., the initial tachycardia during exercise was augmented as the baseline HR decreased.
the presence of hexamethonium, the increases in HR and MAP were almost abolished, and the increases in brachial blood flow and vascular conductance were attenuated to 25–33% of their control responses in the absence of hexamethonium. The attenuated responses in MAP and brachial blood flow and vascular conductance were partly recovered 18–65 min after administration of hexamethonium (Fig. 2). Particularly, it was of interest that the initial increases in brachial blood flow and vascular conductance at the beginning of static exercise were blunted by hexamethonium.

**Relationship between the baseline level and the response during voluntary static exercise.** The responses in HR and brachial vascular conductance during voluntary static exercise were superimposed on each of the postoperative 2–6 days in the same cat (Fig. 3). In progress of postoperative recovery, the baseline levels of HR and brachial vascular conductance were reduced, and the baseline variation of brachial vascular conductance became much less. Even though the preexercise brachial conductance changed considerably during the postoperative recovery, the average magnitude of the initial increase during static exercise seemed constant (Fig. 3). The baseline value and exercise response of brachial blood flow had the same tendency as brachial vascular conductance. In contrast, the HR response to exercise was augmented in progress of postoperative recovery as the preexercise level of HR decreased.

The relationships between the preexercise levels of HR, MAP, and brachial blood flow and vascular conductance and their responses to static exercise are redrawn in Fig. 4. The responses in brachial blood flow and vascular conductance and MAP did not significantly \( P > 0.05 \) correlate with their preexercise baseline values, while the initial tachycardia was inversely related to the preexercise level \( (R = -0.57, P < 0.05) \). Such inverse relationship between the preexercise level and the response of HR was observed in four of the five cats \( (R = -0.47 \text{ to approximately } -0.71, P < 0.05) \). In contrast, the responses in brachial blood flow and MAP did not significantly correlate with their baseline levels in all five cats tested. The response in brachial vascular conductance also did not correlate with the baseline level in four of the five cats; however, there was a significant correlation \( (R = -0.51, P < 0.05) \) in one cat.

**Effects of atropine and L-NAME on the blood flow response during voluntary static exercise.** The effects of atropine and L-NAME on the cardiovascular and brachial blood flow responses at the onset of voluntary static exercise in the same cat are exemplified in Fig. 5. Atropine increased the baseline HR and abolished the HR increase at the onset of static exercise. Also, atropine blunted the increase in brachial blood flow at the onset of static exercise, although it did not alter the baseline values of AP and brachial blood flow (Fig. 5A). On the other hand, L-NAME increased the baseline AP but decreased the baseline values of HR and brachial blood flow. In the presence of L-NAME, the initial increase in HR at the onset of static exercise was attenuated, but the increase in brachial blood flow was preserved (Fig. 5B).

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**Fig. 4.** The relationships between the preexercise baseline levels of HR (A), MAP (B), brachial blood flow (C), and brachial vascular conductance (D) and their responses at the onset of voluntary static exercise in a conscious cat. The relationships, which are redrawn from the data presented in Fig. 3, provide the Pearson correlation coefficients \( (R) \) and \( P \) values. The initial increase in HR was inversely correlated with the preexercise baseline level \( (R = -0.57, P < 0.05) \), although the responses in MAP, brachial blood flow, and brachial vascular conductance did not significantly \( (P > 0.05) \) correlate with their preexercise baseline values.
The effects of atropine and L-NAME on the cardiovascular and brachial blood flow responses at the onset of voluntary static exercise are summarized in Fig. 6. Atropine had a significant \( P < 0.05 \) main effect and interaction for the responses in all hemodynamic variables; all responses at the onset of static exercise were blunted in the presence of atropine. Especially, the peak increases in brachial blood flow and vascular conductance during voluntary static exercise were decreased by atropine to 54–55% of the control responses (Fig. 6), which could not be attributed to changes in the baseline levels (Table 1). The time courses of the increases in the cardiovascular and blood flow responses were relatively similar before and after atropine (Fig. 6). On the other hand, the relative increase in brachial vascular conductance was not affected \( P > 0.05 \) by L-NAME, although L-NAME had a significant \( P < 0.05 \) main effect and interaction for the responses in HR, MAP, and brachial blood flow. It was suggested that nitric oxide-mediated vasodilatation might not contribute to the brachial vasodilatation at the onset of static exercise. Moreover, a combined administration of L-NAME and atropine significantly \( P < 0.05 \) blunted the increases in brachial blood flow and conductance to 43–52% of the control responses, which was almost the same as the sole effect of atropine on their responses.

Blood flow response during naturally occurring body movement. The responses in HR, AP, and brachial blood flow velocity during grooming behavior in a cat are shown in Fig. 7. When the animal performed grooming behavior, rhythmically scratching with the left forelimb alone, HR and AP increased, but the right brachial blood flow was decreased, indicating vasoconstriction in the nonexercising forelimb. On the other hand, as soon as the exercising limb was switched to the right forelimb, the right brachial blood flow was increased, although no further increases...
in HR and AP were observed (Fig. 7). On average, the right brachial blood flow and vascular conductance increased by 83 ± 12% and 69 ± 10% of the baseline values (P < 0.05) during grooming with the right forelimb, respectively; in contrast, brachial blood flow was unchanged during grooming with the left forelimb and brachial vascular conductance decreased by 15 ± 6% (P < 0.05). When the cats performed eating behavior without any body trunk movement, brachial blood flow and vascular conductance decreased by 10 ± 4% and 24 ± 4% (P < 0.05), respectively. It was noted that, when the animals took food to the mouth using the right forelimb, the right brachial blood flow and vascular conductance were increased.

Blood flow response during evoked, intermittent static contraction under anesthesia. The time course of the metabolically evoked increases in brachial blood flow and vascular conductance during intermittent contraction of the forelimb triceps brachial muscle under anesthesia is demonstrated in Fig. 8. The baseline values of brachial blood flow and vascular conductance were not significantly different from those in the conscious condition. The time course and magnitude of the increases in brachial blood flow and vascular conductance during evoked intermittent contraction were quite different from their increases during voluntary static exercise. The increases in brachial blood flow and vascular conductance in response to intermittent contraction were slowly developed with a much longer time delay of 12–14 s and peaked following the cessation of muscle contraction. The peak values of the increases in brachial blood flow and vascular conductance were 40 ± 5% and 37 ± 5%, respectively, which were smaller than the increases during voluntary static exercise.

DISCUSSION

We examined using conscious cats whether the sympathetic cholinergic mechanism is involved in regulation of blood flow...
in the exercising skeletal muscle during voluntary static exercise with a forelimb. The major new findings of this study are that 1) brachial vascular conductance of the exercising forelimb rapidly increased at the onset of voluntary static exercise, leading exercise hyperemia; 2) the initial increase in brachial vascular conductance, which developed much faster and greater than metabolic vasodilatation due to evoked intermittent muscle contraction, was blunted by both hexamethonium and atropine but was not influenced by L-NAME; 3) the attenuating effect of atropine on the increase in brachial vascular conductance was almost comparable to that of hexamethonium. Taken together, the rapid forelimb vasodilatation at the onset of exercise was partly mediated by a neurogenic mechanism via activation of sympathetic cholinergic nerve rather than withdrawal of sympathetic vasoconstrictor activity (22) and/or functional sympatholysis (39, 45, 46). It is likely that a feed-forward control by descending output from higher brain centers (termed central command) may activate sympathetic cholinergic nerve fibers to skeletal muscle, which may, in turn, dilate blood vessels and increase upstream blood flow (32, 33).

**Relationship between the baseline level and the response during voluntary static exercise.** Brachial blood flow and vascular conductance are controlled by sympathetic adrenergic vasoconstrictor nerve activity and nitric oxide released from endothelial cells, as indicated in Table 1. The baseline levels of brachial blood flow and vascular conductance reduced, and their variation became smaller in progress of postoperative recovery (Fig. 3). Because hexamethonium almost doubled the vascular conductance (Table 1), sympathetic adrenergic vasoconstrictor activity is the most important factor to regulate brachial vascular tone. This implies that sympathetic adrenergic vasoconstrictor nerve activity may increase and become stabilized in the course of postoperative recovery. Even though the baseline brachial blood flow and vascular conductance changed during postoperative recovery, their responses at the onset of voluntary static exercise were well maintained, irrespective of the preexercise levels (Figs. 3 and 4). This finding suggests that a postoperative increase in sympathetic adrenergic vasoconstrictor activity elevates resting brachial vascular tone but does not restrain the vasodilator response at the onset of static exercise. In other words, activation of sympathetic cholinergic nerve appears to be independent of regulation of sympathetic adrenergic vasoconstrictor nerve.

In contrast to the responses in brachial blood flow and vascular conductance, the HR response at the onset of static exercise inversely correlated with the preexercise level (Fig. 4). Recently, we have reported that central command inhibits the cardiac component of arterial baroreflex, which, in turn, contributes to the initial tachycardia at the onset of voluntary static exercise (27, 34). If the resting HR is baroreflexly decreased by augmented baroreceptor input with a rise in MAP, a substantial increase in HR will be expected by disinhibition of the augmented baroreceptor input. Inversely, if the resting HR is increased by diminished baroreceptor input with a decrease in MAP, a smaller increase in HR will be expected by disinhibition of the diminished baroreceptor input. This explains why the size of the initial tachycardia at the onset of voluntary static exercise is inversely related to the preexercise level of HR (Fig. 4).

**Possible mechanisms for exercise hyperemia at the onset of voluntary static exercise.** Because brachial blood flow was not altered by the same passive forelimb movement as static exercise (Fig. 1), the increase in brachial blood flow during exercise was not produced by a mechanical artifact in association with limb movement but by voluntary static exercise itself. To understand whether the exercise hyperemia was generated either neurally or nonneurally, we examined the effect of hexamethonium on the increases in brachial blood flow and vascular conductance. Since hexamethonium did not abolish but blunted the increases in brachial blood flow and vascular conductance, it can be concluded that the forelimb vasodilatation during voluntary static exercise is generated partly by the sympathetic nervous system and partly by a nonneurogenic factor, such as locally released metabolites. Indeed, evoked intermittent contraction of the forelimb triceps brachial muscle slowly increased brachial blood flow and vascular conductance (Fig. 8). Furthermore, the attenuating effect of atropine on the initial increases in brachial blood flow and vascular conductance was almost comparable to that of hexamethonium, suggesting that activation of sympathetic cho-
behavior with the ipsilateral forelimb. On the other hand, as soon as the grooming limb was switched to the left forelimb, the right forelimb vasodilatation turned into vasoconstriction (Fig. 7). Also, vasoconstriction of the brachial vascular bed was usually observed during eating behavior following static exercise (Fig. 1). Since vasodilatation and vasoconstriction of the brachial vascular bed were able to alternate rapidly, it is suggested that the sympathetic nervous system, including vasodilator and vasoconstrictor nerve fibers, plays a role in control of skeletal muscle vascular conductance during natural behavior in conscious animals. In particular, central command may contribute to control of sympathetic effector nerve activity to skeletal muscle in association with naturally occurring voluntary behavior.

**Limitations.** Several potential limitations are involved in the present study. First, since the maximal voluntary contraction (MVC) during static exercise in a given cat was uncertain, we could not determine exercise intensity. The force development of ~0.3–0.4 kg during static exercise recorded in some cats was almost comparable with that obtained in previous studies (14, 21, 27, 29). Since the maximally developed force was nearly 1.0 kg in the previous studies using the same animal preparation, it is considered that the present cats might produce ~30–40% of MVC, and the intensity of static exercise was sufficient to produce the significant cardiovascular and brachial blood flow responses. Another problem regarding force development is that each of the drugs (hexamethonium, atropine, and L-NAME) used in this study tended to shorten the exercise duration and reduce the peak force during static exercise (Table 1). Thus it cannot be ruled out that each drug would weaken static exercise performance and in turn diminish the cardiovascular and brachial blood flow responses. Second, when converting the brachial blood flow velocity data measured with a pulsed Doppler flowmetry into the volume flow data, we assumed that the cross-sectional area of the brachial artery was kept constant during exercise. Our laboratory’s previous finding (32, 33), that stimulation of sympathetic cholinergic nerve increases the internal diameter of intramuscular small arteries but not extramuscular larger arteries, supports this assumption, although we did not measure the diameter of the brachial artery in this study. Many studies (5, 25, 44, 47) reported that the diameter of the brachial or axillary artery were unchanged or slightly increased during rhythmic handgrip or elbow flexion exercise with 10–30% of MVC in humans. Therefore, it is likely that the diameter of the conduit brachial artery may remain constant during static exercise in the present study. Third, an increase or decrease in the baseline values of brachial blood flow and vascular conductance following administration of a given drug might influence their relative changes during static exercise. Indeed, hexamethonium increased the baseline brachial blood flow and vascular conductance, whereas L-NAME decreased them (Table 1). On the other hand, atropine affected neither baseline brachial blood flow nor vascular conductance. It cannot be neglected that the changes in brachial blood flow and vascular conductance during exercise may be underestimated, according to the increased baseline values following hexamethonium, and that their changes may be overestimated according to the decreased baseline values following L-NAME. Fourth, the dose of atropine (0.05–0.1 mg/kg) in this study was less than a conventional dose of atropine given to anesthetized or decerebrate animals. To use this dose...
of atropine was unavoidable, because the cats stopped performing voluntary exercise whenever a higher dose of atropine was administered. The atropine dose, however, is still higher than the atropine dose (0.005–0.04 mg/kg) intravenously injected in human studies (6, 17, 48). Moreover, we always observed substantial tachycardia and mydriasis following injection of atropine to the similar extent as in the case of muscarinic blockade with a higher dose of atropine. From these points of view, the present does of atropine is adequate to cause muscarinic blockade in conscious cats.

**Perspectives and significance.** We have, for the first time, demonstrated that activation of sympathetic cholinergic nerve contributes partly to exercise hyperemia during voluntary static exercise in conscious cats. The sympathetic cholinergic vasodilatation at the onset of exercise may have a beneficial role in meeting the initial oxygen demand in exercising skeletal muscle, because the neurogenic vasodilatation is expected to operate faster and greater than locally induced vasodilatation due to metabolites released in contracting muscle, as mentioned from the data in Fig. 8. Atropine-sensitive vasodilatation in skeletal muscle has been reported during fighting behavior or a classical conditioning task (19). Based on the previous and present findings, it is important for central command to activate sympathetic cholinergic nerve to skeletal muscle in conscious cats. In humans, sympathetic cholinergic vasodilatation has not been demonstrated during exercise, although sympathetic cholinergic mechanism has been suggested, because emotional stress increases forearm blood flow, which is blocked by atropine or sympathectomy (7, 8, 37). Similar to the present experimental design, a more voluntary type of exercise with smaller muscle mass should be utilized to reexamine sympathetic cholinergic vasodilatation during exercise in humans.

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**REFERENCES**


