Aerobic muscle contraction impaired by serotonin-mediated vasoconstriction

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Dora, Kim A., Stephen Rattigan, Eric Q. Colquhoun, and Michael G. Clark. Aerobic muscle contraction impaired by serotonin-mediated vasoconstriction. *J. Appl. Physiol.* 77(1): 277–284, 1994.—Vasoconstriction mediated by serotonin (5-HT) inhibits muscle metabolism in resting constant-flow-perfused rat hindlimb and may do so by vascular shunting. In the present study, the effects of 5-HT on tension development and contraction-induced oxygen uptake by the sciatic nerve-stimulated gastrocnemius-plantaris-soleus muscle group of the perfused rat hindlimb and tension development by electrically stimulated isolated incubated soleus and extensor digitorum longus muscles were examined. In both erythrocyte and erythrocyte-free perfusions, 0.25 μM 5-HT increased perfusion pressure and markedly decreased contraction-induced tension, oxygen uptake, and lactate release. The release of metabolic vasodilators from exercising skeletal muscle did not appear to affect 5-HT-mediated vasoconstriction; rather, vascular resistance increased during the period of muscle contraction. In contrast, vasoconstriction during muscle contraction mediated by α-adrenoceptor stimulation did not impair tension and was partially overcome by metabolic vasodilators. In addition, contraction of isolated incubated soleus and extensor digitorum longus muscles was not affected by 5-HT addition to the incubation medium. We conclude that 5-HT impairs contractility of working muscle during the aerobic phase by limiting oxygen delivery through redistributing perfusate flow. The results are consistent with a vasoconstrictor action of 5-HT on larger vessels, perhaps at feed arteries external to the working muscle. When constricted by 5-HT, these vessels are apparently insensitive to metabolic vasodilatation.

vascular shunting; impaired muscle contractility; muscle oxygen delivery; lactate output; metabolic vasodilatation

BLOOD FLOW to exercising muscle may be both locally and centrally controlled by the release of vasomodulators. During low-intensity exercise, local release of vasodilators leads to hyperemia of the exercising muscle by a combination of flow-dependent and endothelial cell-mediated relaxation (23). Evidence suggests that the locus of flow control shifts from the microvessels up the resistance network, by an as yet undetermined method of cell-to-cell communication involving the endothelium (24), to encompass the feed arteries, which are external to the active muscle (6, 9). In addition to vasodilatation, exercise in vivo increases sympathetic nervous system activity, predominantly to redistribute cardiac output away from inactive areas. However, as exercise intensity increases, the maximal flow to contracting muscle can be limited by sympathetic vasoconstriction (11, 12, 16), apparently at the level of the feed arteries (14). Vasoconstriction of these larger vessels overrides the ascending vasodilatation (6), thereby overcoming the "functional sympatholysis" (18).

Several studies have shown that changes in blood flow can alter the force exerted by a muscle contracting intermittently during an extended period. This has been demonstrated in normal humans, in humans with cardiac impairment, and in the isolated cat gastrocnemius-soleus (34). Recent experiments from our laboratory suggest that infusion of serotonin (5-HT) into the constant-flow-perfused rat hindlimb causes a redistribution of perfusate flow away from metabolically active tissue while constant flow is maintained (4). Thus, 5-HT was found to cause a marked decrease in both oxygen uptake ($\dot{V}O_2$) (4, 5) and insulin-mediated glucose uptake (17). These effects appeared to be due to an action on vascular smooth muscle rather than skeletal muscle (17). It was proposed that 5-HT mediates a marked constriction of vascular site-specific large vessels (32) that exhibit different metabolic characteristics than smooth muscle stimulated by other vasoconstrictors (5).

Thus, in the present study we examined the interaction between 5-HT-mediated vasoconstriction, skeletal muscle tension development, and skeletal muscle contraction-induced increases in $\dot{V}O_2$ by the constant-flow-perfused rat hindlimb. Direct effects of 5-HT on tension development by isolated electrically stimulated muscles that were incubated, rather than perfused, were also assessed.

MATERIALS AND METHODS

Animal Care

Experiments were performed using male (60–70 or 180–200 g) hooded Wistar rats. Rats were housed in groups under temperature-controlled (22°C) conditions (12:12-h light-dark cycle) and were provided with a commercial diet (Gibson's, Hobart) and water ad libitum. The experiments and procedures were approved by the University of Tasmania Animal Experimentation Ethics Committee.

Isolated Rat Hindlimb Preparation

Animals (180–200 g) were anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/100 g body wt). Surgery was performed as previously described (21). Flow was restricted to the left limb by ligation of the right common iliac artery. After cannulation and commencement of the perfusion, the rat was placed ventral side down. The skin was removed from the thigh of the left hindlimb, and the sciatic nerve was exposed in the flank and cut to allow the positioning of the distal cut end in a suction electrode. The knee was secured by the tibiopatellar ligament, and the Achilles tendon was attached to a Harvard Apparatus isometric transducer, thereby allowing transmission of tension development from the gastrocnemius-plantaris-soleus (G-P-S) muscle group.

Perfusions were performed in a temperature-controlled cabinet, and arterial perfusion medium was temperature equilibrated by passage through an in-line heat exchanger and water-jacketed arterial line. In addition, the oxygen electrode was water jacketed. In preliminary experiments, in-line thermistors monitored perfusion temperatures.

The perfusate samples for net lactate output determination were taken at 2-min intervals before and during commencement of treatments. Lactate was determined as described previously (8). The rates of lactate release and $\dot{V}O_2$ were calculated from arteriovenous difference and flow rate.

Arterial perfusion pressure was corrected for the pressure fall due to the resistance of the cannula.

Erythrocyte-Free Perfusions

The erythrocyte-free perfusions were performed at 25°C using modified Krebs-Henseleit buffer containing 8.3 mM glucose and 1.27 mM $CaCl_2$ with 2% bovine serum albumin. The buffer reservoir was gassed with 95% O_2-5% CO_2 at 4°C to enable full oxygenation. At equilibration (after 30 min of perfusion at 27 ± 1 ml · min^{-1} · 100 g^{-1}) and before electrical stimulation, the flow rate was increased to 108 ± 2 ml · min^{-1} · 100 g^{-1} (n = 20). The resting length of the muscle was adjusted to attain maximal active tension on stimulation (this normally required 4 or 5 tetanic stimulations of 0.2 s duration using the stimulation parameters below). After attainment of a new steady state for $\dot{V}O_2$ and perfusion pressure (10 min) at the new flow rate, electrical (tetanic) stimulation delivered from a Nerv-Muskel-Reizgerät stimulator (Hugo Sachs Elektroniks) was commenced using 200-ms trains of 100 Hz with 0.1-ms pulses applied every 2 s and adjusted (3–5 V) to attain full fiber recruitment. Tension development, perfusion pressure, and venous PO_2 were recorded continuously on OmniScribe (Activon) chart recorders. Effluent perfusate samples were regularly collected for lactate analysis.

5-HT [0.25 μM; approximately half-maximal response (4)] was continuously infused via a peristaltic pump (LKB, Bromma) for 6 min before and throughout the sciatic nerve stimulation. This allowed assessment of steady-state 5-HT-induced effects and of the effects of 5-HT on contraction and vice versa. In other experiments, 5-HT (0.25 μM) was infused for a brief period during the 20-min period of skeletal muscle contraction. This allowed determination of the reversibility of the 5-HT effects during contraction. In separate control experiments, vehicle (0.9% NaCl) was infused in place of 5-HT.

Erythrocyte Perfusions

Erythrocyte perfusions were performed to ensure that the responses mediated by 5-HT were also present under more physiological conditions. The presence of erythrocytes in the perfusion medium improved oxygen delivery and allowed the temperature to be raised to 37°C.

Fresh bovine erythrocytes were washed three times using saline (0.9% NaCl) and three times using Krebs-Henseleit buffer and were added to modified Krebs-Henseleit medium containing 2.5 mM $CaCl_2$ and 4% bovine serum albumin (35% hematocrit). Perfusion was initially commenced at 26 ± 1 ml · min^{-1} · 100 g^{-1} for 30 min until equilibration, and then the flow rate was increased to 98 ± 2 ml · min^{-1} · 100 g^{-1} (n = 10). The stimulation parameters and procedures were identical to those used in the erythrocyte-free perfusions. Samples of venous perfusate were collected in gastight syringes at basal conditions (~2 min before electrical stimulation), 4 min after stimulation (before infusions) when plateau tension development was reached, 3 min after vehicle or 5-HT infusion (when maximal changes due to 5-HT were attained), and 19 min after commencement of electrical stimulation. To determine the times for collecting these samples, a continuous qualitative assessment of venous effluent oxygen content was made during initial perfusions using an in-line Clark-type oxygen electrode. Arterial samples were taken from an in-line arterial septum at basal conditions and at the completion of the perfusion, which corresponded to ~25 min after commencement of electrical stimulation. Samples of perfusate were analyzed for total oxygen content with use of a galvanic cell oxygen analyzer (TasCon oxygen content analyzer, manufactured by the Physiology Department, University of Tasmania). Duplicate analysis of each collected sample took an average of 15 min.

From estimates conducted by others (21) of 0.5 μmol lactate produced · ml^{-1} · h^{-1} by bovine erythrocytes and an estimated passage time of 1 min for the perfusion media through the apparatus and hindlimb at 15.3 ± 0.2 ml/min, it was calculated that the erythrocytes would contribute only 0.5 μmol · h^{-1} · g^{-1}. This small contribution to lactate release by erythrocytes was confirmed in this study (0.6 μmol · h^{-1} · g^{-1}) and was therefore ignored.

Incubated Muscles

Male hooded Wistar rats (60–70 g) were anesthetized with an intraperitoneal injection of aqueous pentobarbital sodium (6–8 mg/100 g body wt) containing heparin (100 U/100 g body wt). The soleus and extensor digitorum longus (EDL) muscles from each leg were quickly and carefully removed and were placed unsplinted in 95% O_2-5% CO_2 gassed incubation buffer until use.

A modified Krebs-Henseleit buffer was used, which consisted of (in mM) 118 NaCl, 4.74 KCl, 1.19 KH_2PO_4, 1.18 $MgSO_4$, 25 $NaHCO_3$, 1.27 $CaCl_2$, 5 glucose, 5 pyruvate, 35 mannitol, and 5 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid. After the gassing, pH was 7.35 at 25°C.

Incubations were conducted in a thermostatically controlled chamber. The incubation temperature was 25°C to improve the endurance of contraction (25). The incubation chamber was gassed with 95% O_2-5% CO_2, which also served to mix the medium. For each experiment, the proximal and distal tendons of the muscles were secured to the metal support and the force transducer, respectively, and then were tensioned to give maximal force during stimulation.

After 10 min of preincubation, electrical stimulation was commenced using 200-ms trains of 100 Hz with 0.1-ms pulses applied every 2 s and adjusted (10–12 V) to attain full fiber recruitment. Except for the greater voltage, these stimulation parameters were the same as those used in the hindlimb perfusions.

Tension development was recorded continuously during electrical stimulation. After the initial anaerobic phase, the contraction reached a sustained aerobic plateau level (15 min of stimulation). At this point, either vehicle (control) or 1 μM 5-HT + 0.2 mM sucrose {including tracer amounts of 5-[^{3}H]HT (sp act 1.06 Ci/mmol) and [^{14}C]sucrose (sp act 0.26 Ci/mmol)} were added to the incubation chamber and contraction was continued for a further 10 min. After cessation of stimulation, 5-HT-treated muscles were rapidly removed, weighed, and freeze-dried. Freeze-dried muscles were homogenized in H_2O, and after centrifugation an aliquot of supernatant was taken for radioactive counting to determine the total content of 5-[^{3}H]HT and [^{14}C]sucrose. A further aliquot of supernatant was used for high-performance liquid chromatography analysis, which separated 5-HT from its major metabolite 5-hydroxyindole acetic acid (C_{18} reverse phase, mobile phase methanol-H_2O 90:10), to determine the metabolism of 5-[^{3}H]HT.

Statistical Analysis

A repeated-measures analysis of variance was used to test the hypothesis that there was no difference between the effects of vehicle and 5-HT infusion throughout the time course. When a significant difference was found, pairwise comparisons based on the t test were used to determine at which individual times the differences were significant. These tests were

performed using the SAS statistical package (SAS Institute, Cary, NC).

RESULTS

Electrical stimulation of the sciatic nerve contracts ~30% of the perfused hindlimb mass, including the G-P-S muscle group, hamstring muscles, the deep portion of the crural muscles, and the lateral crural muscles, consistent with the results of others (19). Thus, during the experiments, changes in perfusion pressure and metabolism were due to a combination of contracting lower limb and uncontracting upper hindlimb tissue. Values for contraction-stimulated increases in $\dot{V}O_2$ and lactate release were obtained using the entire hindlimb muscle mass; thus, actual values per gram of contracting tissue may be three times greater. Attempts at selectively perfusing the contracting mass via the popliteal artery and vein resulted in collateral circulation to the upper hindlimb, thus making determination of lower leg metabolism quantitatively inaccurate.

Each muscle contraction (1 every 2 s) had a small transient effect (~1–2 mmHg) on perfusion pressure. Pressure values obtained during the stimulation period are averages.

Erythrocyte-Free Perfusions

Under basal conditions, before electrical stimulation (flow rate 108 ± 2 ml · min^{-1} · 100 g^{-1}), mean arterial and venous P_{O_2} were 678.1 ± 8.4 and 593 ± 11.1 Torr, respectively (n = 10), equating to a $\dot{V}O_2$ of 8.0 ± 0.5 μmol · h^{-1} · g^{-1} (pooled value for control and 5-HT groups). Basal perfusion pressure was 58.8 ± 1.2 mmHg (n = 10), and lactate release was 6.5 ± 1.2 μmol · h^{-1} · g^{-1}. The value for $\dot{V}O_2$ under basal conditions was similar to that reported by this laboratory previously at flow rates of >8 ml/min (35).

As shown in Figs. 1 and 2, stimulation of the lower hindlimb muscle groups via the sciatic nerve caused an initial peak in tension development of 898 ± 50 g. The initial strong tension rapidly decreased to a plateau level of 208 ± 18 g after 6 min of stimulation (n = 5). The time taken to attain the plateau level of tension development coincided with the attainment of steady state for $\dot{V}O_2$ (~4 min). After 6 min, $\dot{V}O_2$ had increased to 12.3 ± 0.7 μmol · h^{-1} · g^{-1} and lactate release had increased to 24.0 ± 1.7 μmol · h^{-1} · g^{-1}. In the absence of 5-HT, there was no significant effect of contraction on perfusion pressure (59.5 ± 1.0 mmHg; n = 5). Lactate release increased 4.4-fold with electrical stimulation and then decreased steadily during stimulation to a level approximately two-fold greater than the basal prestimulation value.

Effect of prior infusion of 5-HT on skeletal muscle contraction. Infusion of 0.25 μM 5-HT for 6 min increased perfusion pressure by 44.7 ± 6.6 mmHg and decreased $\dot{V}O_2$ and lactate release by 3.1 ± 0.4 and 3.5 ± 0.4 μmol · h^{-1} · g^{-1}, respectively (Fig. 1; n = 5). Initial muscle contraction by sciatic nerve stimulation was not affected by prior infusion of 5-HT; initial peak tension was 1,006 ± 59 g (Fig. 1; n = 5). However, prior treatment with 5-HT significantly ($P < 0.05$) inhibited plateau tension (6 min) development by 42.3 ± 15.3% and contraction stimulated rises in $\dot{V}O_2$ and lactate release by 75 and 21%, respectively (Fig. 1; n = 5). During the stimulation period, there appeared to be no vasodilatation of the 5-HT-mediated rises in arterial resistance. Indeed, perfusion pressure began to increase steadily after the onset of contraction (Fig. 1). After 6 min of contraction stimulation, perfusion pressure had risen a further 33.0 ± 9.4 mmHg (n = 5).

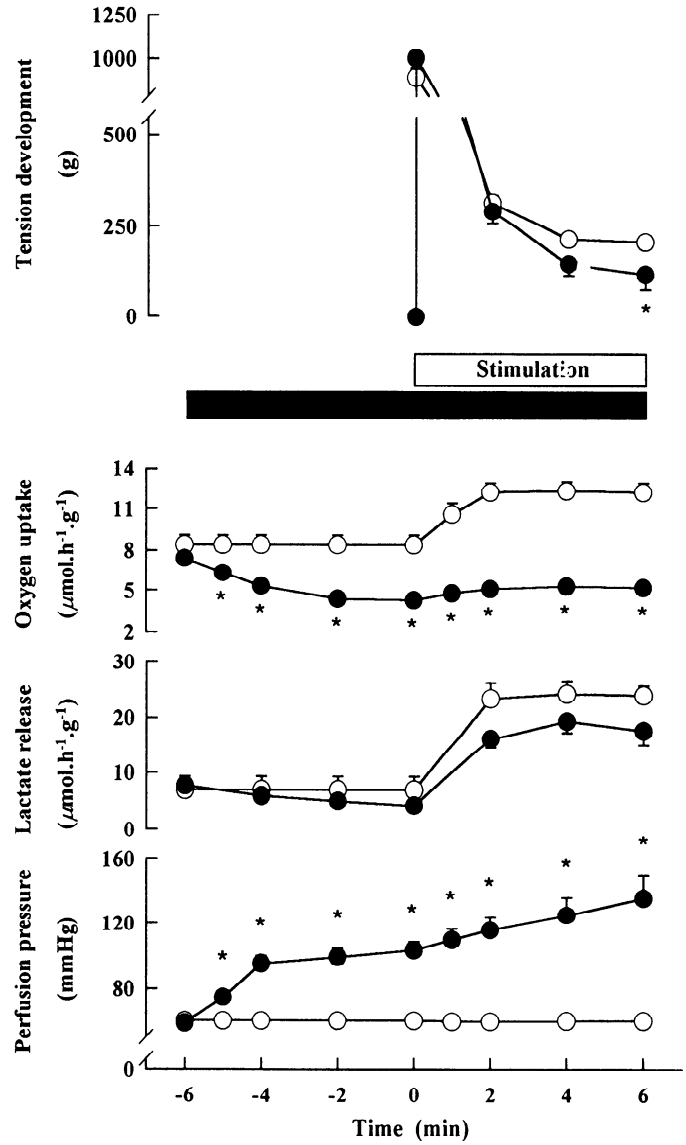

FIG. 1. Effect of serotonin (5-HT) on tension development, oxygen uptake, lactate release, and perfusion pressure before and during skeletal muscle contraction. Rat hindlimb was perfused with constant flow in nonrecirculating mode without erythrocytes at 25°C as described in MATERIALS AND METHODS. Lower hindlimb muscle groups were made to contract during period indicated by open bar by electrical stimulation of severed sciatic nerve (flank) with use of suction electrode. A 200-ms train of 100 Hz with 0.1-ms pulses was applied every 2 s and was adjusted (3–5 V) to attain full fiber recruitment. Tension transmitted from gastrocnemius-plantaris-soleus muscle group was recorded using isometric force transducer. 5-HT (0.25 μM; ●) or its vehicle (control; ○) was infused for period indicated by filled bar. Values are means ± SE; n = 5 control perfusions and 5 5-HT perfusions. When not visible, error bars are within symbols. *Significantly different ($P < 0.05$) from control.

In separate experiments, the effect of skeletal muscle contraction on the α-adrenergic vasoconstrictor re-

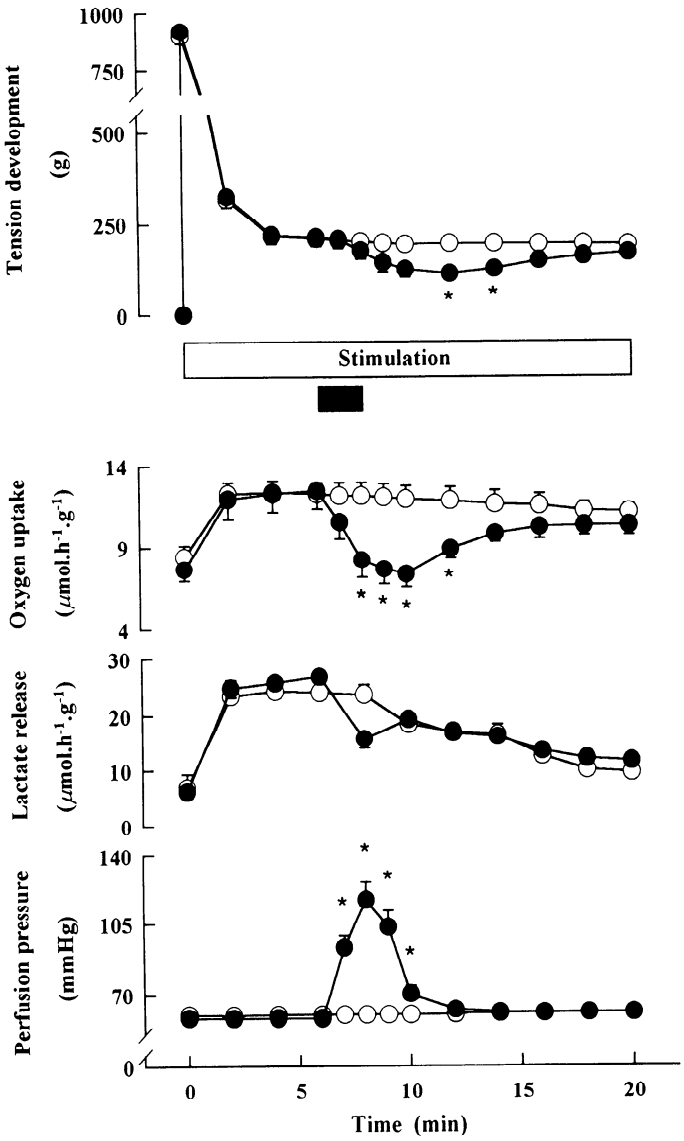

FIG. 2. Effect of 5-HT on tension development, oxygen uptake, lactate release, and perfusion pressure during skeletal muscle contraction. Rat hindlimb was perfused with constant flow in nonrecirculating mode without erythrocytes at 25°C, and lower hindlimb muscles were contracted during period indicated by open bar as described in Fig. 1. 5-HT (0.25 μM; ●) or its vehicle (control; ○) was infused for period indicated by filled bar. Values are means ± SE; n = 5 control perfusions and 5 5-HT perfusions. When not visible, error bars are within symbols. *Significantly different ($P < 0.05$) from control.

sponse to norepinephrine (NE) was investigated. Infusion of 20 nM NE in the presence of 10 μM (±)-propranolol for 6 min increased perfusion pressure by 72.5 ± 6.0 mmHg (n = 5), a response greater than that observed with 5-HT. Commencement of contraction stimulation rapidly caused a vasodilatory response, decreasing perfusion pressure by 14.2 ± 2.5 mmHg (n = 5) after 1 min. Despite a gradual rise in pressure, the vasodilatory response was maintained for the period of contraction stimulation (Table 1). On cessation of contraction, pressure rapidly increased by approximately the same amount as that caused by contraction-induced dilatation.

Effect of 5-HT during skeletal muscle contraction. 5-HT inhibited plateau tension (6 min of stimulation) development by 40% (Fig. 2). The maximal inhibitory effect of 5-HT on tension development occurred 4 min after 5-HT infusion had ceased and slowly returned to control levels by the end of the stimulation period. When 5-HT was infused for >2 min, contraction did not return to control values even 12 min after 5-HT removal (data not shown).

Continuous recording of $\dot{V}O_2$ showed that the 5-HT-mediated inhibition of $\dot{V}O_2$ preceded the inhibition of tension development (Fig. 2). In addition, 5-HT infusion almost totally inhibited the electrical stimulation-induced increase in $\dot{V}O_2$ with the inhibition occurring rapidly and coinciding with the increase in pressure (Fig. 2). However, the inhibition reversed slowly and $\dot{V}O_2$ did not attain pre-5-HT infusion values until 6 min after the infusion of 5-HT had ceased.

Contraction-mediated lactate release was transiently inhibited by 41% after 2 min of 5-HT infusion, but within 2 min of 5-HT removal it had returned to control values.

5-HT infusion during contraction increased perfusion pressure by 59.7 ± 8.8 mmHg (n = 5) after 2 min of infusion (greater than but not significantly different from 5-HT alone), representing an increase of 103% above basal pressure. The time course for the change in pressure indicated that the overall effect lasted ~5 min, and the pressure returned to control untreated levels.

When infused before or during infusion of 5-HT, the 5-HT_2-receptor antagonist ketanserin (0.1 μM) markedly reduced the effects of 5-HT on perfusion pressure, $\dot{V}O_2$ lactate release, and tension development (data not shown).

Erythrocyte Perfusions

Effect of 5-HT during skeletal muscle contraction. Under basal conditions at high flow rate (98 ± 2 $ml \cdot min^{-1} \cdot 100\ g^{-1}$), mean arterial oxygen content was 7.89 ± 0.12 mmol/l, mean venous oxygen content was 6.88 ± 0.13 mmol/l (n = 10) with a $\dot{V}O_2$ of 59.6 ± 3.1 $\mu mol \cdot h^{-1} \cdot g^{-1}$, and lactate release was 12.3 ± 2.1 $\mu mol \cdot h^{-1} \cdot g^{-1}$ (pooled data for control and 5-HT groups). The values for $\dot{V}O_2$ and lactate release under basal conditions were somewhat higher than those reported by Ruderman et al. (21) but may have resulted from the higher flow rate used in the present studies and

TABLE 1. *Comparison of effects of skeletal muscle contraction on 5-HT- and NE-mediated vasoconstriction in perfused hindlimb*

	Perfusion Pressure, mmHg		
Time, min	Vehicle	5-HT (0.25 μM)	NE (20 nM)
0	59.6±1.1	103.0±5.3	128.4±6.9
1	59.1±1.1	109.4±7.1*	114.2±8.1*
6	59.5±1.0	136.0±14.2*	121.2±9.3

Values are means ± SE; n = 5 control perfusions. Data for serotonin (5-HT) were taken from Fig. 1. Rat hindlimb was perfused with constant flow in nonrecirculating mode without erythrocytes at 25°C as described in MATERIALS AND METHODS following protocol of Fig. 1. Lower hindlimb muscle groups were made to contract by electrical stimulation of severed sciatic nerve (flank) (time = 0 to 6 min) in presence of 5-HT or norepinephrine (NE; time = −6 to 6 min). Experiments involving NE were performed in presence of β-adrenoceptor antagonist propranolol (±10 μM). * Significantly different ($P < 0.05$, paired 2-tailed Student's t test) from *time 0*.

the test stimulations of the muscles to set muscle tension at maximum. $\dot{V}O_2$ (22, 35) and lactate release (35) are flow rate dependent in the perfused rat hindlimb. Basal perfusion pressure was 64 ± 1 mmHg (n = 10), which was higher than that in erythrocyte-free perfusions despite similar flow rates. This was presumably due to the higher viscosity of the erythrocyte medium.

Stimulation of the sciatic nerve to contract the lower hindlimb muscle groups produced an initial tension in the Achilles tendon of 1,109 ± 127 and 1,082 ± 95 g for the control and 5-HT-treated groups, respectively (both n = 5; Fig. 3). These values were similar to that initially developed by the erythrocyte-free perfused hindlimb at 25°C. Tension then rapidly decreased to a plateau level of 335 ± 28 and 312 ± 40 g for the control and 5-HT-treated groups, respectively (n = 5), which was 154% of that attained using erythrocyte-free perfused hindlimbs at 25°C, and remained constant for the duration of the perfusion if vehicle alone was added (Fig. 3). Lactate release initially increased fivefold with electrical stimulation and then decreased to twofold basal levels (Fig. 3).

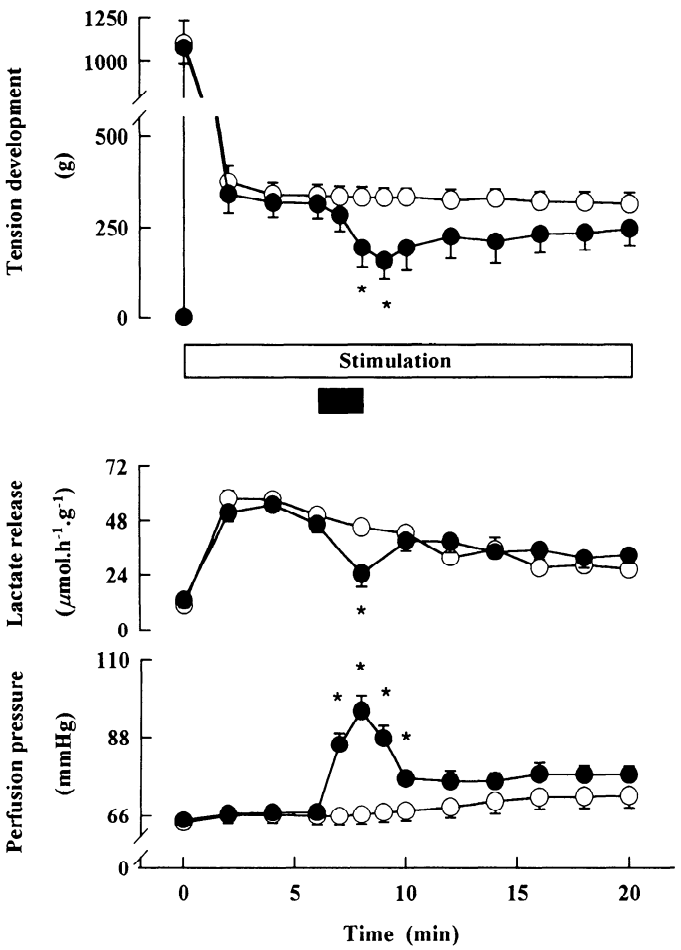

FIG. 3. Effect of 5-HT on tension development, lactate release, and perfusion pressure during skeletal muscle contraction. Rat hindlimb was perfused with constant flow and medium containing erythrocytes at 37°C, and lower hindlimb muscles were contracted during period indicated by open bar as described in Fig. 1. 5-HT (0.25 μM; ●) or its vehicle (control; ○) was infused for period indicated by filled bar. Values are means ± SE; n = 5 control perfusions and 5 5-HT perfusions. When not visible, error bars are within symbols. *Significantly different ($P < 0.05$) from control.

TABLE 2. *Effect of 5-HT on $\dot{V}O_2$ by erythrocyte-perfused hindlimb during skeletal muscle contraction*

Perfusion Condition	Time, min	$\dot{V}O_2$, μmol·h⁻¹·g⁻¹ Vehicle	$\dot{V}O_2$, μmol·h⁻¹·g⁻¹ 5-HT (0.25 μM)
Basal	0	61.1±4.1	58.1±5.1
Plateau contraction	6	92.5±5.9	98.4±12.4
Maximal response to treatment	9	95.1±9.6	64.6±9.5*
End contraction period	19	71.8±6.9	73.1±15.1

Values are means ± SE; n = 5 control (vehicle) perfusions and 5 5-HT perfusions. Rat hindlimb was perfused with constant flow, and lower hindlimb muscles were contracted as described in Fig. 3. Venous effluent was sampled before and during contraction stimulation at times indicated and were analyzed as described in MATERIALS AND METHODS. 5-HT or its vehicle was infused for 2 min after plateau tension was obtained (Fig. 3). Samples for plateau contraction and maximal response to treatment were collected at times corresponding to changes in venous P_{O_2} observed with in-line oxygen electrode. $\dot{V}O_2$, oxygen uptake. * Significantly different ($P < 0.05$) from control.

The perfusion pressure rose gradually by ~8 mmHg in the course of the control perfusions. Stimulation per se appeared to have no immediate effect on perfusion pressure (Fig. 3). The initial effect of electrical stimulation on $\dot{V}O_2$ could not be quantitatively assessed, as timing of sampling to correspond precisely with peak tension development was not possible. However, continuous recording of the venous P_{O_2} in some of the erythrocyte perfusions suggested that there was no immediate change during this period. Table 2 shows that $\dot{V}O_2$ increased in the control group 1.5-fold during stimulation from 61.1 ± 4.1 to 92.5 ± 5.9 $\mu mol \cdot h^{-1} \cdot g^{-1}$. Again, continuous recordings suggested that this took 2–3 min to reach steady state (data not shown). The contraction-stimulated rises in $\dot{V}O_2$ in erythrocyte-perfused hindlimbs when expressed per gram of contracting muscle (119 $\mu mol \cdot h^{-1} \cdot g$ contracting muscle^{-1}) are comparable to values obtained by others (15, 21, 27), which range from 91 to 194 $\mu mol \cdot h^{-1} \cdot g$ contracting muscle^{-1}. When expressed as $\dot{V}O_2$ of the G-P-S per gram of tension developed by the G-P-S, the value in this study (0.11 $\mu mol \cdot h^{-1} \cdot g$ G-P-S$^{-1} \cdot g$ tension^{-1}) falls between the values of others (15, 28).

Figure 3 shows that a brief (2 min) infusion of 0.25 μM 5-HT, commenced 6 min after the start of electrical stimulation, increased perfusion pressure by ~30 mmHg. Continuous recording of data showed that the effect was evident within seconds after commencement of infusion and began to rapidly reverse as soon as infusion ceased. However, a residual pressure increase was consistently present for the remainder of the stimulation period. This was in contrast to the complete reversal of pressure effects in the absence of erythrocytes (Fig. 2). When 5-HT was infused for >2 min, the reversal of pressure after 5-HT removal was slower and remained further elevated above control (data not shown). The increase in pressure was less than the change in pressure in the erythrocyte-free-perfused hindlimb at 25°C (Fig. 2) and may be partly due to temperature difference (7).

Figure 3 also shows that, at 1 min after 5-HT removal, there was a significant decrease (54%) in developed ten-

sion during the plateau aerobic phase from 329.3 ± 25.2 to 156.5 ± 47.9 g (both $n = 5$). Tension slowly returned toward pre-5-HT levels but remained below control values for the remainder of the stimulation period (Fig. 3).

Contraction-mediated lactate release in erythrocyte-containing perfusions was transiently inhibited by 39% during 5-HT infusion from corresponding control values of 45.1 ± 2.3 to 24.4 ± 5.6 $\mu mol \cdot h^{-1} \cdot g^{-1}$ after 2 min of 5-HT infusion (Fig. 3). Within a further 2 min, lactate release had returned to control values, which continually declined for the remainder of the stimulation period (Fig. 3).

Contraction-induced $\dot{V}O_2$ was markedly inhibited by 5-HT (Table 2), but, 13 min after 5-HT removal, $\dot{V}O_2$ returned to control levels (Table 2).

Incubated muscles. Comparison of all the data from both control and 5-HT groups showed that before addition of vehicle or 5-HT electrical stimulation of the soleus and EDL muscles produced significantly different ($P < 0.01$) initial tensions of 22.8 ± 1.1 and 35.3 ± 2.7 g, respectively ($n = 6$). After 15 min of stimulation, the tension rapidly decreased to significantly different (soleus vs. EDL) plateau levels of 11.4 ± 0.6 and 7.3 ± 0.5 g, respectively ($P < 0.001$; $n = 6$), and remained constant for the remainder of the incubation.

Figure 4 shows that 5-HT (1 μM) had no significant effect on the tension developed by either the soleus or EDL muscles. To ascertain whether the 5-HT successfully penetrated the isolated incubated muscles, 1 μM 5-[^{3}H]HT was used in conjunction with 0.2 mM [^{14}C]-sucrose, a marker for the extracellular space. For the soleus and EDL muscles, respectively, after 10 min the spaces for 5-HT for three muscles were 331 ± 29 and 230 ± 12 μl/g wet wt and those for sucrose were 175 ± 10 and 131 ± 4 μl/g wet wt. It was also found that 15.9 ± 1.3% ($n = 8$) of the added 5-HT was degraded. These data suggest that 5-HT filled the extracellular space and was also taken up into the muscle cells and partly degraded. Because only 16% of the 5-HT was degraded, the remaining concentration would have been at least that used in the hindlimb perfusions.

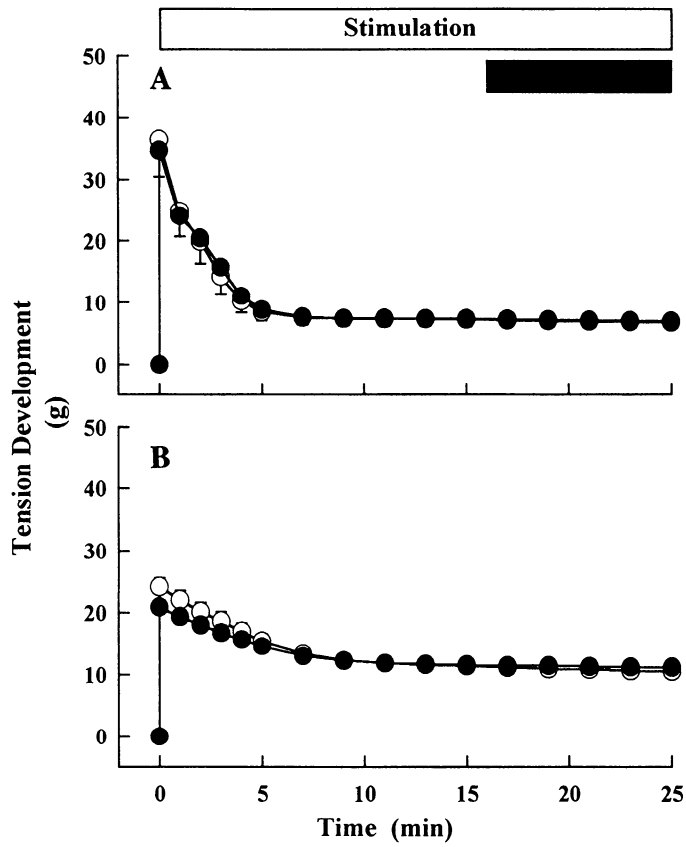

FIG. 4. Effect of 5-HT on muscle tension development by electrically stimulated isolated extensor digitorum longus (*A*) and soleus (*B*) muscles. Muscles were dissected and incubated as described in MATERIALS AND METHODS. Except for greater voltage, electrical stimulation parameters were identical to those used with perfused lower hindlimbs as outlined in Fig. 1. Muscles were stimulated during period indicated by open bar during presence of 5-HT (1 μM; ●) or its vehicle (control; ○) for period indicated by filled bar. Values are means ± SE; $n = 3$ incubations for each treatment comprised of addition of vehicle or 5-HT. When not visible, error bars are within symbols.

DISCUSSION

Two main observations were made from this study. First, 5-HT was found to inhibit the aerobic contractility of working muscle in the constant-flow-perfused rat hindlimb but not that of incubated hindlimb muscles. This effect appeared to have occurred as a result of impaired nutrient delivery associated with vasoconstriction and was evident whether 5-HT was infused before and/or during the stimulation period. Second, the vasoconstrictor action of 5-HT was not counteracted by the vasodilatory action of metabolites released by contracting skeletal muscle in proximity.

The observation that 5-HT inhibited aerobic contractility of working muscle in the constant-flow-perfused rat hindlimb was not unexpected. Previous studies from this laboratory showed that 5-HT inhibited $\dot{V}O_2$ (4, 5) and insulin-mediated glucose uptake (17) in a similar preparation without erythrocytes. To ensure that oxygen delivery to working muscle was adequate in the present study, erythrocytes were included in one series of perfusions. However, despite the differences in potential oxygen delivery, the effects of both sciatic nerve stimulation and 5-HT were qualitatively similar for erythrocyte and erythrocyte-free perfusions. Whereas peak tension development of the muscle group was similar in the two preparations, plateau tension development differed considerably. This difference may have resulted from the superior oxygen delivery afforded by erythrocytes, or it may have reflected lower efficiency of excitation-contraction coupling and/or muscle performance at the lower temperature (29). In both perfusion systems, sciatic nerve stimulation alone led to marked increases in $\dot{V}O_2$ and lactate release in association with tension development but little change in perfusion pressure. Infusion of 5-HT increased perfusion pressure and markedly decreased tension, contraction-induced $\dot{V}O_2$, and, to a lesser extent, lactate release in both systems. The latter suggests a more important role for anaerobic metabolism during 5-HT-impaired contraction. The rapid reversal of the lactate release to control levels may also be due, at least in part,

to the washout of lactate from flow-impoverished zones. A transient rise in lactate release has previously been observed both after 5-HT removal and on reestablishment of flow to physically occluded regions (4).

Time course studies using erythrocyte-free perfused hindlimbs at 25°C (Fig. 2) showed that the sequence of changes induced by 5-HT was increased perfusion pressure (vasoconstriction) closely associated with an inhibition of $\dot{V}O_2$ and lactate release. In contrast, the maximal impairment of contraction was somewhat delayed, occurring 1–2 min after the maximal 5-HT-mediated rise in perfusion pressure. This sequence and the lack of an effect in incubated muscles suggest that 5-HT does not impair contractility directly by receptor-mediated effects on skeletal muscle. Rather, the sequence is consistent with our previous findings (4, 5, 17) in which the effects of 5-HT in inhibiting $\dot{V}O_2$ in the perfused rat hindlimb have been attributed to functional vascular shunting. So far, the evidence supporting the notion of 5-HT-mediated vascular shunting in perfused rat hindlimb is indirect. Studies involving efflux kinetics showed that a significant fraction of a bolus injection of Evans blue dye in the presence of 5-HT washed out of the hindlimb more rapidly than in control muscles (4). In addition, it was observed that 5-HT inhibited the uncoupling effect of sodium azide on $\dot{V}O_2$ by the perfused hindlimb (5). The proportion of this inhibitory effect by 5-HT was similar to its inhibitory effect on basal $\dot{V}O_2$ (i.e., ~30%; Ref. 5). Although uncertain at present, the effects of 5-HT may be attributed to a reduction in capillary surface area for nutritional exchange (20). Rippe and Folkow (20), using a similar rat hindlimb preparation, noted that increasing doses of 5-HT produced a progressive decrease in both capillary diffusion capacity for Cr-EDTA and capillary filtration capacity.

Our present findings together with the effects of ketanserin, a 5-HT_2-receptor antagonist that increases oxygen availability to hyperoxemic tissue (30), support the notion of 5-HT receptor involvement in modulation of skeletal muscle oxygenation via a partial redistribution of flow away from the working muscles such that their perfusate and thus nutrient supply are diminished. This effect is likely to be fiber type specific, presumably with flow decreasing to slow-oxidative fibers, which have less capacity for anaerobic contraction metabolism. A recent study by Wilson and Maughan (33) has shown that administration of a 5-HT reuptake inhibitor reduces the capacity of male athletes to perform prolonged exercise. Thus, it is possible that 5-HT-mediated changes in flow within muscle may have physiological relevance.

The feed arteries have been shown to be active sites of blood flow control to contracting muscle with soleus muscles exhibiting a greater range of flow control than EDL muscles (31). The general finding that 5-HT constricts large arterioles (13, 32) but not small arterioles (2, 10) is consistent with the action of 5-HT on feed arteries. Because these vessels can be found external to the muscle fibers, the local release of exercise-related vasodilators within the microvasculature is less likely to have a strong influence at these sites. However, it has been demonstrated that vasodilator signals acting on microvessels can communicate upstream to cause vasodilatation of the feed arteries (24). Thus, depending on the ability of cells to communicate in the isolated perfused system used in this study, the vasoconstriction mediated by 5-HT may have overridden the conducted vasodilatation. This possibility is consistent with the findings of Joyner and co-workers (11, 12) and O'Leary et al. (16), who demonstrated that in vivo the functional sympatholysis observed during low-intensity exercise can be overcome during intense exercise when an increase in sympathetic nervous system activity causes constriction of feed arteries (14). Alternatively, the apparent difference in metabolic characteristics of vasoconstriction (5), in which energy for 5-HT- but not NE-mediated rises in pressure could be generated solely from anaerobic metabolism, may be also related to susceptibility to metabolic vasodilators.

The question of whether exercise releases vasodilators in the perfused hindlimb is partially addressed by comparing the action of 5-HT with that of other vasoconstrictors. Although basal pressure in the almost fully dilated preparation was not affected, the pressure responses to angiotensin II (3) and NE (Table 1) were rapidly and continually decreased during exercise stimulation. Presumably, the vasoconstriction was not totally opposed by metabolic vasodilators because of the nonrecirculated perfusion and the large mass of perfused tissue not stimulated to contract. Anderson and Faber (1) demonstrated that α_1- and α_2-adrenoceptor-mediated vasoconstriction was markedly reduced by exercise stimulation at frequencies of >4 Hz. These adrenoceptors are found on large and small arterioles, respectively (1). Thus, it appears that vasoconstriction within, but not external to, the vascular bed of contracting skeletal muscle is susceptible to functional sympatholysis.

An interesting observation from this work that warrants future investigation is the effect of contraction of steadily increasing 5-HT-mediated vasoconstriction (Fig. 1). A decrease in oxygen availability in working muscle has been shown to cause the accumulation of a pressor substance (26). Thus, the rise in pressure may have been due to the release of an endogenous constrictor from the working muscle rendered oxygen limited by 5-HT.

We conclude that, under the perfusion conditions chosen, vasoconstriction by 5-HT causes an impairment of muscle contraction because of a diminution of flow and thus oxygen delivery. It is proposed that this constriction occurs on larger vessels, perhaps feed arteries, which are external to the muscle and either insensitive or inaccessible to metabolic vasodilators. In future studies, it will be determined whether 5-HT gives rise to qualitatively similar effects under conditions more closely resembling in vivo and whether the vasoconstriction mediated by 5-HT results in the secondary release of an endogenous contraction-associated constriction factor.

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REFERENCES

1. **Anderson, K. M., and J. E. Faber.** Differential sensitivity of arteriolar α_1- and α_2-adrenoceptor constriction to metabolic inhibition during rat skeletal muscle contraction. *Circ. Res.* 69: 174–184, 1991.
2. **Blackshear, J. L., C. Orlandi, J. D. Garnic, and N. K. Hollenberg.** Differential large and small vessel responses to serotonin in the dog hindlimb in vivo: role of the 5-HT_2 receptor. *J. Cardiovasc. Pharmacol.* 7: 42–49, 1985.
3. **Colquhoun, E. Q., M. Hettiarachchi, J.-M. Ye, S. Rattigan, and M. G. Clark.** Inhibition by vasodilators of noradrenaline and vasoconstrictor-mediated, but not skeletal-muscle contraction-induced oxygen uptake in the perfused rat hindlimb; implications for non-shivering thermogenesis in muscle tissue. *Gen. Pharmacol.* 21: 141–148, 1990.
4. **Dora, K. A., E. Q. Colquhoun, M. Hettiarachchi, S. Rattigan, and M. G. Clark.** The apparent absence of serotonin-mediated vascular thermogenesis in perfused rat hindlimb may result from vascular shunting. *Life Sci.* 48: 1555–1564, 1991.
5. **Dora, K. A., S. M. Richards, S. Rattigan, E. Q. Colquhoun, and M. G. Clark.** Serotonin and norepinephrine vasoconstriction in rat hindlimb have different oxygen requirements. *Am. J. Physiol.* 262 (*Heart Circ. Physiol.* 31): H698–H703, 1992.
6. **Folkow, B., R. R. Sonnenschein, and D. L. Wright.** Loci of neurogenic and metabolic effects on precapillary vessels of skeletal muscle. *Acta Physiol. Scand.* 81: 459–471, 1971.
7. **Harker, C. T., L. M. Taylor, Jr., and J. M. Porter.** Vascular contractions to serotonin are augmented by cooling. *J. Cardiovasc. Pharmacol.* 18: 791–796, 1991.
8. **Hettiarachchi, M., K. M. Parsons, S. M. Richards, K. A. Dora, S. Rattigan, E. Q. Colquhoun, and M. G. Clark.** A role for vascular smooth muscle in vasoconstrictor-mediated release of lactate from the perfused rat hindlimb. *J. Appl. Physiol.* 73: 2544–2551, 1992.
9. **Hilton, S. M.** A peripheral arterial conducting mechanism underlying dilatation of the femoral artery and concerned in functional vasodilatation in skeletal muscle. *J. Physiol. Lond.* 149: 93–111, 1959.
10. **Hollenberg, N. K.** Large and small vessel responses to serotonin in the peripheral circulation. *J. Cardiovasc. Pharmacol.* 7, *Suppl.* 7: S89–S91, 1985.
11. **Joyner, M. J., R. L. Lennon, D. J. Wedel, S. H. Rose, and J. T. Shepherd.** Blood flow to contracting human muscles: influence of increased sympathetic activity. *J. Appl. Physiol.* 68: 1453–1457, 1990.
12. **Joyner, M. J., L. A. Nauss, M. A. Warner, and D. O. Warner.** Sympathetic modulation of blood flow and O_2 uptake in rhythmically contracting human forearm muscles. *Am. J. Physiol.* 263 (*Heart Circ. Physiol.* 32): H1078–H1083, 1992.
13. **Lamping, K. G., H. Kanatsuka, C. L. Eastham, W. M. Chilian, and M. L. Marcus.** Nonuniform vasomotor responses of the coronary microcirculation to serotonin and vasopressin. *Circ. Res.* 65: 343–351, 1989.
14. **Lind, A. R., and C. A. Williams.** The control of blood flow through human forearm muscles following brief isometric contractions. *J. Physiol. Lond.* 288: 529–547, 1979.
15. **McAllister, R. M., and R. L. Terjung.** Training-induced muscle adaptations: increased performance and oxygen consumption. *J. Appl. Physiol.* 70: 1569–1574, 1991.
16. **O'Leary, D. S., L. B. Rowell, and A. M. Scher.** Baroreflex-induced vasoconstriction in active skeletal muscle of conscious dogs. *Am. J. Physiol.* 260 (*Heart Circ. Physiol.* 29): H37–H41, 1991.
17. **Rattigan, S., K. A. Dora, E. Q. Colquhoun, and M. G. Clark.** Serotonin-mediated acute insulin resistance in the perfused hindlimb but not in incubated muscle: a role for the vascular system. *Life Sci.* 53: 1545–1557, 1993.
18. **Rememsnyder, J. P., J. H. Mitchell, and S. J. Sarnoff.** Functional sympatholysis during muscular activity. *Circ. Res.* 11: 370–380, 1962.
19. **Rennie, M. J., and J. O. Holloszy.** Inhibition of glucose uptake and glycogenolysis by availability of oleate in well-oxygenated perfused skeletal muscle. *Biochem. J.* 168: 161–170, 1977.
20. **Rippe, B., and B. Folkow.** Simultaneous measurements of capillary filtration and diffusion capacities during graded infusions of noradrenaline (NA) and 5-hydroxytryptamine (5-HT) into the rat hindquarter vascular bed. *Acta Physiol. Scand.* 109: 265–273, 1980.
21. **Ruderman, N. B., C. R. S. Houghton, and R. Hems.** Evaluation of the isolated perfused rat hindquarter for the study of muscle metabolism. *Biochem. J.* 124: 639–651, 1971.
22. **Ruderman, N. B., F. W. Kemmer, M. N. Goodman, and M. Berger.** Oxygen consumption in perfused skeletal muscle. *Biochem. J.* 190: 57–64, 1980.
23. **Segal, S. S.** Communication among endothelial and smooth muscle cells coordinates blood flow control during exercise. *News Physiol. Sci.* 7: 152–156, 1992.
24. **Segal, S. S., D. N. Damon, and B. R. Duling.** Propagation of vasomotor responses coordinates arteriolar resistances. *Am. J. Physiol.* 256 (*Heart Circ. Physiol.* 25): H832–H837, 1989.
25. **Segal, S. S., J. A. Faulkner, and T. P. White.** Skeletal muscle fatigue in vitro is temperature dependent. *J. Appl. Physiol.* 61: 660–665, 1986.
26. **Sheriff, D. D., C. R. Wyss, L. B. Rowell, and A. M. Scher.** Does inadequate oxygen delivery trigger pressor response to muscle hypoperfusion during exercise? *Am. J. Physiol.* 253 (*Heart Circ. Physiol.* 22): H1199–H1207, 1987.
27. **Spriet, L. L., M. I. Lindinger, G. J. F. Heigenhauser, and N. L. Jones.** Effects of alkalosis on skeletal muscle metabolism and performance during exercise. *Am. J. Physiol.* 251 (*Regulatory Integrative Comp. Physiol.* 20): R833–R839, 1986.
28. **Spriet, L. L., C. G. Matsos, S. J. Peters, G. J. F. Heigenhauser, and N. L. Jones.** Muscle metabolism and performance in perfused rat hindquarter during heavy exercise. *Am. J. Physiol.* 248 (*Cell Physiol.* 17): C109–C118, 1985.
29. **Stephenson, D. G., and D. A. Williams.** Calcium-activated force responses in fast- and slow-twitch skinned muscle fibres of the rat at different temperatures. *J. Physiol. Lond.* 317: 281–302, 1981.
30. **Thorborg, P., and N. Lund.** Serotonin as a modulator of skeletal muscle oxygenation: effects of ketanserin and ritanserin on oxygen pressure distributions. *Int. J. Microcirc. Clin. Exp.* 8: 191–203, 1989.
31. **Williams, D. A., and S. S. Segal.** Feed artery role in blood flow control to rat hindlimb skeletal muscles. *J. Physiol. Lond.* 463: 631–646, 1993.
32. **Wilmoth, F. R., P. D. Harris, and F. N. Miller.** Differential serotonin responses in the skeletal muscle microcirculation. *Life Sci.* 34: 1135–1141, 1984.
33. **Wilson, W. M., and R. J. Maughan.** Evidence for a possible role of 5-hydroxytryptamine in the genesis of fatigue in man: administration of paroxetine, a 5-HT re-uptake inhibitor, reduces the capacity to perform prolonged exercise. *Exp. Physiol.* 77: 921–924, 1992.
34. **Wright, D. L., and R. R. Sonnenschein.** Relations among activity, blood flow, and vascular state in skeletal muscle. *Am. J. Physiol.* 208: 782–789, 1965.
35. **Ye, J.-M., E. Q. Colquhoun, M. Hettiarachchi, and M. G. Clark.** Flow-induced oxygen uptake by the perfused rat hindlimb is inhibited by vasodilators and augmented by norepinephrine: a possible role for the microvasculature in hindlimb thermogenesis. *Can. J. Physiol. Pharmacol.* 68: 119–125, 1990.