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Keywords

muscle, skeletal, contracting, resting, consumption, oxygen, vasoconstrictors, effect, hindlimb, perfused, rat, pump, autologous

Disciplines

Medicine and Health Sciences

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THE EFFECT OF VASOCONSTRICTORS ON OXYGEN CONSUMPTION IN RESTING AND CONTRACTING SKELETAL MUSCLE OF THE AUTOLOGOUS PUMP-PERFUSED RAT HINDLIMB

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This study used a novel *in vivo* model to test the hypothesis that nutritive and non-nutritive blood flow distribution can still be observed under conditions of high vascular tone and oxygen delivery at rest and in metabolically active (twitch contracting) skeletal muscle. Experiments were performed in a constant flow autologous pump-perfused hindlimb in anaesthetised male Wistar rats. Agonists were tested at rest with a flow rate of $1\text{ml}\cdot\text{min}^{-1}$, and during hindlimb muscle twitch contractions (sciatic nerve stimulation: 6V, 1Hz, 0.05ms, 3min) at a flow rate of $2\text{ml}\cdot\text{min}^{-1}$. Oxygen consumption was determined from hindlimb venous and arterial blood samples. Resting perfusion pressure at $1\text{ml}\cdot\text{min}^{-1}$ was 92 ± 3 mmHg (N=15) and oxygen consumption was 0.41 ± 0.05 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$. Serotonin increased perfusion pressure and significantly decreased basal hindlimb oxygen consumption at rest. During acute muscle contraction this effect on oxygen consumption was diminished. Noradrenaline significantly increased perfusion pressure but had no significant effect on basal hindlimb oxygen consumption. Vasoconstriction that impacts upon muscle metabolism occurs *in vivo*, which potentially could be due to selective redistribution of blood flow. However, during muscle contraction local release of vasodilatory regulation can overcome exogenously-induced vasoconstriction. These results support the hypothesis that dual vascular pathways may explain differential vasoconstriction and how it impacts upon muscle metabolism.

Key words: *oxygen consumption, skeletal muscle, contraction, vasoconstriction*

INTRODUCTION

The delivery of nutrients to skeletal muscle *via* the vascular system is a tightly regulated process with the precise mechanism of regulation unknown. A lack of histological evidence for classical shunts such as large arterio-venous anastomoses (1, 2) has tended to contradict physiological evidence supporting parallel dual circulation. Redistribution of blood flow brought about by an increase in vascular resistance is thought to play an oxygen-saving role. However, this will not protect from oxygen consumption by the tissue at the peripheral level. Hence, the existence of dual circulation, with one route having a low metabolic demand, could play a crucial role to minimise nutrient exchange (3). It has been proposed that there are alternative parallel vascular pathways within skeletal muscle that enable blood flow to be directed to the muscle cells when they are active (nutritive pathway) and to bypass the muscle cells when they are inactive (non-nutritive pathway) (4). It has been postulated that preferential tissue blood flow results from heterogeneity of perfusion, which occurs at the capillary level of the vascular network and that specific anatomical structures that shunt skeletal muscle blood flow are not required (5, 6).

Early evidence concluded that skeletal muscle has two circulations, one being concerned with the delivery of nutrients to the muscle fibres (nutritive), and the other delivering nutrients to the intramuscular septa and tendons (non-nutritive) (7-9).

Skeletal muscle blood flow distribution and the existence of parallel dual vascular pathways has been proposed as to play a regulatory role in muscle metabolism and insulin resistance (4, 10, 11). The effects of various vasoconstricting agents on perfusion pressure and oxygen uptake in the constant flow non-recirculating perfused rat hindlimb show differing results. While all agents increase perfusion pressure, some increase oxygen consumption whilst others reduce it. Clark and colleagues have grouped vasoconstrictors that increase perfusion pressure in the hindlimb into two categories depending on their effects on metabolic activity (12). Type A vasoconstrictors such as noradrenaline (NAd) increase oxygen uptake and metabolite efflux (changes consistent with an increase in muscle metabolism) whilst type B vasoconstrictors such as serotonin (5-HT) decrease oxygen uptake. Furthermore, the ability to increase the number of perfused capillaries (capillary recruitment) by such metabolic stimulators as insulin is thought to play a role in the regulation of skeletal muscle metabolism (13). These studies were performed in a constant flow, non-recirculating, *in situ* perfused hindlimb or isolated muscle preparations using either cell free, human erythrocyte or bovine erythrocyte perfusates.

The perfused hindlimb models used to study skeletal muscle metabolism have many non-physiological parameters such as low perfusion pressure and oxygen consumption resulting from a lack of resting vascular tone and choice of perfusate. A majority of studies have been performed at 25°C under

conditions of constant flow with a 95% oxygen-5% carbon dioxide gas phase. Qualitatively similar changes have been observed in other studies when the hindlimb is perfused at 37°C under constant flow, with a perfusate using bovine red blood cells with the same gas phase (14, 15). These gassed saline solutions have low oxygen content and provide poor oxygen delivery to the muscle compared to *in vivo* conditions. The importance and physiological relevance of using blood in perfusion when investigating oxygen consumption, tissue function and metabolic response has been demonstrated in heart and liver perfusion systems (16, 17). Furthermore, because these models often have high perfusion rates to overcome the poor perfusion pressure, the resultant decrease in RBC transit time increases hindlimb oxygen consumption, reflecting increased oxygen delivery (18-20). Overall, these perfused hindlimb models are limited by their low oxygen delivery, incapable of vasodilation to increase blood flow, and thus are rarely able to provide data on the relationship of oxygen consumption to muscle activity. While oxygen delivery that is too low to support skeletal muscle contraction can be overcome by using a red blood cell perfusate (21), all require high flow rate, reflecting lack of neural vascular tone. Since nutritive/non-nutritive theory relies on the provision of oxygen for metabolism, the capacity to vasodilate is important under conditions of high metabolic rate, as in contracting muscle.

This study aimed to establish a model of selective vasoconstriction (seen before *in-situ*) and investigate changes in blood flow distribution during active metabolism. We have developed a constant flow autologous pump-perfused rat hindlimb model, adapted from the canine model (22, 23), which allows for sampling of both arterial and venous blood. The animal remains alive and with intact autonomic nervous system throughout, ensuring vascular tone and maintained perfusion pressure at low perfusion rates. This model provides optimal physiological tissue oxygenation during times of increased metabolic demand.

MATERIALS AND METHODS

Experiments were approved by the University of Wollongong Animal Ethics Committee. Male Wistar rats were obtained from Gore Hill Animal Research Laboratory (Royal North Shore Hospital, Sydney, NSW, Australia) and housed in the University of Wollongong's Animal facility. Room temperature was maintained at 23°C and there was a 12 hour light cycle. Animals had free access to rat chow and water.

Chemicals

Noradrenaline and 5-Hydroxytryptamine (serotonin, 5-HT) were prepared at a 10 mM stock with isotonic saline and 0.1% ascorbic acid, and stored at -18°C. Drugs were prepared fresh before each protocol using defrosted stock and isotonic saline. All drugs were purchased from Sigma-Aldrich Diagnostics (Sydney, NSW, Australia).

Hindlimb perfusion

Autologous pump-perfused rat hindlimb was adapted from the previously well described canine model (22, 23). Briefly, rats (12-14 weeks) were anaesthetised with sodium pentobarbitone (6 mg•100 g⁻¹ body weight i.p.) with maintenance doses given as required. Rats were artificially ventilated (7025, Ugo Basile, Italy) according to animal body weight (1 ml•200 g⁻¹) *via* a tracheal cannulation with room air and core body temperature was maintained at 37°C with an external heat source.

All cannulae (0.58 mm ID, 0.96 mm OD; Dural Plastics, Australia) were fluid filled with saline containing 6% Dextran70 (w/v) and 50 U•ml⁻¹ heparin. Systemic blood pressure was measured by a cannula inserted into the left common carotid artery and connected to a pressure transducer (Argon CDXIII, Maxxim Medical, USA). Arterial (oxygenated) blood was accessed *via* the cannulation of the right femoral artery (non-perfused leg) which was passed through a flow controlled a peristaltic roller pump (Miniplus 3, Gilson, France) connected to a cannula that was inserted into the left femoral artery (perfused leg) towards the foot. The cannula passed well down the femoral artery towards the knee and restricted perfusion to only the muscle beds of the lower hindlimb. Other side branches were ligated. Hindlimb perfusion pressure was measured *via* a T-junction inserted in the blood flow line on the perfused hindlimb side of the pump connected to a pressure transducer. A Windkessel pressure dampener was inserted into the blood flow line. The left femoral vein was cannulated towards the foot of the perfused leg for venous sampling of the hindlimb with the other end inserted in the right external jugular vein for passive venous return to the circulation for re-oxygenation.

Passive flow was allowed through the pump and when all tubes had filled with the animal's own blood (~1min) the pump was engaged to provide a constant flow rate of 1 ml min⁻¹ through the hindlimb vasculature for 30 min to allow for mean perfusion pressure to reach steady state (92±3 mmHg, N=15) similar to the systemic arterial pressure.

Hindlimb muscle contraction was achieved *via* sciatic nerve stimulation. A small incision was made in the skin approximately 1cm below the iliac crest. The gluteal muscles were separated to expose the sciatic nerve trunk. A bipolar electrode (Grass Instrument Division, USA) was placed under the nerve trunk supplying the muscles of the gastrocnemius-plantaris-soleus muscle bundle for direct electrical stimulation. The left leg was secured at the knee and foot to prevent movement during stimulation. The gastrocnemius-plantaris-soleus muscle group tendons were tied with non-stretch silk and connected to a force transducer (FT03C, Grass Instrument Division, USA) to measure force and rates of contraction and relaxation.

Resting protocol

Basal hindlimb oxygen consumption was determined by sampling arterial and venous blood taken at the end of the 30 min equilibrium perfusion. To test the effects of the vehicle on hindlimb pressure and oxygen consumption, 0.2 ml saline was infused in the arterial perfusion with venous sampling. Agonist drugs were infused in a constant volume (0.2 ml) into the arterial perfusion through the self-sealing silicon tubing. Drugs were infused in an increasing molar concentration sequence with a 10 min rest period in between doses to allow for washout (natural clearance or uptake processes) to occur.

Arterial haemoglobin saturation and oxygen content was monitored from arterial samples, taken an average of four times during the course of an experiment and remained consistent throughout the experiment. During the basal protocols, venous samplings were taken at 2, 3, 4 and 5 min post-agonist infusion.

Contraction protocol

Muscle contraction protocols commenced at the end of the 30 min equilibrium perfusion. Perfusion rate was increased to 2 ml min⁻¹ and hindlimb muscle stimulation commenced (1Hz, 6V, 0.05 ms, for 3 min). Agonist drugs were infused after achievement of maximum peak tension, 10 seconds after the commencement of the hindlimb stimulation. Venous sampling occurred 2.5 min and 3.5 min after infusion of a drug. After 3

min, pump speed was returned to $1 \text{ ml}\cdot\text{min}^{-1}$ and muscle stimulation terminated. A minimum of 10 min of resting perfusion was allowed between protocols to reduce the likelihood of fatigue and to allow for drug washout. One arterial and two venous blood samples were taken to determine contracting hindlimb oxygen consumption and the effects of the vehicle on hindlimb pressure and oxygen consumption determined.

Blood variables

Arterial and venous blood was sampled (100 μl) from the external lines of the hindlimb through in-line, thick walled, self-sealing silicone tubing. Measurement of pH, Po_2 , Pco_2 , haematocrit (Hct), and haemoglobin were performed using a blood gas and electrolyte analyser (ABL77 Radiometer, Denmark) using 70 μl of sampled blood. The remaining sample volume was re-suspended in an equal volume of heparinised isotonic saline (1:1 heparin to saline) and re-injected into the venous side of the perfusion. A maximum of 710 μL of whole blood was lost to sampling during any experiment and it was replaced by an equal volume of heparinised saline. Haemoglobin levels were maintained above $12.5 \text{ g}\cdot 100 \text{ ml}^{-1}$ whole blood.

Data collection and statistical analysis

Perfusion pressure and twitch force data was collected using Labview for Windows (National Instruments, USA) simultaneously at a sampling rate of 200 Hz. The data was displayed in real time and stored for later analysis. Data are expressed as mean \pm S.E.M. Group means were compared using Student's paired *t* test with differences judged significant at $P \leq 0.05$.

RESULTS

Baseline parameters

Table 1 shows that all blood parameters were maintained within the normal physiological range both under basal conditions and during muscle contraction. Basal venous Po_2 was decreased and Pco_2 was increased compared to arterial levels. The (a-v) O_2 difference was 29% of the initial arterial oxygen content, resulting in a basal oxygen consumption of $0.41 \pm 0.05 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ at $1 \text{ ml}\cdot\text{min}^{-1}$ perfusion ($n=15$). During the contraction protocol ($2 \text{ ml}\cdot\text{min}^{-1}$ with hindlimb muscle contraction) oxygen consumption was significantly increased to $0.57 \pm 0.08 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$.

Mean systemic blood pressure ($85 \pm 2 \text{ mmHg}$) was not influenced by changes in hindlimb perfusion rate. The mean

Table 1. Baseline conditions and measures for all groups taken after 30min perfusion at a constant flow rate of $1 \text{ ml}\cdot\text{min}^{-1}$ (Basal).

Variable	Arterial	Venous
n	19	
pH	7.37 ± 0.02	7.25 ± 0.02 *
Po_2 (mmHg)	100.6 ± 2.8	44.1 ± 3.2 *
Pco_2 (mmHg)	36.6 ± 1.6	55.5 ± 2.8 *
Hb ($\text{g}\cdot 100\text{ml}^{-1}$)	14.5 ± 0.3	15.0 ± 0.2 *
Hct (%)	44.5 ± 0.7	46.1 ± 0.8 *
Co_2 ($\text{ml}\cdot 100\text{ml}^{-1}$)	19.9 ± 0.3	14.2 ± 0.9 *
So_2 (%)	97.4 ± 0.1	67.2 ± 0.1 *

mean \pm S.E.M. * $P < 0.05$ vs Arterial.

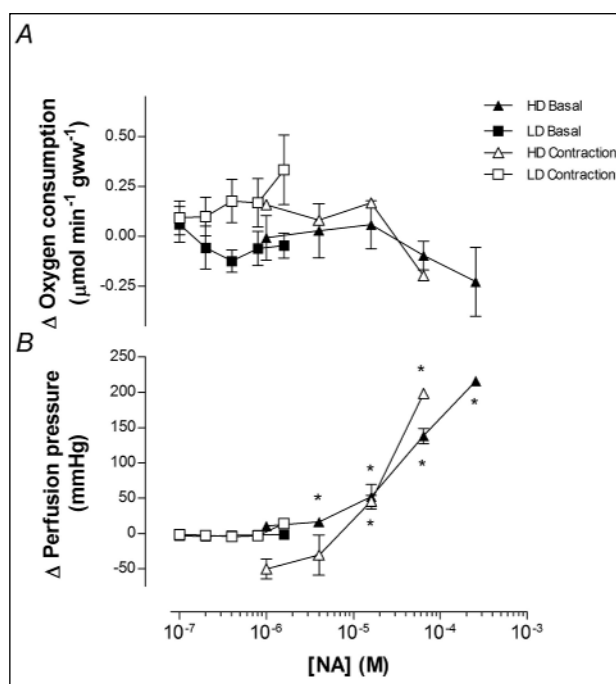


Fig. 1. The effect of Noradrenaline on hindlimb perfusion pressure and oxygen consumption. **A:** The effect of low dose (basal \blacksquare $n = 3$, contraction \square , $n = 6$) and high dose (basal \blacktriangle , $n = 6$, contraction \triangle , $n = 3$) Noradrenaline infusion on oxygen consumption and **B:** perfusion pressure during basal and contraction conditions. * $P < 0.05$ vs pre-infusion levels. Mean \pm S.E.M.

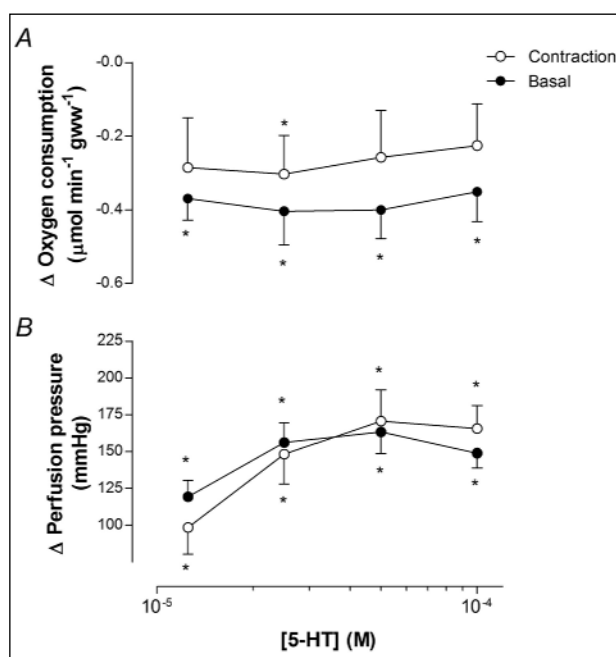


Fig. 2. The effect of Serotonin on mean hindlimb perfusion pressure and oxygen consumption. **A:** Change in oxygen consumption and **B:** mean hindlimb perfusion pressure during basal (\bullet) and contraction (\circ) protocols. * $P < 0.05$ vs pre-infusion levels. $n = 6$ per group. Mean \pm S.E.M.

hindlimb perfusion pressure was 92.3 mmHg at a basal flow rate of $1 \text{ ml}\cdot\text{min}^{-1}$ and increased to 166.6 mmHg when the flow rate was increased to $2 \text{ ml}\cdot\text{min}^{-1}$ for the contraction protocol. During stimulation the mean peak force production (T_{max}) was 0.24 ± 0.02

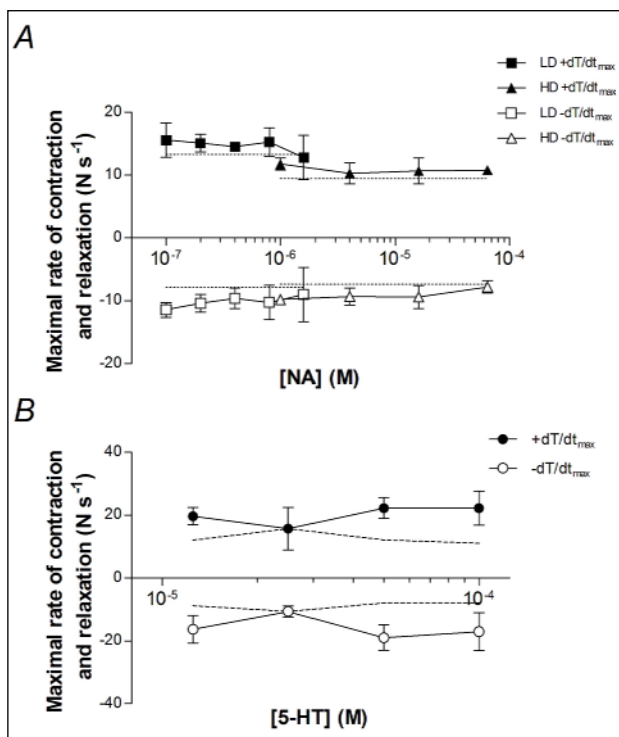


Fig. 3. A: The effect of Noradrenaline on maximal rate of contraction and relaxation during muscle contraction. Hindlimb response to Noradrenaline on maximal rate of contraction (low dose: ■, $n = 3$; high dose: ▲, $n = 6$) and relaxation (low dose: □; high dose: △) during 1Hz sciatic nerve stimulation. **B:** The effect of Serotonin on maximal rate of contraction and relaxation during muscle contraction. Hindlimb response to Serotonin on maximal rate of contraction (●) and relaxation (○) during 1Hz sciatic nerve stimulation. The dashed line indicates pre-infusion maximal rate of contraction and relaxation. * $P < 0.05$ vs pre-infusion levels. Mean \pm S.E.M.

N with a mean force production of 0.15 ± 0.01 N, a maximal rate of contraction ($+dT/dt_{max}$) of 10.2 ± 1.3 N \cdot s⁻¹ and a maximal rate of relaxation ($-dT/dt_{max}$) of -9.4 ± 1.4 N \cdot s⁻¹ ($n = 8$).

Vasoconstriction and skeletal muscle metabolism

There was no significant difference between NAd and 5-HT groups in pre-drug mean hindlimb perfusion pressure or mean hindlimb Vo_2 (data not shown). Under resting conditions infusion of NAd produced dose-dependent increases in mean hindlimb perfusion pressure with a threshold observed at 16μ M (Fig. 1B). No significant change in hindlimb Vo_2 was observed during the basal protocol at any dose of NAd (Fig. 1A). Infusion of 5-HT produced a dose-dependent increase in mean hindlimb perfusion pressure under resting conditions with the maximal vasoconstrictor effect on perfusion pressure at 50μ M (Fig. 2B). Furthermore, there was a significant decrease in hindlimb oxygen consumption after 5-HT infusion at all doses (Fig. 2A).

Effect of increased metabolic demand upon agonist effects

There was no significant difference between NAd and 5-HT groups in pre-drug mean hindlimb perfusion pressure or mean hindlimb Vo_2 prior to stimulation (data not shown). Infusion of NAd produced dose-dependent increases in mean hindlimb perfusion pressure with a threshold observed at 1.6μ M during

muscle contraction (Fig. 1B). There was no significant effect of NAd infusion on Vo_2 during the 3 min muscle twitch contraction (Fig. 1). No significant effects were observed on either $+dT/dt_{max}$ or $-dT/dt_{max}$ following either low dose or high dose NAd infusion (Fig. 3A).

Perfusion pressure was significantly increased after 5-HT infusion for all doses during contraction, with a similar dose-dependent trend as seen during the basal protocol (Fig. 2A). Mean hindlimb perfusion pressure was maximally increased by 163 ± 15 mmHg after 50μ M infusion with higher doses resulting in no further increase. 5-HT decreased Vo_2 during the contraction protocol with a similar trend to that observed during the basal protocol (Fig. 2B) however the decrease was only significant at 25μ M 5-HT. At 100μ M, 5-HT infusion produced only 64% ($P = 0.054$) of the reduction in oxygen consumption during muscle contraction compared to that observed under basal conditions. Despite the reduction in Vo_2 , 5-HT infusion had no significant effect on either $+dT/dt_{max}$ or $-dT/dt_{max}$ in the autologous pump-perfused hindlimb (Fig. 3B).

DISCUSSION

Blood flow distribution *via* parallel vascular pathways has been previously demonstrated to impact upon skeletal muscle metabolism. These studies were performed in perfused rat hindlimbs which are characterised by a lack of vascular tone and high flow rates to reach physiological perfusion pressure (24). The data presented herein extend these previous studies and clearly demonstrate that oxygen consumption is reduced in resting autologous pump-perfused hindlimb skeletal muscle by administration of the vasoconstrictor 5-HT and that during muscle contraction, which itself increases oxygen consumption (21) this reduction is diminished. However, no significant effect on oxygen consumption was observed after NAd administration, which has been previously shown to increase Vo_2 in perfused rat hindlimbs. These results are in support of previous findings which showed that in resting skeletal muscle 5-HT changes in perfusion pressure were closely associated with changes in oxygen consumption at rest and during muscle contraction following 5-HT infusion (10).

Previous studies have suggested a role for nutritive/non-nutritive blood flow in the regulation of skeletal muscle metabolism *via* selective distribution of nutrients and substrates (4, 10). This hypothesis has come about from studies where the use of various vasoconstrictors were categorised into two groups; type A, which increase metabolic parameters and; type B, which decrease these metabolic parameters (4). However, the majority of these studies were performed in perfused hindlimbs of sacrificed animals in the absence of normal vascular tone using high flow rates to achieve physiological perfusion pressure. Results from this study clearly demonstrate that the autologous pump-perfused rat hindlimb model is more physiologically relevant.

The type B vasoconstrictor 5-HT has been shown to decrease oxygen uptake, insulin-mediated glucose uptake, free fatty acid efflux and lactate production, which can be blocked by 5-HT₂-antagonists (25-29). Furthermore, 5-HT administration in the perfused hindlimb reduced the total number of perfused capillaries and that those perfused were of greater diameter (3). We report here that in our model 5-HT reduced oxygen consumption whilst increasing hindlimb perfusion pressure. This decrease in hindlimb metabolism by type B vasoconstrictors is thought to occur through selective vasoconstriction of the nutritive bed redirecting flow into the non-nutritive bed, thought to be associated with low metabolic demand connective tissue capillary beds (29). At higher doses, we observed that 5-HT-

mediated increases in perfusion pressure were not further increased which is similar to previous reports (26, 27, 30). Whilst our results support the notion of dual parallel vascular pathways, further studies to identify the precise location of 5-HT₂ receptors within the microvasculature are necessary to link the vasculature and metabolic changes occurring during 5-HT infusion.

Dora *et al.* have demonstrated that skeletal muscle aerobic contractility was inhibited during 5-HT infusion due to diminished nutrient delivery through redirection of blood flow by selective vasoconstriction (28). This occurred without an effect of local vasoactive metabolites released during skeletal muscle contraction to overcome the vasoconstrictor action of 5-HT. In contrast, we demonstrated that 5-HT had no significant effect on the maximal rate of muscle contraction or relaxation in our model. The effect of 5-HT on hindlimb oxygen consumption was diminished during the contraction protocol. If nutritive flow was reduced it may follow that the twitch characteristic would differ due to reduced oxygen availability, however we observed no effect on twitch characteristics. This suggests that the reduced nutritive flow might still provide sufficient oxygen for the twitch contractions in the presence of relatively high oxygen content during acute (3 min) muscle contraction. The differing observations may lie with the fact that Dora *et al.* used a tetanic protocol in the perfused hindlimb using cell-free and bovine RBC in Krebs-Henseleit buffer (low O₂ availability) whilst this study used a twitch protocol and the animal's own blood. Furthermore the duration of the muscle contractions (3 min) or the pharmacological effect (bolus dose) used in this study may not be sufficient to induce changes in twitch characteristics by detrimental changes in muscle metabolism.

Previous studies have reported that NAd at low doses (< 1 μ M) increases a variety of metabolic parameters whilst at high doses (> 1 μ M) inhibits metabolism in the perfused hindlimb but not in isolated muscle preparations (29, 31). NAd-induced metabolic changes are thought to occur by selective vasoconstriction of the non-nutritive route to redistribute blood flow towards the myocytes (32, 33). However, in the autologous pump-perfused rat hindlimb used in this study the metabolic effects of NAd administration are in contrast with those reported by Clark and colleagues (24, 34). The absence of NAd-induced increases in oxygen consumption during vasoconstriction in our model has been reported previously in a variety of models (35-38). Our results suggest that during adequate oxygen delivery to the myocytes, a further increase in oxygen availability, possibly *via* selective vasoconstriction, does not result in an increase in oxygen uptake. The different observations may lie in the experimental conditions.

The results from the current study may suggest that selective vasoconstriction by NAd to increase perfusion of muscle fibre associated capillaries does not increase oxygen consumption during adequate oxygen delivery. Hence, increased availability of oxygen when basal metabolic rate is matched does not increase oxygen consumption. On the other hand, 5-HT decreased oxygen consumption, which could be a result of selective vasoconstriction of the nutritive bed to increase perfusion of the connective and adipose tissue. It should be remembered that physiological control of skeletal muscle blood flow *in vivo* occurs predominantly *via* vasodilation. Therefore, further investigations into the effects of vasodilators in our autologous pump perfused rat hindlimb are required to determine whether blood flow distribution (regulated by vasodilatory mechanisms) can alter hindlimb metabolism.

The existence of a dual vascular arrangement in skeletal muscle is a distinct possibility. Clearance rate studies, pharmacological studies and direct observation studies all support the notion of a dual circulation (10), although definitive evidence in weight bearing muscles (unlike the

tenuissimus, cremaster muscles) is required. Functionally, the redistribution of blood flow by 5-HT resulting in reduced oxygen consumption could play a part in nutrient retention when released from platelets during times of aggregation such as haemorrhage (39).

In summary, this study has demonstrated that skeletal muscle metabolism can be influenced by exogenous vasoconstrictor administration in the autologous pump-perfused rat hindlimb. These changes in metabolism in this model provide further evidence of the existence of parallel vascular pathways within skeletal muscle. The ability to greatly increase hindlimb oxygen consumption by increasing metabolic demand through muscle contraction but not through increasing muscle blood flow suggests that *in vitro*, muscle oxygen delivery is adequate and redistribution of perfusate to increase oxygen consumption is largely an artefact of the high flow, low oxygen delivery preparations used in other models. Considering the ability of muscle metabolism to override the exogenously produced flow distribution, a role for NAd *in vivo* might be in the expectant rise in sympathetic nervous activity that is associated with exercise to provide an initial redistribution prior to the onset of muscle activity. While not producing any increase in oxygen consumption, it would provide increased delivery in preparation for or anticipation of the impending muscle activity.

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