Marsupials are a diverse group of mammals with well-developed thermoregulation. The Tasmanian bettong (Bettongia gaimardi) is a rat kangaroo closely related to the larger kangaroos and wallabies. At birth, the bettong is ectothermic. The young becomes progressively endothermic before it finally vacates the pouch (25). Intramuscular injection of norepinephrine (NE) elicited a significant increment in oxygen consumption (VO₂) in vivo in the potoroo Potorous tridactylus (Kerr), a close relative of the bettong, and this increase was blocked by propranolol but not by phenoxybenzamine (23). Hayward and Lisson (15) have presented strong anatomic and microscopic evidence that marsupials, including bettongs, do not possess brown adipose tissue (BAT), which is a major contributor to nonshivering thermogenesis in most newborn and small eutherian mammals. This characteristic makes the bettong a valuable animal for the study of nonshivering thermogenesis occurring in tissues other than BAT, such as skeletal muscle. The perfused rat hindlimb preparation has proved to be a useful model in defining the controlling influences for thermogenesis originating from skeletal muscle, particularly under resting conditions. Thus in the perfused rat hindlimb, we have found that vasoconstrictors (excluding serotonin [5-HT]) such as NE, epinephrine (Epi), vasopressin (VP), and angiotensin (ANG) I, II, and III, increase VO₂ within a limited dose range while causing vasoconstriction in a constant-flow preparation (8, 9, 32, 33). In addition, vasodilators, such as nitroprusside, nifedipine, and isoproterenol, blocked the vasoconstrictor-mediated VO₂ (7–9). Increasing perfusion flow also increased VO₂, which could be further augmented by NE but depressed by nitroprusside (33).

Thus the concept has emerged (7) that either increased flow to, or vasoconstriction within, the rat hindlimb led to enhanced thermogenesis from this vascular bed. However, the findings that 5-HT (11) and high, but not low, concentrations of NE decreased VO₂ by the constant-flow perfused rat hindlimb have led to a modification of the above proposal (6) and suggested the possibility that preexisting vascular tone, particularly in vivo, might be under some circumstances inhibitory. If so, then vasodilators, such as β-adrenergic agonists, could also exert a positive thermogenic response by decreasing the preexisting inhibitory effect on VO₂. Alternatively, β-activation, by increasing cardiac output and hence flow to muscle, may increase thermogenesis (33). These two explanations are not mutually exclusive and could coexist.

No study on VO₂ in a perfused marsupial hindlimb has been found in the literature. As a result of a successful breeding program, a small number of bettongs, which are legally protected under state law, became available for experimentation. Accordingly, the present study attempted to examine whether the vasoconstrictive hormones, NE, VP, and 5-HT, have any effect on VO₂ and on lactate and glycerol efflux from the perfused hindlimb of the bettong, as an example of marsupial muscle metabolism.

METHODS

Animals. Bettongs of either sex were obtained from the University of Tasmania breeding colony (by R. W. Rose). The experiments were approved by the Ethics Committee of the University of Tasmania under the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The animals were housed in room-size cages (approximately 6 × 2 m) in an outdoor animal compound with free access to food and water. During the period of experiment, the seasonal temperature ranged between 18 and 28°C during the daytime and between 10 and 20°C at night. Diet was a choice of apples, bread, and Pig dog pellets (Uncle Ben's of Australia), containing 15% crude protein and 10% fat.

Hindlimb perfusion. The animals (mass 1.2 ± 0.11 kg) were anesthetized with an intraperitoneal injection of pentobarbital sodium at a dose of 60 mg/kg. Additional injections were given to maintain the anesthesia when required. An incision was made distal to the inguinal ligament. The femoral artery and
vasoconstrictors and rat kangaroo metabolism

vein were exposed. Heparin (1,000 IU) was injected into the femoral vein at the level of the inguinal ligament 1 min before cannulation, and an arterial cannula and a venous cannula were then inserted into the respective vessels just below the branch points for the superficial iliac circumflex artery and vein. A tight ligature was placed around the circumference of the thigh ~1 cm above the two cannulas, and another tight ligature around the tarsus, so that only the muscle bed distal to the inguinal ligament of the limb was perfused. The mass of muscle perfused was determined for the first three animals by excising and weighing dye-containing muscle from the contralateral limb that had been infused with perfusion medium containing Evans blue (1% wt/vol) after the same surgical procedures and heparinization of the animal but before the perfusion commenced on the remaining hindlimb. A regression formula was then calculated (perfused muscle mass [g] = 0.035 x muscle wt. kg~1~ + 0.012, r = 0.99), to be applied to the following experimental animals, on the assumption that both hindlimbs were identical in muscle mass. A similar surgical strategy was adopted when muscle samples were collected from the contralateral hindlimb for the in vivo muscle lactate and phosphagen samples. After such sampling the femoral artery and vein were ligated together with a circumferential ligature around the thigh to prevent any hemorrhage before perfusion of the remaining hindlimb.

The perfusion method was essentially that previously described for the rat hindlimb by Colquhoun et al. (9). The perfusate consisted of Krebs-Ringer bicarbonate buffer containing 1.27 mM CaCl~2~, 2% bovine serum albumin, and 8.3 mM glucose. The perfusate was pumped from a reservoir kept at 4°C through a Silastic tube oxygenator continuously equilibrated against a gas mixture of 95% O~2~, 5% CO~2~ and a heat exchanger connected in series at 25°C. The VO~2~ was calculated from arteriovenous difference of oxygen partial pressure measured with a Clark-type oxygen electrode as follows:

\[ \text{VO}_{2} (\mu \text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}) = 1.508 \times (P_{a_{\text{O}}_{2}} - P_{v_{\text{O}}_{2}}) \text{(mmHg)} / [1,000 (\text{ml} / \text{L}) \times \text{muscle mass (g)}] \times 60 \text{ (min/h)} \times \text{flow rate (ml/min)} \]

where 1.508 (μmol·l⁻¹·mmHg⁻¹) is the Bunsen coefficient for plasma at 25°C (5), and PaO~2~ and PvO~2~ are arterial and venous oxygen partial pressures, respectively.

The PaO~2~ was measured at the beginning and end of each perfusion by connection of arterial and venous lines without passing through the hindlimb, so that diffusional losses from the tubing would be the same during perfusion. Pvo~2~ was continuously monitored throughout the perfusion by passing the effluent through a Clark-type oxygen electrode with a chamber volume of ~0.5 ml. The perfusion pressure was also continuously monitored, the pressure-transducer tubing connected to the perfusion line before the arterial cannula and recorded via a pressure transducer. To avoid the perturbation to metabolite assays by erythrocytes and to allow comparisons with results already obtained in our laboratory from other species (8, 9, 11, 16), the perfusion was conducted with an erythrocyte-free medium at 25°C. The perfusion flow rate per mass of hindlimb muscle (0.28 ± 0.02 ml·g⁻¹·min⁻¹) was adjusted to be as close as possible to the flow rate used previously for these perfused rat hindlimbs (0.27 ml·g⁻¹·min⁻¹) (8, 9, 11, 16). For all perfusions the Pvo~2~ remained >200 mmHg, indicating hindlimb flow was adequate.

Calorimetric measurement. The system was composed of a sealed respiratory chamber sitting in a water bath, a coil of copper piping placed in the water bath to act as a heat exchanger for the inlet air, a vacuum pump for controlling air flow through the chamber, a sampling pump, and a modified O~2~–CO~2~ gas analyzer (Doxex, Labtech, Helsinki, Finland). The experiments were performed between 10 A.M. and 6 P.M., which is in the animal’s normal sleep and quiescent phase.

Bettongs were placed in well-ventilated coarse-woven jute bags to reduce ambient light influences to a minimum and were then moved into the respiratory chamber maintained at 25°C. The flow rate of air through the chamber was adjusted to between 6.5 and 8.01/min to keep the expired CO~2~ below 0.3%. The expired air was continuously monitored with the gas analyzer, and the data were collected and stored in a computer for every 1-min interval during the period of experiment. The resting metabolic rate was taken as the minimum 5-min average when the animals stayed quiet. Normally, animals settled well within 15 min, but 30 min were allowed to elapse before data were collected. For comparison, metabolic rates were also determined on rats (male Wistar, 180–200 g) in the same system. Heat production was calculated from the oxygen consumed at rest under conscious condition with the method by Weir (30).

Metabolite assay. For the measurement of muscle metabolites, the calf muscle group (gastrocnemius-plantaris-soleus group) was freeze-clamped with liquid N~2~- precooled aluminum tongs. The freeze clamped muscle samples were taken under anesthesia before perfusion from the contralateral leg. The perfused samples were taken at the end of the perfusion. Samples, after storage at ~80°C, were processed by pulverizing under liquid N~2~, and a portion (200 ± 20 mg) was homogenized in 2.0 ml of perchloric acid (0.42 M), which was then neutralized with dipotassium hydrogen phosphate (1.0 M) to pH 6.5. The neutralized perchloric acid extract was then used for the assay muscle lactate (0.2 ml) and high-energy phosphate compounds (10–20 μl). For the measurement of lactate and glycerol release, 2.0 ml of venous effluent were collected either at each steady state or at nominated times for the time-response curves and were frozen (~20°C) until assay. The arterial perfusate was used as the blank in the assays. Lactate was assayed according to the method of Gutmann and Wahlfeld (14). Glycerol was measured enzymatically, essentially as described by Wieland (31), using the change in fluorescence of NADH measured with an Amino-Bowman spectrophotofluorometer set at 340 nm (excitation) and 460 nm (emission). High-energy phosphate compounds were determined by high-performance liquid chromatography (26) with a Nova Pak C~18~, 4-μm column (Waters Associates).

Chemicals. The enzymes, NAD, AMP, ADP, and ATP were purchased from Boehringer Mannheim (Germany). NE, VP, 5-HT, phenolamine, and propranolol were obtained from Sigma (St. Louis, MO). Bovine serum albumin (fraction V) was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN) and dialyzed five times against distilled water. Sodium nitroprusside was purchased from Merck. All other chemicals were analytic grade from Ajax Chemicals (Australia).

Statistics. Student’s t-test was used for the statistics, and P < 0.05 was considered significantly different. All values are presented as means ± SE.

RESULTS

Validation of perfusion. Hindlimb perfusions were performed on eight bettongs (7 male, 1 female). Table 1 shows the mean values for body weight of these young animals aged 6–8 mo, which have left the pouch for some time. The mass of perfused skeletal muscle, as well as the PaO~2~, perfusion pressure, hindlimb VO~2~, and rate of release of lactate and glycerol under basal conditions are also shown in Table 1. Perfusion conditions ap-
peared stable; \( V_0_2 \) as well as lactate and glycerol efflux remained constant for 3–4 h. Measurement of metabolites in the frozen gastrocnemius-plantaris-soleus muscle group at the end of the perfusion period showed that there were no significant statistical differences of adenine nucleotides between the muscle samples obtained in vivo and after perfusion. In fact, the concentration of lactate and creatine decreased \( (P < 0.01) \), and the phosphocreatine-to-ATP ratio was improved \( (P < 0.05) \) after the perfusion (Table 2). These results show an essentially normal metabolite profile of the perfused muscle without evidence of hypoxia.

**Actions of vasoconstrictors.** As shown in Fig. 1, NE, VP, and 5-HT each exerted a qualitatively similar dose-dependent vasoconstrictive effect in the perfused bettong hindlimb. Their other effects on \( V_0_2 \) and on lactate and glycerol efflux aggregated into two different sets of responses. Thus qualitatively, NE and VP induced increases in \( V_0_2 \) and in lactate and glycerol efflux, which appeared to be closely associated with the changes in perfusion pressure. NE-induced changes attained a maximal value at \( \sim 1.0 \mu M \) NE, with increases >100% over the basal in the three variables. Above this dose, values of \( V_0_2 \) tended to decline, although the perfusion pressure continued to rise. Similarly, VP stimulated, but to a lesser degree, the \( V_0_2 \) and the efflux of lactate and glycerol, with maximal values occurring at \( \sim 25 \) nM. Perfusion pressure continued to increase with dose. In contrast to NE and VP, 5-HT significantly inhibited \( V_0_2 \) \( (P < 0.05) \) in the concentration range 0.1–25 \( \mu M \) during vasoconstriction. However, the inhibition of lactate and glycerol efflux by 5-HT was not large in magnitude and reached statistical significance only for lactate efflux. After termination of infusion of each of the vasoconstrictors, values for \( P_0_2 \) and perfusion pressure returned to basal values throughout the periods of perfusion, which lasted at least 3–4 h.

**Effects of \( \alpha \) - and \( \beta \) -adrenergic antagonists on NE-mediated effects.** Changes in perfusion pressure, \( V_0_2 \), and lactate and glycerol release in response to NE were rapid in onset, sustained, and rapidly reversible (Fig. 2). In the presence of the \( \beta \)-adrenergic antagonist propranolol (10 \( \mu M \)) in the perfuse, \( V_0_2 \) and lactate and glycerol release in the presence of NE and propranolol were not statistically different from values with NE alone. However, the additional infusion of the \( \alpha \)-adrenergic antagonist, phentolamine (10 \( \mu M \)), completely blocked the rise in perfusion pressure as well as the increases in \( V_0_2 \) and in lactate and glycerol release elicited by NE.

**Effect of nitroprusside.** The nitrodiol sodium nitroprusside (1.0 \( mM \)) completely blocked the rise in perfusion pressure and the changes in \( V_0_2 \) stimulated by NE, VP, and 5-HT (Fig. 3). Nitroprusside also blocked the increased release of lactate and glycerol elicited by NE and VP and reversed the inhibitory effects elicited by 5-HT on lactate and glycerol efflux (data not shown).

**Metabolic rate.** The bettongs studied by indirect calorimetry were slightly larger than those killed for perfusion studies. The weight (mean ± SE) of the animals was \( 1.7 ± 0.1 \) kg (n = 7). The \( V_0_2 \) was \( 23 ± 2 \) \( \mu M \) · h\(^{-1}\) · g body wt\(^{-1}\), which is equivalent to a resting heat production of 249 ± 21 \( \mu J \) · kg\(^{-1}\) · day\(^{-1}\). Under these conditions, the rat \( V_0_2 \) amounted to 60 ± 1 \( \mu M \) · h\(^{-1}\) · g body wt\(^{-1}\) (i.e., \( 656 ± 10 \) \( \mu J \) · kg\(^{-1}\) · day\(^{-1}\)).

**DISCUSSION**

In the present study, bettong hindlimbs were perfused at 25°C at flow rates chosen to give a constant flow per unit mass of muscle of 0.28 ml · min\(^{-1}\) · g\(^{-1}\) (Table 1). This constant flow rate was the same as that employed by us previously for rat hindlimb perfusions at the same temperature (8, 9, 11, 16). Perfusions were performed without red blood cells to prevent their contribution to lactate production (16). This necessitated a lower temperature of 25°C to allow delivery of sufficient oxygen to the preparation, which was stable throughout the experimental period. The characteristics of the perfused hindlimb preparation, as well as incubated muscle at 25°C for the study of muscle metabolism, have been recently reviewed, and the data were generally comparable to

<table>
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<tr>
<th>Table 1. Basal measurements for perfused bettong muscle</th>
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<td><strong>Body Mass, kg</strong></td>
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Values are means ± SE; n = 7–8 animals.

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<th>Table 2. Metabolites of bettong gastrocnemius-plantaris-soleus muscle group</th>
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<td><strong>Lactate</strong></td>
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Values are means ± SE; n = 5 samples/group. Muscle samples were freeze-clamped with liquid N\(_2\)- precooled aluminum tongs. The in vivo group was taken under anesthesia, whereas the perfused samples were taken at the end of the perfusion. Among the perfused group, 3 muscle samples were taken after the removal of vasoconstrictors, 1 in the presence of norepinephrine (0.1 \( \mu M \)) and the other in the presence of 5-hydroxytryptamine (1 \( \mu M \)). As no significant differences in the metabolites were found in the presence or absence of the vasoconstrictors, data were pooled in 1 group. \( \Sigma \)AN, total adenine nucleotides; Cr, creatine; PCr, phosphocreatine; EC, energy charge. EC = (\( \Sigma \)AMP + [ATP])/([AMP] + [ADP] + [ATP]), where [AMP], [ADP], and [ATP] refer to concentrations of AMP, ADP, and ATP, respectively. * \( P < 0.01 \), \(^{†} P < 0.05 \) vs. in vivo.
those obtained at 37°C by applying a Q10 of 2.5 (2), where Q10 refers to the rate of change of muscle metabolism per 10°C increase in temperature.

While resting hindlimb perfusion pressures are at first sight low, arterial pressures will necessarily be lower than in vivo when a cell-free, low-viscosity perfusate is employed. In addition, there is neither resting sympathetic tone nor circulating vasoconstrictors in the perfused hindlimb. Thus the arterial tree is almost fully relaxed as shown by the fact that vasodilators (e.g., nitroprusside) do not lower the perfusion pressure (Fig. 3). The in vivo blood flow rates to the skeletal muscle of the bettong’s larger relative, the red kangaroo (Macropus rufus), at rest under different environmental temperatures ranged from 0.010 to 0.053 ml min⁻¹ g⁻¹ (22). Thus if similar flow rates apply to the bettong, the flow rate we employed is approximately 5 to 28 times greater than in vivo. Furthermore, because there is little diminution of pressure in the dilated arterial and arteriolar beds, the capillary exchange pressures will be sufficient. Adequate perfusion and oxygen delivery to the hindlimb preparation at rest and under the maximal vasoconstriction or VO2 are indicated by the normal values for intracellular lactate, high-energy phosphate compounds, and the calculated energy charge in perfused muscle (Table 2). This is further supported by values for PV02 that at no stage fell below 200 mmHg.

The basal VO2 of the perfused bettong hindlimb, which for calculation we have attributed to the mass of skeletal muscle present, was 4.18 ± 0.35 μmol g⁻¹ h⁻¹, which is ~65% that of perfused rat hindlimb of 6.4 μmol g⁻¹ h⁻¹ (8, 16). When the whole body resting metabolic rates were measured under the same conditions, the metabolic rate of 249 ± 21 kJ kg⁻¹ day⁻¹ (n = 7) for the bettong was ~38% that of the rat at 656 ± 10 kJ kg⁻¹ day⁻¹ (n = 7). These latter metabolic rates were similar to those obtained by others in a closely related species of bettong (29) and in the rat (20).

Although there are a number of reports on the effect of infused NE on VO2 in the perfused muscle in eutherian mammals such as the rat (8, 13, 32) and the dog (24), to the best of our knowledge this is the first report on the effects of vasoconstrictors (i.e., NE, VP, and 5-HT) on VO2 and on lactate and glycerol efflux in perfused marsupial hindlimb and hence in a noneutherian homeothermic species.

In the perfused bettong hindlimb, increases in VO2 and in both lactate and glycerol efflux were observed in response to NE and VP, whereas decreases in VO2 and in lactate and glycerol efflux were noted with 5-HT (Figs. 1 and 2). The changes in VO2 and in lactate in response to these vasoconstrictors were qualitatively similar to those observed previously in the perfused rat hindlimb (8, 9, 11). A new observation for muscle perfusion was that changes in the glycerol efflux closely paralleled the changes in the hindlimb VO2 and lactate (Figs. 1 and 2). The production of lactate and glycerol will have thermogenic potential in vivo if they are subsequently taken up by other tissues such as the liver and resynthesized to carbohydrate or lipid, as these processes would require additional energy expenditure.

Analysis of the dose-response data (Fig. 1) for the perfused bettong hindlimb shows that the thermogenic response to NE started at 25 nM, which is almost 25 times higher than that required for the perfused rat hindlimb under the same conditions (9). The increase in VO2 stimulated by NE of ~100% above the basal value was greater than that of the perfused rat hindlimb, which increases by approximately 60–70% at the same flow rate (9, 16, 33). At present, no data on circulating NE are available for the bettong, and in general, data are sparse for all marsupials. However, a small marsupial, the sugar glider (27), and the platypus (21) show resting and stressed values of NE approximately 3- to 10-fold higher than those of the rat; thus the present observations may be relevant to field conditions.

Marsupials appear to have more skeletal muscle than eutherians. Red and gray kangaroos have 32% of body mass as skeletal muscle, which is 6–8% more than in the wildebeest or Thomson’s gazelle and ~20% more than in domestic eutherian species (28). The blood flow to the marsupial skeletal muscle varies considerably in re-
response to the environmental temperature. For example, distribution of cardiac output to skeletal muscle increased from 4% at 37°C to 23% at 7°C (22). When exposed to the cold, the maximal heat production of small marsupials can match or exceed that of rodents of similar size (10). Thus the present study, when taken in conjunction with these observations, suggests that marsupial skeletal muscle may contribute a larger proportion to whole body thermogenesis than the skeletal muscle in eutherian species. As the bettong and other marsupials have been shown not to possess histologically apparent BAT (15), the bigger relative increase in NE (or other vasoconstrictor)-induced energy metabolism and the greater proportion of skeletal muscle may compensate for the lack of BAT in these species.

Unexpectedly, the NE-induced glycerol release was found to be mediated through α-adrenergic receptors as shown by its blockade by phentolamine plus propranolol but not by propranolol alone (Fig. 2). Activation of lipolysis by Epi has been reported in the perfused mouse hindlimb, and β-adrenergic receptors were thought to be responsible (3). We are unaware of any study so far that characterizes increased glycerol efflux mediated by α-adrenergic receptors, VP, or its inhibition by 5-HT. On the contrary, inhibition of adipose tissue lipolysis has been associated with activation of the α2-adrenergic receptor (17). A recent autoradiographic study indicates that β-receptors were predominantly distributed on skeletal muscle cells while the α-receptors were much more

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**Fig. 2.** Effects of α- and β-adrenergic antagonists on NE-mediated perfusion pressure, VO₂, and lactate and glycerol efflux. Prop, propranolol (10 μM racemic); Ph, phentolamine (10 μM); NE (0.1 μM). Data are means ± SE from 3 experiments.

**Fig. 3.** Representative tracing of inhibitory effect (n = 2–3) of nitroprusside (NP, 1.0 mM) on partial pressure of venous oxygen (PVO₂; top) and perfusion pressure (bottom) mediated by NE (0.1 μM), 5-HT (2.5 μM), and VP (25 nM).
sparse and were 30 times higher on arterioles than in the surrounding skeletal muscle (19). Nevertheless, the NE-mediated VO₂ and lactate and glycerol efflux in the present experiment is α-receptor dependent rather than β-receptor dependent (Fig. 2), suggesting blood vessels are somehow involved in the increased metabolism. The close parallel relationship between vasoconstrictor-mediated glycerol release and VO₂ change implies that the vasoconstrictors may play a role in modulating lipid mobilization either directly by receptor activation or indirectly by association with smooth muscle contraction to adjust the fuel sources for skeletal muscle thermogenesis. We have recently made similar preliminary findings of NE-mediated increases in glycerol efflux in the perfused rat hindlimb in the presence of propranolol (6). Chin et al. (4) have shown that infused lactate can lead to increased glycerol production in the perfused rat hindlimb. Hence in the present study, which uses a once-through perfusion, another potential source of glycerol would be the conversion of a small proportion of lactate to glycerol downstream from the site of lactate production.

The lack of a β-adrenergic stimulation of VO₂ in perfused blood flow to skeletal muscle. Increased flow to skeletal muscle has been shown to increase VO₂ by perfused skeletal muscle in the rat (33). In addition, other thermogenic tissues, such as liver, might also contribute to β-adrenergic stimulation of the whole body metabolic rate (18). Alternatively, β-stimulation leading to vasodilatation in vivo may oppose a preexisting pattern of vasoconstriction that inhibits VO₂ as observed with 5-HT and high concentrations of NE in the perfused rat hindlimb (6).

The action of the nitric oxide generator, nitroprusside, to block both vasoconstriction and the changes in VO₂, whether they be increased or decreased (Fig. 3), strongly suggests that vasoconstriction is tightly linked in some manner to these phenomena. Yet the mechanism for the vasoactive hormone-induced increase in VO₂ is still controversial. A priori, to explain the increased VO₂, there must either be an increased hydrolysis and subsequent synthesis of ATP or a stimulated mitochondrial uncoupling within the hindlimb analogous to that occurring in BAT. Four general possibilities are apparent for the bettong and other species that could explain the vasoconstrictor-mediated control of such processes in the perfused hindlimb.

First, the vasoconstrictors may act directly on skeletal muscle receptors to cause the above metabolic changes independent of the increases in perfusion pressure because some vasoconstrictor hormones have receptors on skeletal muscle. This possibility appears to be unlikely because for NE, β-receptors are predominantly distributed on skeletal muscle cells while α-receptors are much higher on arterioles than on surrounding skeletal muscle (19). Moreover, results from Fig. 2 show α-rather than β-receptors are responsible for NE-induced metabolism in the perfused bettong hindlimb. Further evidence suggesting that the vasoconstrictor receptors coupled to VO₂ appear not to be present on skeletal muscle comes from the observations that NE had little or no effect on VO₂ from perfused mouse skeletal muscle (12), nor did NE or angiotensin stimulate VO₂ or lactate production from perfused rat skeletal muscle (16).

The second possibility is that the rise in perfusion pressure associated with vasoconstriction may redistribute the perfusate within the hindlimb to previously underperfused zones and thus, by relieving regions of hypoxia, stimulate increased VO₂. This is unlikely because, as discussed earlier, there is no evidence of regional hypoxia as the high-energy nucleotides are normal (Table 2). Furthermore, such a pressure-induced redistribution of flow to relieve hypoxia does not accord with the inhibition of VO₂ associated with the vasoconstriction induced by 5-HT (Figs. 1 and 3).

The third possibility is that arterial vasoconstriction is energetically expensive and uses the observed VO₂. We have previously hypothesized vascular thermogenesis in the perfused rat hindlimb (7). The possibility of direct blood vessel smooth muscle VO₂ during constriction is consistent with the close association of vasoconstriction and increases in VO₂ and lactate and glycerol efflux and the block of all these changes by the vasodilator nitroprusside (Figs. 1–3). It is not readily apparent how vascular thermogenesis could explain the inhibition of VO₂ and lactate efflux by 5-HT that accompanies a rise in vascular resistance in the bettong hindlimb (Fig. 1) or the rat hindlimb (11). However, when infused into the isolated perfused rat mesenteric artery, 5-HT (as well as NE and VP) caused vasoconstriction and increased VO₂ (11, 32). These observations led to the idea that 5-HT initiates a state of functional shunting of perfusate within the muscle vascular bed away from actively metabolizing cells (11). Similarly, functional shunting may occur in the bettong hindlimb in response to 5-HT.

The fourth possibility is that vasoconstrictors by selective actions redirect flow to particular thermogenic skeletal muscle cells or other thermogenic cells in the hindlimb. This would provide more oxygen to those cells to allow activation of increased levels of uncoupling, ion pumping, or increased substrate cycles. So far, such specialized muscle cells have only been described in billfish, which heat blood going to the retina and brain by presumptive calcium ion cycling (1). However, data from the present experiments cannot discriminate between these two mechanisms of controlling or contributing for the involvement of blood vessels in the vasoconstrictor-altered VO₂ and lactate and glycerol efflux in the perfused bettong hindlimb. They may not be necessarily mutually exclusive.

In summary, the present experiments show that vasoconstrictive hormones play an important role in controlling and/or contributing to VO₂ and lactate and glycerol efflux in the perfused bettong muscle. There appears to be a greater contribution from skeletal muscle vascular bed to the whole body thermogenesis in
the betting than in the eutherians both at rest and in response to NE, which may in vivo compensate for its lack of BAT. The responses, however, do not relate in a simple way to the degree of vasoconstriction because there are both increases and decreases in the metabolic parameters depending on the vasoconstrictor employed. The present findings on perfused bettong muscle when taken together with other studies on perfused rat muscle raise the possibility of vasoconstrictor control of VO2 and metabolism as an entrenched and widespread physiological mechanism in evolution.

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