Altered muscle metabolism associated with vasoconstriction in spontaneously hypertensive rats

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Ye, Ji-Ming, and Eric Q. Colquhoun. Altered muscle metabolism associated with vasoconstriction in spontaneously hypertensive rats. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E1007–E1015, 1998—In the rat muscle vascular bed, vasoconstrictors either increase or decrease oxygen consumption (VO₂). The present study compared the effects of norepinephrine (NE), angiotensin II (ANG II), and 5-hydroxytryptamine (5-HT) on vasoconstriction-associated metabolism in the constant-flow perfused hindlimb of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) in the absence of insulin. Basal perfusion pressure VO₂, glucose uptake, and lactate production were increased by 21.4, 11.9, 46.4, and 44.9% (P < 0.05 for all), respectively, in SHR, which had also higher blood pressure and metabolic rate (P < 0.05) in vivo. Dose-response curves for NE-induced perfusion pressure, VO₂, and lactate production in SHR were shifted to the left compared with WKY. Associated with the increased perfusion pressure, NE-induced VO₂ and glucose uptake were both decreased (P < 0.01), particularly at high concentrations. These differences were unaffected by 10 µM propranolol but were all diminished by further addition of prazosin (2.5 nM). ANG II stimulated VO₂, glucose uptake, and lactate production in both strains, but the increased lactate production was smaller in SHR (P < 0.05) with a proportional decrease (P < 0.05) in glucose uptake. Conversely, 5-HT decreased VO₂ in both strains (P < 0.01), and this effect was greater in SHR (P < 0.01). These data suggest that SHR muscle thermogenesis and glucose uptake are impaired during vasoconstriction, especially in response to NE.

Hypertension; oxygen consumption; glucose uptake; lactate production; norepinephrine; angiotensin II; 5-hydroxytryptamine; hindlimb

STUDIES IN BOTH HUMANS and animals have revealed a close correlation between hypertension and obesity as well as hyperinsulinemia, hyperglycemia, and hyperlipidemia, now often referred to as syndrome X (11, 18, 22, 24, 36). Although obesity is an important risk factor for the pathogenesis of hypertension, the effect of hypertension on metabolic disorders such as energy balance and glucose disposal is presently of great interest (22, 26–28, 31–35).

Skeletal muscle is a major thermogenic (or oxygen-consuming) source (4, 7, 9) and the primary tissue for glucose disposal (18, 33). Muscle insulin resistance is a prerequisite for hyperglycemia seen in obesity and hypertension (18, 23, 36). Both metabolic alterations in muscle are, at least in part, controlled by the vascular system (7, 10, 19, 33, 35, 40). Compared with other organs such as gut and kidney, both vasoconstriction and oxygen consumption (VO₂) in perfused rat hindlimbs are far more sensitive to norepinephrine (NE) and vasopressin (VP; see Ref. 41), implying that the muscle vascular bed is one of the first organs affected by vasoconstrictors in vivo.

In the constant-flow perfused rat hindlimb, a muscle bed with many characteristics similar to those in vivo (4, 7), all vasoconstrictive hormones tested so far either increase or decrease VO₂ and lactate production, indicators for muscle metabolism. Furthermore, according to their metabolic effects, vasoconstrictors may be classified as type A, which induces a positive stimulation of both VO₂ and lactate production, and type B, which inhibits VO₂ and lactate production (Ref. 7 and references therein). Type A vasoconstrictors include low concentration NE (LNE, <1 µM) and other α₁-adrenoceptor agonists at low doses, angiotensin II (ANG II), VP, and low-frequency sympathetic nerve stimulation (<4 Hz), whereas type B vasoconstrictors include high concentration NE (HNE, >1 µM) and all α₁-adrenoceptor agonists at high doses, 5-hydroxytryptamine (5-HT), and high-frequency sympathetic nerve stimulation (>4 Hz). Despite the disparity of their metabolic effects, vasoconstriction appears crucial because both metabolic and pressure changes are reversed by infusion of vasodilators such as nitroprusside for both type A and type B vasoconstrictors (7, 10, 13, 19, 42).

Essential hypertension is characterized by increased peripheral vascular resistance. Spontaneously hypertensive rats (SHR) have been widely used to delineate the mechanisms for abnormalities in the cardiovascular system (1, 5, 6, 38) and metabolic disorders in hypertension (3, 8, 25, 27, 28, 31–33). In SHR, there is an elevated peripheral vascular resistance in muscle caused by either increased vascular tension due to a higher sensitivity of resistance blood vessels or/and elevated vasoconstrictor levels (1, 3, 5, 6, 25, 39). At the level of microcirculation, vascular rarefaction has been found in skeletal muscle (3, 26, 38, 39). These alterations are likely to affect muscle metabolism controlled by vasoconstrictors. To test this idea, we compared the effects of NE, ANG II, and 5-HT on perfusion pressure and associated changes in VO₂, glucose uptake, and lactate production in the perfused hindlimbs of SHR and their normotensive counterparts, Wistar-Kyoto rats (WKY).

MATERIALS AND METHODS

Animals. Age-matched (11 wk) male SHR and WKY used for the experiments were purchased from the Animal Resources Center of Western Australia. Twenty SHR and 20 WKY were used for this study. Hypertension has been shown by others (1) to be well developed at this age in the SHR from the same source. The animals were housed at 20°C with a 12:12-h light-dark cycle and allowed free access to water. The
diet consisted of 20.4% protein, 4.6% lipid, 69% carbohydrate, and 6% crude fiber with added vitamins and minerals (Gibson, Hobart, Australia). All 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.

Whole body metabolic rate. Metabolic rates were measured in pairs (SHR–WKY, or WKY–SHR) each day between 10:00 AM and 6:00 PM by an indirect calorimetry system under conscious conditions at 25 ± 0.5°C as previously described (42). To minimize stress, the animals had been exposed to human handling for 2 wk before the experiment. The respiratory chamber was covered with black cloth to prevent any visual disturbance from the environment. The flow rate of air through the chamber was adjusted to 3 l/min to keep the expired CO2 below 0.3% and monitored by a mass flowmeter (Hartings, Hampton, VA). The expired air was dried by passing it through a column (50 × 5 cm) filled with CaSO4 placed before a modified O2/CO2 gas analyzer (Datex; Labtech, Helsinki, Finland) and continuously monitored throughout the experiment. The inlet air was measured before and after each experiment for the calculation of V˙O2 and CO2 emission on the basis of the difference between the inlet and expired airs as previously described (42). The measurement for each rat lasted 3 h, and the resting metabolic rate was calculated from the average of minimal readings for a period of 10 min.

Blood pressure and interscapular brown adipose tissue. Eight animals from each strain were used for these measurements using the method described by Sexton et al. (39) under anesthesia with pentobarbital sodium (60 mg/kg body weight ip). Blood pressure was measured in the anesthetized state from a cannulated carotid artery by the use of a sphygmomanometer (ALPK2) placed at the level of the heart. Three consecutive steady-state blood pressure recordings for each rat were obtained and averaged. After the measurement of blood pressure, the carotid artery was tied off, and the interscapular brown adipose tissue (IBAT) was removed and weighed.

Hindlimb perfusion. The surgical procedures were similar to those described before (10) under anesthesia (mentioned above) with modifications of additional occlusions of abdominal vessels to improve the leg muscle perfusion (17) and restrict flow distribution to the contralateral trunk region. In brief, a midline abdominal incision was made to expose the abdominal cavity. The lower part of the anterior abdominal wall was removed, and the cut edges were ligated with sutures. Gut, seminal vesicles, and testes were removed after appropriate ligations. The right common iliac artery and vein were ligated, and a string was firmly placed around the tail at its root. To prevent any perforate spillover when perfusion pressure increased, blood vessels connected with tissues other than the left hindlimb were carefully tied off. These included the right hypogastric vessels, the left inferior and superficial epigastric vessels, the inferior mesenteric vessels and superior vesicle vessels, and the iliolumbar and saphenous vessels in both sides. The perfused areas (as confirmed by perfusion with Evan’s blue) included both the left leg, the whole left trunk, and a small part (usually 0.3–0.5 cm) of the right trunk along the spine. This flow distribution pattern was more comparable to the single hindquarter perfusion preparation with hypogastric occlusion as described by Gorski et al. (17). After heparin (0.5 ml, 1,000 U/ml) was injected into the vena cava, two cannulas (Ohmeda) were inserted caudally into the abdominal aorta (16-gauge) and vena cava (18-gauge) between the left renal and iliolumbar vessels. The animal was then immediately placed on a perspex platform to begin the perfusion. The animal was killed with an overdose of the anesthetic. Ligatures were placed firmly around the lumbar trunk approximately between L3–L4 vertebrate, the right thigh (near the inguinal ligament), and the genitalia (above the penis).

A temperature-regulated cabinet (25°C) contained the perfusion system, which consisted of a perfusion pump (Masterflex, Chicago, IL), microinfusion infusion pump (model 11; Harvard Apparatus), an in-line Clark-type oxygen electrode, sphygmomanometer, pressure transducer, artificial lung, heat exchange, and polyethylene tubing. The oxygen electrode and pressure transducer were connected to a dual channel chart recorder (Omniscribe, series D5000; Houston Instrument). The oxygen electrode was calibrated against 100% oxygen, room air, and 99.9% nitrogen. The perfusate consisting of a cell-free Krebs-Ringer bicarbonate buffer (pH 7.4) containing 8.3 mM glucose, 1.27 mM CaCl2, and 2% bovine serum albumin was equilibrated by the artificial lung with a mixture of 95% O2 and 5% CO2. The perfusion flow was set at 6 ml/min by adjusting the perfusion pump speed and confirmed by intermittent collection of the venous efflux from the hindlimb in a measuring cylinder. The hindlimb was perfused in a nonrecirculating manner at 25°C. The hindlimb perfused under these conditions gives qualitatively similar results to those perfused with erythrocyte-containing media at 37°C in response to various vasoconstrictors (4, 7, 33). The venous oxygen partial pressure was always >150 mmHg even when a maximal oxygen extraction took place while the arterial oxygen measured was 684 ± 33 mmHg (n = 58). Adequate oxygen delivery at this flow rate had been confirmed in our earlier studies (10). The perfusion was completed within 180 min, and our previous experiments under similar conditions have shown that this preparation was stable for at least 180 min with similar muscle metabolic characteristics as those in vivo (10). The heart was weighed after perfusion.

Perfusion pressure was monitored via the pressure transducer from a side arm of the arterial line immediately before the arterial cannula. Oxygen partial pressure of the perfusate was measured by the oxygen electrode, which was calibrated before and after each perfusion with oxygen and air. The oxygen content in the perfusate was calculated according to the partial pressures using a Bunsen coefficient at 25°C for plasma as described previously (10). V˙O2 by the perfused hindlimb was then calculated from the arteriovenous difference of oxygen contents multiplied by flow rate and divided by the mass of perfused muscle. The perfused muscle mass was measured by weighing dye-containing muscle dissected from hindlimbs (11 SHR and 11 WKY) that had been infused with Evan’s blue (1% wt/vol) under the same perfusion conditions. The average values were used for the calculation of the rest of the rats for each strain of rat. The gastrocnemius, plantaris, and soleus muscle groups in the right hindlimb were dissected from eight animals of each strain for the measurement of weight, assuming that the weight of both hindlimbs was equal. Both glucose and lactate were measured with a glucose analyzer (YSI 2300 STAT plus). The venous perfusate was sampled in the steady state as indicated by a constant venous partial oxygen pressure record for each dose of the vasoconstrictors. Arterial perfusate was also taken before and after each perfusion to calculate glucose uptake and lactate production in the same way as for V˙O2.

Experimental protocols. After the perfusion began, an equilibration period of 30 min was allowed to elapse during which the partial pressure of venous oxygen reached the same constant value. The basal values for perfusion pressure, V˙O2, glucose uptake, and lactate production were obtained between 30 and 40 min. The experiments in each strain
were assigned to three equal groups with four rats in each group scheduled to receive either NE, ANG II, or 5-HT infusion. With the effects of NE, the involvement of β- and α2-adrenoceptors was assessed in the presence of 10 μM prazosin as well as a combination of prazosin with 2.5 nM prazosin. The antagonists were infused 20 min before NE and then were confused with NE throughout the perfusion. Drugs were infused from a port in the arterial line at a rate <1% of the perfusion flow rate and were mixed by a magnetic stirrer in a small bubble trap immediately before entering the hindlimb. Dose-response curves were constructed for each vasoconstrictor in a cumulative fashion. Data were collected at the steady-state levels for each dose of vasoconstrictors, which usually took 5–10 min with weak vasoconstriction and 20–30 min with strong vasoconstriction, based on the continuously recorded perfusion pressure and VO2. Earlier studies using the same preparation showed sustained changes in vasoconstriction and metabolism in response to different vasoconstrictors (7, 10, 13). The preliminary experiments in this study showed no differences in the development between SHR and WKY rats when compared with WKY. How- ever, no significant differences were found in the basal perfusion pressure, V˙O2, glucose uptake, and lactate production. The basal values of the perfused hindlimb in the absence of vasoconstrictor are summarized in Table 2. At similar perfusion flow rates, perfusion pressure, V˙O2, glucose uptake, and lactate production were increased by 21.4% (P 0.01), 44.9% (P 0.05). In SHR, respectively, in SHR when compared with WKY. However, no significant differences were found in the basal perfused muscle mass or weight of the individual muscle groups measured (P 0.05).

Effects of NE on perfusion pressure, V˙O2, glucose uptake, and lactate production. Infusion of NE led to a dose-dependent sigmoidal increase in perfusion pressure in both strains of rats (P 0.001). In WKY, NE-induced increases in perfusion pressure started at 33 nM and reached a plateau at 10 μM with a maximal increment of 193.3 ± 3.1 mmHg. In SHR, the NE-induced increases were increased by 21.4% (P 0.01), 44.9% (P 0.05), and 44.9% (P 0.01), respectively, in SHR when compared with WKY. However, no significant differences were found in the basal perfused muscle mass or weight of the individual muscle groups measured (P 0.05).

RESULTS

Blood pressure, heart weight, and whole body metabolic rate. Table 1 shows the body weight, blood pressure, whole body VO2, and weight of the heart and IBAT in SHR and WKY. Compared with the age-matched WKY rat, SHR showed significantly higher mean blood pressure under anesthesia (94%, P < 0.01) and increased heart weight (20%, P < 0.01). The measured resting metabolic rate (expressed as VO2) was also increased by 11.6% (P < 0.05) in SHR. However, there were no significant differences in body weight and IBAT mass between these two strains (P > 0.05).

Table 2. Basal values of perfusion pressure, V˙O2, glucose uptake, lactate production, and muscle mass of the perfused hindlimb

<table>
<thead>
<tr>
<th>Flow Rate, ml·min⁻¹·g⁻¹ (n = 11)</th>
<th>PP, mmHg (n = 11)</th>
<th>VO2, μmol·g⁻¹·h⁻¹ (n = 11)</th>
<th>Glucose Uptake, μmol·g⁻¹·h⁻¹ (n = 11)</th>
<th>Lactate Production, μmol·min⁻¹·g⁻¹ (n = 12)</th>
<th>Muscle Mass, g (n = 12)</th>
<th>Soleus, g (n = 8)</th>
<th>Plantaris, g (n = 8)</th>
<th>Gastrocnemius, g (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>0.26 ± 0.03</td>
<td>36.5 ± 0.38</td>
<td>6.96 ± 0.11</td>
<td>1.27 ± 0.07</td>
<td>3.85 ± 0.40</td>
<td>23.02 ± 0.56</td>
<td>0.09 ± 0.003</td>
<td>0.24 ± 0.006</td>
</tr>
<tr>
<td>SHR</td>
<td>0.27 ± 0.02</td>
<td>44.3 ± 0.63*</td>
<td>7.79 ± 0.11*</td>
<td>1.86 ± 0.21†</td>
<td>5.58 ± 0.36*</td>
<td>22.18 ± 0.79</td>
<td>0.10 ± 0.002</td>
<td>0.23 ± 0.004</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. PP, perfusion pressure. *P < 0.01 and †P < 0.05 vs. WKY.

Table 1. Body weight, blood pressure, metabolic rate, and heart and interscapular brown adipose tissue weight

<table>
<thead>
<tr>
<th>Body Wt, g (n = 20)</th>
<th>BP, mmHg (n = 8)</th>
<th>Heart, g (n = 12)</th>
<th>Heart/Body Wt, % (n = 11)</th>
<th>VO2, μmol·g⁻¹·h⁻¹ (n = 8)</th>
<th>IBAT, g (n = 8)</th>
<th>IBAT/Body Wt, % (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>276.6 ± 1.2</td>
<td>94 ± 3.0</td>
<td>0.90 ± 0.01</td>
<td>0.328 ± 0.006</td>
<td>41.4 ± 2.2</td>
<td>0.36 ± 0.03</td>
</tr>
<tr>
<td>SHR</td>
<td>273.3 ± 1.3</td>
<td>182 ± 3.0*</td>
<td>1.10 ± 0.01*</td>
<td>0.395 ± 0.005*</td>
<td>46.2 ± 2.5†</td>
<td>0.39 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; BP, blood pressure under anesthesia; VO2, oxygen consumption; IBAT, interscapular brown adipose tissue. *P < 0.01 and †P < 0.05 vs. WKY.
interaction between NE doses and rat groups, P
and 5-HT in the perfused hindlimbs
P
the left in SHR by more than twofold (indicated by the values of pEC 50 and pIC50 (Table 3).

Table 3. pEC50 or pIC50 values for NE, ANG II, and rat groups was also highly significant for NE-

dose-response curves were distinctly different between SHR and WKY (P < 0.002, Fig. 1). In WKY, the increased glucose uptake reached a plateau at 0.1 µM and remained at that level with further increases in NE dose. In contrast, a bell-shaped dose-response curve of glucose uptake was observed in SHR, with the maximal increase in glucose uptake only one-half of that in WKY. Above 0.33 µM, NE-induced glucose uptake started to decline in SHR with further increases in NE dose.

Overall, there was a highly significant interaction (P < 0.001) between SHR and WKY in vasoconstriction, VO2, and glucose uptake in response to NE.

Effects of propranolol and prazosin on NE-induced changes in perfusion pressure, VO2, glucose uptake, and lactate production. Although appearing somewhat smaller, a similar pattern of increased vasoconstriction (P < 0.01) and reduced VO2 (P < 0.01) in SHR also occurred at different doses of NE in the presence of 10 µM propranolol. Compared with WKY, NE-induced glucose uptake in SHR was still lower at the high dose range (P < 0.05). The interaction between NE doses and rat groups was also highly significant for NE-mediated dose-response curves of perfusion pressure, VO2, and glucose uptake (P < 0.01 for all 3 parameters, Fig. 2).

NE caused dose-dependent changes in VO2 and lactate production (P < 0.001 for both) characterized by stimulatory action at low doses and inhibitory action from the maximal increase to values near or below basal levels at high NE doses (Fig. 1). The dose-dependent biphasic change in VO2 was also shifted to the left in SHR by more than twofold (P < 0.05), as indicated by the values of pEC50 and pIC50 (Table 3). Compared with WKY, the bell-shaped VO2 curves in SHR were downshifted significantly with different NE doses (P < 0.01).

Glucose uptake was also increased by NE during vasoconstriction (P < 0.001) in both strains, and the dose-response curves were distinctly different between SHR and WKY (P < 0.002, Fig. 1). In WKY, the increased glucose uptake reached a plateau at 0.1 µM and remained at that level with further increases in NE dose. In contrast, a bell-shaped dose-response curve of glucose uptake was observed in SHR, with the maximal increase in glucose uptake only one-half of that in WKY. Above 0.33 µM, NE-induced glucose uptake started to decline in SHR with further increases in NE dose.

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Table 3. pEC50 or pIC50 values for NE, ANG II, and 5-HT in the perfused hindlimbs

<table>
<thead>
<tr>
<th></th>
<th>Perfusion Pressure</th>
<th>VO2</th>
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<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>NE (type A)</td>
<td>pEC50</td>
<td>6.27 ± 0.047</td>
</tr>
<tr>
<td>NE (type B)</td>
<td>pIC50</td>
<td>5.57 ± 0.077</td>
</tr>
<tr>
<td>ANG II</td>
<td>pEC50</td>
<td>8.81 ± 0.095</td>
</tr>
<tr>
<td>5-HT</td>
<td>pEC50</td>
<td>6.16 ± 0.094</td>
</tr>
<tr>
<td></td>
<td>pIC50</td>
<td>6.15 ± 0.123</td>
</tr>
</tbody>
</table>

Values are means ± SE. NE, norepinephrine; 5-HT, 5-hydroxytryptamine. pEC50 is denoted as the −log value of EC50 (M); pIC50 represents the negative log value of IC50 (designated here as the inhibitory effect of NE on VO2). Data are obtained from 4 perfusions for each of the vasoconstrictors. *P < 0.01 and †P < 0.05 vs. WKY.

Fig. 1. Effects of norepinephrine (NE) on perfusion pressure, oxygen consumption (VO2), glucose uptake, and lactate production in the perfused hindlimb of spontaneously hypertensive rats (SHR; ●) and Wistar-Kyoto rats (WKY; ○). Basal values of perfusion pressure, VO2, glucose uptake, and lactate production were 37.0 ± 0.8 mmHg, 7.3 ± 0.1 mmol·g−1·h−1, 1.3 ± 0.9 mmol·g−1·h−1, and 4.8 ± 1.0 mmol·g−1·h−1 for WKY and 43.0 ± 1.2 mmHg, 8.1 ± 0.2 mmol·g−1·h−1, 2.0 ± 0.4 mmol·g−1·h−1, and 5.4 ± 0.6 mmol·g−1·h−1 for SHR, respectively. Data are means ± SE (n = 4 perfusions). Error bars not visible are within the symbols. ANOVA analysis results: 1) repeated measure of NE doses, P < 0.001 for A-D; 2) differences between SHR and WKY, P < 0.01 for A and B; P < 0.002 for C but P > 0.20 for D; 3) interaction between NE doses and rat groups, P < 0.001 for A-C but P > 0.10 D. [Norepinephrine], NE concentration.

Fig. 2. Effects of propranolol (10 µM) on NE-induced perfusion pressure, VO2, glucose uptake, and lactate production in the perfused hindlimb of SHR (●) and WKY (○). Basal values of perfusion pressure, VO2, glucose uptake, and lactate production were 36.7 ± 0.7 mmHg, 7.2 ± 0.1 mmol·g−1·h−1, 1.8 ± 0.3 mmol·g−1·h−1, and 4.4 ± 0.3 mmol·g−1·h−1 for WKY and 45.7 ± 0.7 mmHg, 8.0 ± 0.3 mmol·g−1·h−1, 2.2 ± 0.6 mmol·g−1·h−1, and 4.3 ± 0.2 mmol·g−1·h−1 for SHR, respectively. Data are means ± SE (n = 4 perfusions). Error bars not visible are within the symbols. ANOVA analysis results: 1) repeated measure of NE doses, P < 0.001 for A-D; 2) differences between SHR and WKY, P < 0.01 for A and B, P < 0.05 for C (between 0.1 and 10 µM range), but P > 0.20 for D; 3) interaction between NE doses and rat groups, P < 0.01 for A-C, and P > 0.10 for D.
However, when the NE-elicted maximal increase in perfusion pressure in SHR was normalized to a similar level as that of WKY by further addition of the α1-adrenergic antagonist prazosin, there were no significant differences between SHR and WKY in the curves for VO₂, glucose uptake, and lactate production (2.5 nM, Fig. 3).

Effects of ANG II on perfusion pressure, VO₂, glucose uptake, and lactate production. ANG II elicited increases in VO₂, glucose uptake, and lactate production during vasoconstriction in both strains at all doses tested (P < 0.001 for all 4 parameters). Whereas ANG II-mediated VO₂ dose-response curves were similar between SHR and WKY (P > 0.5), the curves for glucose uptake and lactate production tended to be lower in SHR (P < 0.12, Fig. 4). When analyzed as percentage changes above the basal levels (Fig. 5), the maximal increments in glucose uptake (P < 0.05) and lactate (P < 0.01) in SHR were less than one-half compared with WKY. The doses ranged between 3.3 and 10 nM with similar pEC₅₀ values in both strains (Table 3).

Effects of 5-HT on perfusion pressure, VO₂, glucose uptake, and lactate production. 5-HT caused dose-dependent increases in perfusion pressure (P < 0.001) in both strains of rats within the range between 0.1 and 10 µM (Fig. 6). 5-HT inhibited VO₂ at all doses during vasoconstriction in both SHR and WKY (P < 0.001), and this inhibition was greater in SHR (P < 0.01). However, there were no significant differences in the dose-response curves for perfusion pressure (P > 0.5), glucose uptake (P > 0.5), and lactate production (P > 0.2) between SHR and WKY (Table 3). The maximal changes in perfusion pressure, VO₂, glucose uptake, and lactate production induced by NE, ANG II, and 5-HT. Because the measured basal metabolites (in the absence of vasoconstrictors) were different between SHR and WKY (as shown in Table 2), we further evaluated the metabolic alterations during vasoconstriction by expressing them as percentages of the basal values. The data in Fig. 5 represent the maximal VO₂ (%) over the basal values obtained from Figs. 1, 4, and 6. The results with NE clearly show marked proportional reductions in VO₂, glucose uptake, and lactate production in SHR during vasoconstriction in SHR. ANG II-induced glucose uptake and lactate production were also proportionally inhibited in SHR. An increased inhibition of VO₂ was found when 5-HT elicted vasoconstriction. However, there were no significant increases in proportion to their basal values of perfusion pressure for NE and 5-HT, whereas the ANG II-elicted maximal perfusion pressure was in fact proportionally smaller.

DISCUSSION

A close correlation between obesity, insulin resistance, and hypertension has been recognized in humans (11, 18, 22, 24, 36). These complex diseases are multifactorial traits with both environmental and genetic determinants. To delineate the mechanisms involved, genetic animal models have proved to be help-
ful. For example, most metabolic disorders seen in humans resemble those observed in genetically obese rodents, although hypertension is not as profound in these animals as in humans (25, 36). In terms of the cardiovascular abnormalities, the SHR is a well-characterized model. Metabolic disorders are also found in this model. For instance, insulin resistance has been indicated with the euglycemic-hyperinsulinemic clamp technique in vivo (33, 36). In the skeletal muscle, altered Na$^+$-K$^+$-ATP pump number and activity have been demonstrated (31). There are also reports showing a transition of muscle fiber from the slow to fast type (3). These changes could potentially affect the thermogenic capacity and glucose utilization rate. On the other hand, higher rates of glycogen synthesis and lactate formation have been observed at certain doses of insulin concentrations in incubated soleus muscle strips of SHR compared with that of WKY (27). These controversial reports on SHR metabolism and glucose utilization may be related to the experimental conditions employed. As elegantly demonstrated by Rao (33), interpretation of insulin resistance in SHR can be very different in glucose clamp studies depending on whether the assessment is based on insulin infusion rate or on plasma insulin levels. Likewise, the influence of vascular function on muscle thermogenesis and glucose uptake can not be ignored (33, 35). Nonetheless, the relationship between vascular function and muscle metabolism has not been emphasized in studies involving SHR.

Several important findings have emerged from the present experiment. First, in the absence of vasoconstrictors, the basal perfusion pressure, $\text{V}O_2$, glucose uptake, and lactate production were all higher in the SHR hindlimb when compared with WKY rats. These results appear to be consistent with the increased blood pressure and increased resting metabolic rate of the whole animal in vivo. Second and most important, our results clearly showed impaired muscle thermogenesis and glucose uptake associated with vasoconstriction elicited by NE. Similar defects were also found with 5-HT or ANG II during vasoconstriction.

Vascular resistance and metabolism in the absence of vasoconstrictors. Whereas resting hindlimb perfusion pressures are at first sight low, arterial pressures will necessarily be lower than in vivo when employing a cell-free, low-viscosity perfusate. In addition, there is neither resting sympathetic tone nor circulating vasoconstrictors in the perfused hindlimb. Thus physiological pressures require high flow rates (40), or, alternatively, physiological flow rates generate subphysiological

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**Fig. 5.** Maximal changes in perfusion pressure, $\text{V}O_2$, glucose uptake, and lactate production in the perfused skeletal muscle of SHR and WKY. Data are drawn from Figs. 1, 4, and 6 in which the maximal or minimal $\text{V}O_2$ was obtained. For low concentrations of NE (LNE), the doses for were 0.1 $\mu$M for SHR and 0.33 $\mu$M for WKY, whereas for high doses of NE (HNE), the dose was 10 $\mu$M for both strains. ANG II and 5-HT were 3.3 $nM$ and 3.3 $\mu$M, respectively, for both SHR and WKY. These maximal changes are expressed as the percentage of their basal values. Error bars not visible are within the symbols. ANOVA analysis results: 1) repeated measure of 5-HT doses, $P < 0.001$ for A and B, $P < 0.05$ for C, $P > 0.20$ for D; 2) differences between SHR and WKY, $P > 0.50$ for A, $P < 0.01$ for B, $P > 0.5$ for C, and $P > 0.2$ for D; 3) interaction between 5-HT doses and rat groups, $P > 0.5$ for A, C, and D and $P < 0.01$ for B.

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**Fig. 6.** Effects of 5-hydroxytryptamine (5-HT) on perfusion pressure, $\text{V}O_2$, glucose uptake, and lactate production in the perfused hindlimb (mb) of SHR (●) and WKY (○). Basal values of perfusion pressure, $\text{V}O_2$, glucose uptake, and lactate production were 37.0 ± 0.0 mmHg, 6.5 ± 0.3 mmol·g$^{-1}$·h$^{-1}$, 1.7 ± 0.7 mmol·g$^{-1}$·h$^{-1}$ and 3.0 ± 0.2 mmol·g$^{-1}$·h$^{-1}$ for WKY and 43.3 ± 0.9 mmHg, 7.2 ± 0.2 mmol·g$^{-1}$·h$^{-1}$, 2.0 ± 0.2 mmol·g$^{-1}$·h$^{-1}$, and 5.6 ± 0.6 mmol·g$^{-1}$·h$^{-1}$ for SHR, respectively. Data are means ± SE (n = 4 perfusions). Error bars not visible are within the symbols. ANOVA analysis results: 1) repeated measure of 5-HT doses, $P < 0.001$ for A and B, $P < 0.05$ for C, $P > 0.20$ for D; 2) differences between SHR and WKY, $P > 0.50$ for A, $P < 0.01$ for B, $P > 0.5$ for C, and $P > 0.2$ for D; 3) interaction between 5-HT doses and rat groups, $P > 0.5$ for A, C, and D and $P < 0.01$ for B.
pressure (4, 10). In the present study, a compromise approach was adopted by using the same constant perfusion flow rather than constant pressure. Even so, the low vascular tone of the hindlimb is unlikely to have had significant bearing on the experimental observations, as a number of studies suggest that the vasodilation is uniform across both nutritive and nonnutritive networks and does not alter the proportion of flow between the two routes (7). Over a range of perfusion pressures, total hindquarter flows are 76% lower in SHR than in WKY regardless of muscle fiber types (39).

Consistent with earlier reports by others (1, 3, 26, 39), the results in Table 2 clearly showed a marked increase in basal perfusion pressure in the SHR hindlimb. This change is probably due to morphological changes in resistance vessels, which could start as early as 4 wk of age in SHR (1, 12). Although it may be different from that of a conscious animal in terms of the absolute value, the blood pressure measured under anesthesia confirmed a fully developed hypertension at this age as reported by others (1).

It is of interest to note that the basal VO$_2$, glucose uptake, and lactate production were higher (11.5, 43, and 41.5%, respectively) in the perfused SHR hindlimb. This appears puzzling at first because the vascular changes resulting from the increased basal perfusion pressure could be argued to restrict the exchange rate between capillaries and muscle cells. However, it is possible that the capillary changes at this stage have not developed to such an extent that basal metabolism is inhibited at a constant flow rate. The observed higher basal metabolism may relate to changes in cellular properties. For example, an increased number of sarcolemmal Na$^+$-K$^+$-ATP pumps and their overall activity have been noted in soleus muscle of young SHR (up to 16 wk) in compensation for expelling increased intracellular Na$^+$ (31). These changes could be expected to consume more ATP, leading to a rise in muscle metabolism. Elevated vascular tone due to increased smooth muscle layers (12) might also use more oxygen (9). Other changes such as increased lactate dehydrogenase activity and slow-to-fast fiber type transition (3) may also contribute to the increased lactate production. Such an explanation is also consistent with an increased ATP turnover in skeletal muscle found in patients with untreated primary hypertension (37). Interestingly, the measured whole body VO$_2$ at rest was also higher in SHR (Table 1). This latter result agrees with earlier studies by others showing a slightly higher metabolic rate (3) and body temperature (8). The data taken together suggest that the muscle vascular bed may not only contribute to the increased peripheral vascular resistance but also to the elevated whole body metabolic rate during hypertension. Because increased VO$_2$ could potentially improve glucose uptake and utilization, impaired muscle metabolic capacity in SHR may not be readily demonstrable under conditions in which the cardiovascular system is not challenged, such as in incubated muscle strips (27). This interpretation would also appear to be consistent with the observations by Rao (33). In the study by Rao, insulin resistance was most convincingly revealed using euglycemic and hyperinsulinemic clamp technique with a series concentration doses of insulin even although neither hyperinsulinemia nor hyperglycemia were present in SHR under basal conditions. This is true for the SHR and WKY from the same source used in the present experiment [the fasted plasma insulin levels were 188 ± 34 and 169 ± 22 pmol/l, respectively (32)].

Changes in vasoconstrictor-controlled muscle thermogenesis and glucose uptake. An increased vascular sensitivity to vasoconstrictors has been shown in muscle vascular beds of SHR (1, 5, 6, 26, 39). The present study also found an augmented vasoconstriction in SHR hindlimb produced by NE (Fig. 1). NE-elicited biphasic changes in VO$_2$ and lactate production in the perfused rat hindlimb have been previously observed in Wistar rats, and both phases are predominantly mediated by $\alpha$$_1$-adrenoceptors (7, 35) presumably by different subtypes (13). In SHR, LNE-induced increases in VO$_2$ were significantly reduced, and the inhibitory phase of VO$_2$ produced by HNE was much greater compared with WKY. This accentuated inhibition of VO$_2$ and glucose uptake still remained in the presence of 10 µM propranolol despite a significant leftward shift of the dose-response curve of the perfusion pressure of WKY together with a slight inhibition of the maximal VO$_2$. However, further addition of 2.5 nM prazosin (which normalized SHR and WKY perfusion pressure without full blockade of the NE effect) abolished the decreased VO$_2$ produced by NE in SHR when compared with WKY. Interestingly, these effects of prazosin on VO$_2$ were associated with a much stronger inhibition of vasoconstriction in SHR than in WKY, suggesting that the altered muscle thermogenesis in SHR is closely associated with changes of vasoconstriction primarily attributed to $\alpha$$_1$-adrenoceptors.

A stimulatory effect of $\alpha$-adrenergic stimulation on glucose uptake by the perfused rat hindlimb has been described by others when epinephrine was administered, and this effect was associated with increased VO$_2$ and perfusion pressure (35). Of particular interest is the striking reduction of glucose uptake in SHR induced by NE compared with that of WKY. In parallel with changes in VO$_2$, this impaired glucose uptake in SHR appeared to be associated with vasoconstriction mediated by $\alpha$$_1$ rather than $\beta$-adrenoceptors because it could only be reversed when the increased vasoconstriction was abolished by prazosin. Coincidentally, Mondon et al. (28) also suggested that the altered vascular system may be responsible for the impaired insulin removal in SHR.

NE does not directly stimulate VO$_2$ in muscle preparations in which nutrients and oxygen are not delivered by the vascular system (7). By contrast, in the muscle preparations in which nutrients and oxygen are delivered via the vascular system, NE elicits changes in VO$_2$, glucose uptake, and lactate production, and these changes are all reversed when vasoconstriction is blocked (7, 10, 35). Therefore, a hemodynamic mechanism for NE-controlled VO$_2$ in perfused muscle preparations is highly probable (7, 34). Such an explanation is
supported by the vasoconstriction-associated metabolic changes produced by ANG II and 5-HT, neither of which showed direct action on muscle metabolism in superfused or incubated muscle preparations (7, 34). However, ANG II-elicted glucose uptake in the perfused SHR hindlimb was proportionally lower with a reduced lactate production, and 5-HT inhibition of VO$_2$ was much greater compared with that in WKY. Hence, in general, vasoconstrictor-controlled metabolism in SHR hindlimb muscle was downregulated although not all three parameters measured were uniformly altered by ANG II and 5-HT.

The hemodynamic mechanism underlying the altered muscle metabolism in SHR may involve both functional and morphological changes in the muscle microvasculature. Julius et al. (22) have proposed that hypertension-related changes in the muscle microcirculation may contribute to the impaired glucose uptake in hypertension by impairing the delivery of insulin and glucose to muscle cells. As reviewed by us earlier (Ref. 7 and references therein), muscle resting VO$_2$ may be largely controlled by the ratio of nutritive to nonnutritive flow. Type A vasoconstrictors LNE and ANG II may preferentially constrict the precapillary arterioles before nonnutritive routes, thereby redirecting to nutritive routes. Accordingly, VO$_2$, lactate production, and glucose uptake are increased. Conversely, type B vasoconstrictors HNE and 5-HT may redistribute flow to nonnutritive routes by closing nutritive capillaries, causing an inhibition of muscle metabolism. The muscle tendon vessels have been shown to be possible nonnutritive routes for type B vasoconstrictors (29). In SHR skeletal muscle, a decreased vascular flow capacity has been shown to correlate with increased vascular resistance and alleviated capillary exchange function (39) due to vascular remodeling such as capillary rarefaction (3, 26). These changes are likely to reduce the nutritive route reserve and thus suppress vasoconstrictor-controlled muscle metabolic capacity in SHR. On the other hand, the transition of muscle fiber types from the slow to fast type, which may be partly related to capillary rarefaction (3), could also contribute to the overall metabolic changes in SHR muscle. It is well known that the slow muscle fiber has higher VO$_2$ and glucose utilization rate with denser capillaries (27).

Comparison of NE, ANG II, and 5-HT. A comparison of the effects of NE, ANG II, and 5-HT in SHR indicates that NE-induced changes in perfusion pressure and altered muscle metabolism were most profound in the perfused SHR hindlimb. The reported plasma ANG II level in SHR is ~100 pM in a low-salt diet and 57 pM in a high-salt diet (5), whereas the plasma 5-HT level in normal humans is ~6 pM, and under some disease conditions such primary pulmonary hypertension it can be increased fivefold (20). These concentrations are below the range of the dose-response curves observed in Figs. 3 and 4. Although plasma NE ranges between ~1 and 3 nM in rats (1, 2) and humans (2, 23) under normal physiological conditions, the measured NE in rat muscle is ~0.12 mg/g wet tissue weight (1, 30), equivalent to 153 mg/l water (or 0.9 µM). Importantly, NE is a major neurotransmitter of the peripheral sympathetic nerves that widely innervate muscle resistance vessels. NE concentration in the neuromuscular junction is estimated to be as high as 10–50 µM (16). Such concentrations would cover the full dose-response range of perfusion pressure and metabolism for NE seen in Fig. 1. Furthermore, sympathetic nerve plexus density in skeletal muscle small arteries is significantly higher (38), with a 40% increased NE turnover in muscle tissue (1) in SHR than in WKY rats. Similarly, both plasma NE levels and the recorded muscle sympathetic nerve activity are elevated in young hypertensive subjects (2, 15). Thus NE-induced changes found in this study may have significant implications in vivo. It has been shown that chronic treatment of SHR with trandolapril, an angiotensin-converting enzyme inhibitor, normalizes blood pressure and improves the response of glycogen metabolism to insulin in isolated soleus muscle (27). It would be interesting to see whether chronic blockage of α-adrenoceptors in SHR will also ameliorate the altered metabolism during vasoconstriction.

Conclusion. Data from the present study have clearly shown an impaired potential of muscle thermogenesis and glucose uptake in SHR associated with vasoconstrictor action, although the resting metabolism in the absence of vasoconstrictors is increased. These data may imply, at least in part, a vascular role in some metabolic disorders such as obesity and glucose intolerance during hypertension.

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