Changes in Functional Expression of Alpha-1 Adrenoceptors in Hindlimb Vascular Bed of Spontaneously Hypertensive Rats and their Effects on Oxygen Consumption

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Accepted for publication April 21, 1998
This paper is available online at http://www.jpet.org

ABSTRACT
Norepinephrine (NE) induces a sigmoidal dose-response curve for perfusion pressure and a bell-shaped curve for oxygen consumption (VO₂) in the constant-flow perfused hindlimb of Wistar rats. These effects are now described in spontaneously hypertensive rats (SHR) and age-matched Wistar-Kyoto rats (WKY). In SHR, the pressure curve was shifted left- and upward compared with WKY. In the presence of propranolol plus yohimbine, the pressure curve was markedly shifted to the right by both the selective alpha-1D antagonist 5-methylurapidil (3.3 nM), and by the alpha-1D antagonist BMY 7378 (0.1 μM) or SK&F 105854 (2 μM) in SHR but not in WKY. With respect to the VO₂ curve, 5-methylurapidil attenuated the ascending limb without affecting the ascending limb. Similar effects were also obtained with another alpha-1A-agonist 1 nM KMD-3213 in both SHR and WKY. In contrast, BMY and SK&F markedly inhibited the ascending limb of the VO₂ curve. These results indicate that both alpha-1A- and alpha-1D subtypes are functionally up-regulated in SHR

Studies in humans and animals have indicated a close link between hypertension and obesity. While one of the major causes for hypertension is an increased peripheral vascular resistance, obesity is due to either an excessive energy intake, a decreased energy expenditure or both. Both peripheral vascular resistance and energy metabolism are regulated by the sympathetic nervous system (Reaven, 1995).

In skeletal muscle, the largest and potentially most important thermogenic tissue, the sympathetic nervous system controls thermogenesis through both α and β ARs by different mechanisms. Whereas β-ARs directly mediate VO₂ (an indirect measure of thermogenesis) in muscle cells, α-ARs appear to control muscle VO₂ by hemodynamic mechanisms (Ye et al., 1995). In the constant-flow perfused hindlimb of Wistar rats, a reliable muscle vascular preparation with many characteristics similar to those in vivo (Bonen et al., 1994), administration of α1-agonists or sympathetic nerve stimulation elicits either positive or negative changes in VO₂ during a sigmoidal increase in perfusion pressure, an indicator of vasoconstriction in this model (Clark et al., 1995; Hall et al., 1997). One of the major features of α-AR mediated VO₂ is its bell-shaped dose-response curve characterized by increases (the ascending limb) at low concentrations of norepinephrine (LNE, <1 μM) and decreases from the maximum to a value below the basal level (the descending limb) at high concentrations of NE (HNE > 1 μM) (Dora, 1993; Rattigan et al., 1995; Clark et al., 1995). Similarly, sympathetic nerve stimulation raises VO₂ at low frequencies (<4 Hz) but reduces VO₂ at high frequencies (>4 Hz) during vasoconstriction (Hall et al., 1997). Both the increase and the decrease in VO₂ are reversed when the vasoconstriction is blocked by either α-AR antagonists or by vasodilators such as nitroprusside (Dora, 1993; Rattigan et al., 1995; Ye et al., 1995, Hall et al., 1997). These findings strongly suggest a close link of muscle VO₂ to vasoconstriction mediated by α-ARs in the perfused rat hindlimb.

In the perfused hindlimb of SHR, NE is known to cause stronger vasoconstriction compared to that in their genetically normotensive counterparts, WKY (Cheng and Shibata, 1980). Similar results were obtained with the α-AR agonist methoxamine (Adams et al., 1989). These data suggest that

ABBREVIATIONS: α₁-ARs, alpha-1 adrenoceptors; NE, norepinephrine; VO₂, oxygen consumption; 5 MU, 5-methylurapidil; KMD, KMD-3213; CEC, chloroethylclonidine; BMY, BMY 7378; SK&F, SK&F 105854; ANOVA, analysis of variance.
functional changes in \( \alpha-1 \)-ARs may occur in the resistance blood vessels of muscle vascular beds in SHR. If so, the muscle \( VO_2 \) (and therefore thermogenesis) controlled by \( \alpha-1 \)-AR-mediated vasoconstriction is also likely to be affected.

At least three \( \alpha-1 \)-ARs have so far been identified in vascular tissues, namely \( \alpha-1A \), \( \alpha-1B \) and \( \alpha-1D \)-subtypes (Hieble et al., 1995a). Functional characterization of these subtypes has been made possible now by using subtype selective antagonists. For instance, all these three subtypes show a high affinity for prazosin and a low affinity for yohimbine (Bylund et al., 1994). Both 5 MU (Perez et al., 1994) and KMD (Shibata et al., 1995) have a higher affinity for the \( \alpha-1A \)-AR than for the other two subtypes, whereas BMY (Goetz et al., 1995) and SK&F (Hieble et al., 1995b) each possess a higher affinity for the \( \alpha-1D \)-AR. The \( \alpha-1B \)-AR is most sensitive to alklylation by CEC (Minneman et al., 1988, Bylund et al., 1994). We hypothesized that the increased sensitivity of SHR muscle vascular bed to NE may be mediated by differrently altered \( \alpha-1 \)-AR subtypes and these alterations may then lead to changes in NE-elicited thermogenesis in this tissue. Therefore, we compared the effects of seven selective \( \alpha \)-AR antagonists on NE-induced vasoconstriction and associated \( VO_2 \) in the perfused hindlimb of SHR and their age-matched WKY in our study.

**Materials and Methods**

**Animals.** Age-matched (11 wk) male SHR (277.7 ± 1.0 g, n = 36) and WKY (276.2 ± 0.9 g, n = 36) used for the experiments were purchased from the Animal Resources Center of Australia. The animals were housed on arrival at 20°C with a 12 hr light/12 hr dark cycle and allowed free access to food and water. The diet consisted of vitamins and minerals (Gibson, Hobart, Tasmania). 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 (1990). Blood pressure determined in the anesthetized state (pentobarbital, 60 mg/kg, i.p.) from a cannu- lated carotid artery by the use of a manometer (ALPK2, Japan) was much higher in SHR compared with that in WKY (182 ± 3.0 vs. 94 ± 3.0 mmHg, P < .001, n = 8).

**Hindlimb perfusion.** The rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). A midline incision was made to expose the abdominal cavity and the cut edges of the abdominal wall were ligated, where necessary, with sutures. Gut, seminal vesicles and testicles were removed after appropriate ligations. The right common iliac artery and vein were ligated so that only the left hindlimb was perfused. To prevent any perfusate spillover when perfusion pressure was 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 and superior veseicle vessels, and the iliolumbar and saphenous vessels on both sides. Before cannulation, heparin (0.5 ml, 1000 U/ml) was injected into the vena cava between the right and left renal veins. Two cannulae (Ohmeda, Sweden) were then inserted caudally into the abdominal aorta (16G) and vena cava (18G) between the left renal and iliolumbar vessels. The rat was then immediately placed on perspex platform for perfusion followed by an overdose injection of the anesthetic to kill the animal. Ligatures were then placed firmly around: the lumbar trunk between \( L_3-L_4 \) vertebrae, right thigh (near the inguinal ligament), and the genitalia (above the penis), respectively.

The perfusate consisting of a modified cell-free Krebs-Ringer bicarbonate buffer (pH 7.4) containing 8.3 mM glucose, 1.27 mM CaCl\(_2\) and 2% bovine serum albumin was equilibrated by an artificial lung with a mixture of 95% \( O_2 \) and 5% \( CO_2 \). The basal perfusion flow was set at 6 ml/min by adjusting the perfusion pump speed and confirmed by intermittent collection of the venous effluent from the hindlimb. The rat hindlimb was perfused in a nonrecirculating manner at 25°C. The hindlimb perfused under these conditions provides qualitatively similar results to those perfused with erythrocyte containing media at 37°C in its metabolic responses to various vasoconstrictors (Bonel et al., 1994; Clark et al., 1995). The venous oxygen partial pressure was maintained above 150 mmHg even at maximal oxygen extraction. Adequate oxygen delivery at this flow rate had been confirmed in our earlier studies (Colquhoun et al., 1990). The perfusion was completed within 180 min and our previous experiments under similar conditions have shown that this preparation was stable for at least this period of time with similar muscle metabolic characteristics as those in vivo (Colquhoun et al., 1990). The heart, weighed after perfusion, showed a 20% increase in SHR compared WKY (1.10 ± 0.01 g vs. 0.90 ± 0.01, P < .01, n = 12).

Perfusion pressure was monitored via a pressure transducer from a side arm of the arterial line immediately before the arterial cannu- lula. Oxygen partial pressure of the perfusate was measured by an in-line Clark-type 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 pressure using Bunsen coefficient for plasma as described previously (Colquhoun et al., 1990). \( VO_2 \) 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 that had been infused with Evan’s blue (1% w/v) at the end of experiment without changing perfusion conditions.

The perfused muscle mass was 23.02 ± 0.56 and 22.18 ± 0.79 g for WKY and SHR, respectively (P > .05, n = 41). When expressed as ml \( min^{-1} g^{-1} \) muscle, the perfusion flow rate was not significantly different between WKY and SHR (0.26 ± 0.03 vs. 0.27 ± 0.02, P > .05, n = 11).

**Experimental protocols.** After commencing the perfusion, 50 min was allowed to elapse before constructing the dose-response curve for NE. The basal values for perfusion pressure and \( VO_2 \) were obtained between 40 and 50 min. Three sets of experiments were performed in both SHR and WKY as follows. Set 1 was designed to assess the involvement of \( \beta \)-ARs in NE-induced changes in vasoconstriction and associated \( VO_2 \) by comparing the effects of NE in the absence and presence of 10 \( \mu \)M propranolol. In set 2, experiments were divided to three groups and conducted in the presence of 10 \( \mu \)M propranolol to evaluate the role of \( \alpha_1 \)- and \( \alpha_2 \)-ARs in altered vasoconstriction and \( VO_2 \) induced by NE: control (from set 1), prazosin (2.5 nM) and yohimbine (0.1 m M). The experiments were assigned to the following groups: control (from set 2), 5 MU (3.3 nM), KMD (1 nM), CEC (10 \( \mu \)M), BMY (0.1 \( \mu \)M) and SK&F (2 \( \mu \)M). The doses of these \( \alpha \)-AR antagonists were chosen to maximize the differentiation of differences between SHR and WKY according to our preliminary experiments within the range of their selectivity. The experiments on SHR and WKY were interspersed randomly. After obtaining the results from set 3, additional experiments were performed with low doses of BMY (10 nM) and SK&F (0.33 \( \mu \)M) in SHR to further examine the role of \( \alpha-1D \)-AR subtype on the descending limb of the \( VO_2 \) response curve. Each antagonist was infused 30 min before and during the period of NE infusion. NE and \( \alpha \)-AR antagonists were infused from a port in the arterial line at a rate less than 1% of the perfusion flow rate and mixed by a magnetic stirrer in a small bubble trap before entering the hindlimb. In experiments with CEC, the alkylating agent was infused for a period of 30 min and then washed out for 20 min before the infusion of NE. Dose-response curves were con- structed in a cumulative fashion. Each hindlimb was used for con- structing the dose-response curves only once to ensure that the
metabolic characteristics of the preparation were valid within the period of time previously established (Colquhoun et al., 1990). The perfusion flow rate was checked (corrected if necessary) each time after changing NE dose.

**Chemicals.** [-]NE bitartrate, dl-propranolol hydrochloride, prazosin hydrochloride and yohimbine hydrochloride were obtained from Sigma (St. Louis, MO). 5-Methylurapidil, BMY 7378 dihydrochloride, chloroethylclonidine dihydrochloride were purchased from RBI (Natick, MA). KMD-3213 (=(-)-1-(3-hydroxypropyl)-5-[2-[(2,2,2-trifluoroethoxy)phenoxyl]ethyl]aminepropyl]indoline-7-carboxamide) was a gift from Dr. Y. Kurashina (Kissei Pharmaceutical Co., Matsumoto, Japan) and SK&F 105584 (furo-5- benzazepine) a gift from Dr. J. P. Hieble (SmithKline Beecham Pharmaceuticals, King of Prussia, PA). NE was dissolved freshly in 9% NaCl containing 0.1% ascorbic acid. Prazosin was initially dissolved in dimethylsulfoxide in a stock solution and then diluted to an appropriate concentration with 0.9% NaCl before use. Other antagonists were dissolved in the normal saline. Bovine serum albumin (fraction V) was obtained from Boehringer Mannheim Corp. (Indianapolis, IN). Other chemicals were analytical grade from Ajax Chemicals (Sydney, Australia).

**Calculation and statistical analysis.** EC\(_{25}\), EC\(_{50}\) and IC\(_{75}\) (designated here as the inhibitory effect of NE on VO\(_2\)) were calculated individually from the best fit dose-response curves by Sigma Plot for Windows on the basis of fractional changes (Kenakin, 1993). The regression coefficient closest to 1 was used to determine the best fitness of a curve. Because NE-induced changes in VO\(_2\) were bell-shaped, EC\(_{25}\) and IC\(_{75}\) were calculated instead of EC\(_{50}\) and IC\(_{50}\) to avoid, where possible, the influence of one side of the response on the other (Szabadi, 1977). The negative log values of EC\(_{25}\), EC\(_{50}\) and IC\(_{75}\) were expressed as pEC\(_{25}\), pEC\(_{50}\) and pIC\(_{75}\), respectively (Jenkinson et al., 1995). pEC\(_{50}\) values were calculated according to the following formula (Kenakin, 1993) using EC\(_{50}\) of perfusion pressure: pEC\(_{50}\) = log (DR-1)-log[B] where DR is the ratio of EC\(_{50}\) in the presence of a given antagonist to EC\(_{50}\) in absence of antagonist, and [B] is the concentration of the antagonist. Because DR could not be obtained from individual hindlimb preparations, pK\(_B\) was calculated using the mean EC\(_{50}\) and was without a S.E. Data are presented as means ± SE. Dose-response curves were determined to differ (P < .05) using ANOVA (Startview SE, Abacus Concept, Berkeley, CA) with dose as a repeated measure. Bell-shaped dose-response curves were firstly tested using all points. Then each side of the curves was further analyzed based on the model of two functionally antagonistic receptor populations activated by the same agonist (Szabadi, 1977). Student's t-tests were used for the comparison between two means values with P < .05 as statistically significant.

**Results**

**Effects of adrenergic antagonists on basal perfusion pressure and VO\(_2\).** None of the antagonists used, when infused alone, had any significant effect on either the basal perfusion pressure or VO\(_2\) in SHR or WKY (table 1). The calculated basal perfusion pressure and VO\(_2\) from the pooled data were 37.3 ± 0.3 and 7.0 ± 0.1 μmol g\(^{-1}\) hr\(^{-1}\), respectively, for WKY (n = 36). In SHR, the basal perfusion pressure (45.5 ± 0.6 mmHg) and VO\(_2\) (7.9 ± 0.1 μmol g\(^{-1}\) hr\(^{-1}\)) were both significantly higher (P < .01, n = 36).

**Effects of NE on perfusion pressure and VO\(_2\).** NE induced a dose-dependent sigmoidal increase in perfusion pressure in both SHR and WKY (fig. 1). Compared with WKY, the perfusion pressure was displaced to the left for more than 2-fold in SHR as indicated by the value of pEC\(_{50}\) (table 2). The maximal increase in pressure (P\(_{max}\)) was greater in SHR (235.7 ± 7.5 mmHg, P < .01) than in WKY (199.3 ± 3.1 mmHg). During vasoconstriction, NE-elicited bell-shaped changes in VO\(_2\) in both strains. Compared with WKY, the change in VO\(_2\) was altered in SHR with the ascending side increased (P < .05) and descending side depressed (P < .01, ANOVA). Furthermore, LNE-induced maximal increment in VO\(_2\) (VO\(_2\) max) was smaller (3.65 ± 0.11 vs. 4.41 ± 0.14 μmol g\(^{-1}\) hr\(^{-1}\), P < .01) and HNE-induced maximal inhibition of VO\(_2\) (VO\(_2\) min) was greater (-3.51 ± 0.37 vs. -0.87 ± 0.43 μmol g\(^{-1}\) hr\(^{-1}\), P < .01) in SHR.

**Effects of β-AR antagonist on NE-induced perfusion pressure and VO\(_2\).** In the presence of propranolol, NE-induced perfusion pressure was shifted to the left in WKY (fig. 2 A and B; table 2). Although VO\(_2\) max was smaller in WKY (5.64 ± 0.18 μmol g\(^{-1}\) hr\(^{-1}\)) than in SHR (6.4 ± 0.14 μmol g\(^{-1}\) hr\(^{-1}\), P < .01, t-test), the entire VO\(_2\) curve was not significantly different (P > .05, ANOVA). Neither pressure nor VO\(_2\) produced by NE was significantly altered by propranolol in SHR (fig. 2 C and D).

**Effects of α\(_1\)-AR and α\(_2\)-AR antagonists on NE-induced perfusion pressure and VO\(_2\).** In WKY, both prazosin (2.5 mM) and yohimbine (0.1 μM) markedly shifted NE-induced dose-response curves of perfusion pressure and VO\(_2\) to the right without changing P\(_{max}\), VO\(_2\) max or VO\(_2\) min (fig. 3 A and B; table 2). In SHR, the antagonistic effect of prazosin was much bigger and that of yohimbine was significant only within the range of LNE (fig. 3 C and D). NE-induced perfusion pressure was shifted to the right more in SHR by prazosin compared with WKY and the differences in associated changes in VO\(_2\) between SHR and WKY disappeared (fig. 4). The pK\(_B\) values for prazosin and yohimbine of NE-induced perfusion pressures for WKY were 9.24 and 7.34, respectively. In comparison, the pK\(_B\) value was higher for prazosin (9.81) and lower for yohimbine (6.89) in SHR.

**Table 1**

<table>
<thead>
<tr>
<th>Propranolol</th>
<th>Yohimbine</th>
<th>5MU</th>
<th>KMD</th>
<th>CEC</th>
<th>BMY</th>
<th>SK&amp;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmHg)</td>
<td>WKY</td>
<td>37.0 ± 0.8</td>
<td>36.7 ± 0.7</td>
<td>37.0 ± 1.5</td>
<td>36.3 ± 1.2</td>
<td>38.0 ± 0.7</td>
</tr>
<tr>
<td>SHR</td>
<td>43.0 ± 1.2</td>
<td>45.7 ± 0.7</td>
<td>43.0 ± 1.8</td>
<td>43.5 ± 1.2</td>
<td>46.5 ± 2.2</td>
<td>46.8 ± 0.6</td>
</tr>
<tr>
<td>VO(_2)</td>
<td>7.3 ± 0.1</td>
<td>7.16 ± 0.1</td>
<td>6.7 ± 1.5</td>
<td>7.1 ± 0.4</td>
<td>7.3 ± 0.2</td>
<td>7.3 ± 0.2</td>
</tr>
<tr>
<td>(μmol g(^{-1}) hr(^{-1}))</td>
<td>8.1 ± 0.2</td>
<td>8.0 ± 0.3</td>
<td>7.8 ± 1.3</td>
<td>8.0 ± 0.4</td>
<td>7.6 ± 0.2</td>
<td>7.9 ± 0.1</td>
</tr>
</tbody>
</table>

Propranolol: 10 μM, prazosin: 2.5 mM, yohimbine: 0.1 μM, 5MU: 3.3 mM, KMD: 1 nM, CEC: 10 μM, BMY: 0.1 μM, SK&F: 2 μM. Data are presented as means ± S.E. Four experiments were performed in each group.

\( ^{a}P < .01\) vs. WKY.

\( ^{b}P < .05\) vs. WKY.
ANOVA). In WKY, pretreatment with 10 μM CEC was only small inhibitory effect on perfusion pressure and VO\textsubscript{2}. Although not significantly reduced in both WKY and SHR by KMD, the VO\textsubscript{2} elicited by CEC had only small inhibitory effects on the perfusion pressure and VO\textsubscript{2}. Associated with the inhibition of vasoconstriction, the VO\textsubscript{2} of the hindlimb was also found to be 13% higher in SHR. The reason for this may be associated with increased numbers of the sarcolemmal Na\textsuperscript{+}-K\textsuperscript{+}-ATP pump and its overall compensatory activity to expel accumulated intracellular Na\textsuperscript{+} at this age (Pickar et al., 1994). Such an explanation is supported by recent findings showing an elevated ATP turnover in skeletal muscle from patients with untreated primary hypertension (Ronquist et al., 1995).

**Roles of β-ARs in altered vasoconstriction and VO\textsubscript{2} induced by NE in SHR.** β-ARs have been shown to be widely distributed in skeletal muscle of normal rats in radioautography (Summers et al., 1995). We have previously found blockade of β\textsubscript{1}/β\textsubscript{2}-ARs by 1 μM propranolol in perfused hindlimb of Wistar rats leads to a leftward shift of vasoconstriction produced by adrenaline (Colquhoun et al., 1990). Consistent with this earlier finding, blockade of β\textsubscript{1}/β\textsubscript{2}-ARs by 10 μM propranolol in our study also enhanced NE-induced vasoconstriction in WKY with a small inhibition of VO\textsubscript{2} max. In comparison, propranolol did not show any significant effect on either vasoconstriction or VO\textsubscript{2} induced by NE in SHR, pointing to a reduced role of β-ARs in the muscle vascular bed. Coincidentally, a loss of β-AR-mediated vasodilation has been noted in portal veins (Doggrell and Surman, 1995) and mesenteric arteries (Blankensteijn et al., 1996) of SHR. Nonetheless, NE-induced bell-shaped VO\textsubscript{2} is not attributable to β-ARs because it was present in the presence of 10 μM propranolol.

**Roles of α\textsubscript{1}- and α\textsubscript{2}-ARs in altered vasoconstriction and VO\textsubscript{2} induced by NE in SHR.** Compared with β-ARs, α-ARs are sparse in skeletal muscle (Rattigan et al., 1986) and predominantly located on small arteries with high affinity for prazosin (Martin et al., 1990). In the constant-flow perfused hindlimb of Wistar rats performed earlier in this laboratory, NE-induced biphasic changes in VO\textsubscript{2} were both completely reversed by prazosin at concentrations more than 1000-fold lower than by yohimbine (Dora, 1993). In our study, the predominant role of α\textsubscript{1}-ARs in NE-induced vasoconstriction and associated bell-shaped changes of VO\textsubscript{2} in the perfused rat hindlimb is supported by a high pK\textsubscript{b} for prazosin (9.24) and low pK\textsubscript{b} for yohimbine (7.34) in WKY. These results are consistent with our recent findings that the α\textsubscript{1}-AR agonist phenylephrine produces bell-shaped VO\textsubscript{2} with a strong vasoconstriction whereas the α\textsubscript{2}-AR agonist UK-14,304 elicits only a small and monophasic increase in VO\textsubscript{2} with a much weaker vasoconstriction in the perfused rat.
TABLE 2
Effect of α- and β-AR antagonists on the pEC$_{50}$ for perfusion pressure and the pEC$_{25}$ and pIC$_{75}$ for VO$_2$ produced by NE in the perfused hindlimb of SHR and WKY

<table>
<thead>
<tr>
<th>Pressure pEC$_{50}$</th>
<th>NE</th>
<th>Prop + NE</th>
<th>Prop + Praz + NE</th>
<th>Prop + Yoh + NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>6.27 ± 0.05</td>
<td>6.67 ± 0.02$^a$</td>
<td>5.94 ± 0.04$^b$</td>
<td>6.17 ± 0.06$^{bc}$</td>
</tr>
<tr>
<td>SHR</td>
<td>6.61 ± 0.11$^{cd}$</td>
<td>6.85 ± 0.04$^{cd}$</td>
<td>5.62 ± 0.07$^{bcd}$</td>
<td>6.62 ± 0.06$^{def}$</td>
</tr>
<tr>
<td>VO$<em>{2p}$pEC$</em>{25}$</td>
<td>WKY</td>
<td>8.04 ± 0.09$^{ef}$</td>
<td>8.21 ± 0.07$^{f}$</td>
<td>7.31 ± 0.04$^b$</td>
</tr>
<tr>
<td>SHR</td>
<td>8.47 ± 0.06$^{f}$</td>
<td>8.47 ± 0.13$^{f}$</td>
<td>7.41 ± 0.05$^b$</td>
<td>7.98 ± 0.09$^{bd}$</td>
</tr>
<tr>
<td>VO$<em>{2p}$pIC$</em>{75}$</td>
<td>WKY</td>
<td>5.35 ± 0.03$^{f}$</td>
<td>5.55 ± 0.03$^{f}$</td>
<td>5.06 ± 0.06$^b$</td>
</tr>
<tr>
<td>SHR</td>
<td>5.64 ± 0.04$^{f}$</td>
<td>5.68 ± 0.05$^{f}$</td>
<td>4.93 ± 0.11$^b$</td>
<td>5.61 ± 0.02$^h$</td>
</tr>
</tbody>
</table>

NE, Norepinephrine, Prop: 10 μM propranolol, Praz: 2.5 nM prazosin, Yoh: 0.1 μM yohimbine. Data are presented as means ± S.E. Four experiments were performed in each group.

$^a$ P < .001 vs. NE alone.
$^b$ P < .001 vs. Prop + NE.
$^c$ P < .05 vs. Prop + NE.
$^d$ P < .01 vs. WKY.
$^e$ P < .05 vs. Prop + NE.
$^f$ P < .001 vs. Prop + Praz + NE.
$^g$ P < .01 vs. NE alone.
$^h$ P < .05 vs. WKY.

Fig. 2. Effects of propranolol on NE-induced perfusion pressure and VO$_2$ in the perfused hindlimb of SHR and WKY. NE-induced dose-response curves were constructed in the absence (squares) or presence (circles) of 10 μM dl-propranolol in both WKY (A and B) and SHR (C and D). Basal values of perfusion pressure and VO$_2$ are shown in table 1. Data are means ± S.E. (n = 4). P < .01 for the pressure curves in A and P > .05 for the curve comparisons in B, C and D (ANOVA).

Fig. 3. Effects of prazosin and yohimbine on NE-induced perfusion pressure and VO$_2$ in the perfused hindlimb of SHR and WKY. Dose-response curves were constructed in the presence of 10 μM dl-propranolol. Basal values of perfusion pressure and VO$_2$ are shown in table 1. A and B represent WKY and C and D represent SHR. Control (circles with solid lines), 2.5 nM prazosin (squares with dash lines), 0.1 μM yohimbine (circles with dash lines). Data are means ± S.E. (n = 4). A, P < .01 for both prazosin and yohimbine; B, P < .01 for prazosin in both sides, P < .01 for the ascending side and P > .05 for the descending side of yohimbine; C, P < .01 for prazosin and P > .05 for yohimbine; D, P < .01 for prazosin in both sides, P < .01 for the ascending side and P > .05 for the descending side of yohimbine Analyses were performed using ANOVA (vs. control).

Roles of α$_1$-AR subtypes in the altered vasoconstriction and associated VO$_2$ in SHR. The effects of α$_1$-AR subtype on NE-induced vasoconstriction and descending limb of the VO$_2$ curve were first suggested by a blockade by 5 MU at 0.25 μM in Wistar rats (Dora, 1993). However, this dose appeared to be too high because the P$_{max}$ at 20 μM of NE was only a quarter of the control. In our study, 5 MU clearly showed antagonistic effects on vasoconstriction in SHR without suppressing the P$_{max}$ at 3.3 nM which had no effect in WKY. The pK$_{B}$ value of 8.94 is similar to those reported for its action on the α$_1$-AR subtype in mesenteric, carotid and caudal arteries of SHR (Villalobos-Molina and Ibarra, 1996). Intriguingly, HNE-elicited descending limb of the VO$_2$ dose-response curve was markedly attenuated, but LNE-induced
ascending limb of VO₂ was unaffected. Similar attenuating effects on HNE-elicited descending limb of the VO₂ curve were also found with 1 nM KMD. These results suggest that HNE-elicited descending limb of the bell-shaped VO₂ dose-response curve appears to be predominantly mediated by the α₁₈-AR subtype.

It was noted, however, that KMD did not differentiate NE-induced vasoconstriction between SHR and WKY. The reason for this disparity between these two α₁-AR-antagonists at these doses tested is not clear, but may be related to some other unknown properties of KMD. For example, KMD has at these doses tested is not clear, but may be related to some other unknown properties of KMD. For example, KMD has been shown to be a competitive α₁-AR-antagonist in human prostate and recombinant human and rat α₁-ARs expressed in Chinese hamster ovary cells (Shibata et al., 1995), whereas it showed an unsurmountable antagonism to NE-induced vasoconstriction in both WKY and SHR in our work. Pharmacological experiments in blood vessels have suggested the presence of α₁-AR subtypes with low affinity for prazosin (the α₁L and α₁N) and 5 MU is known to have low affinity for both α₁L and α₁N (Muramatsu et al., 1995). However, the effect of KMD, whose functionally high affinity for α₁-AR (a putative α₁L-subtype) in human prostate has just been identified (Moriyama et al., 1997), on the α₁L and α₁N subtypes in muscle vascular bed is yet to be clarified. Further studies using other highly selective α₁-AR-antagonists with low affinity for the α₁L-AR, such as the newly-developed RS 17053 (Ford et al., 1996) may resolve this discrepancy.

BMY and SK&F are both α₁D-subtype selective antagonists. The first doses used for both BMY (0.1 μM) or SK&F (2 μM) clearly differentiate the differences between WKY and SHR. In the rat cremaster vascular bed, BMY has been shown to be selective for the α₁D-subtype at this dose (Leech and Faber, 1996). The dose of SK&F is within the range of its selectivity for the α₁D-subtype (Hieble et al., 1995b) and the influence of its higher affinity for all three α₂-AR subtypes was eliminated with the use of yohimbine. Neither BMY nor SK&F caused any significant shift of the dose-pressure curve in WKY, although a slight inhibition of both perfusion pressure and VO₂ was observed at very low concentrations of NE. In contrast, both dose-response curves of perfusion pressure and VO₂ in SHR were markedly shifted to the right by the same doses of either of these α₁D-subtype antagonists. These data suggest that the α₁D-subtype is more likely to be functionally up-regulated in the SHR hindlimb. In normal rats, α₁B-ARs do not contribute to arterial vasoconstriction in rat cremaster (Leech and Faber, 1996) and perfused hindlimb (Zhu et al., 1997) preparations. A small inhibition of 10 μM CEC of perfusion pressure and VO₂ in SHR at one or two low concentrations of NE is consistent with its blocking action on the α₁D-AR (Hieble et al., 1995a).

Fig. 4. Comparison of NE-induced perfusion pressure and VO₂ in the presence of prazosin or yohimbine in the perfused hindlimb of SHR and WKY. The perfusions were performed in the presence of 10 μM propranolol. Open symbols represent WKY and close symbols represent SHR. 2.5 nM prazosin (A and B), 0.1 μM yohimbine (C and D). Data are means ± S.E. (n = 4). A, P = .056; B, P > .05; C, P < .01; D, P < .05 for both sides (ANOVA).

Fig. 5. Effect of α₁A-antagonists on NE-induced perfusion pressure and VO₂ in the perfused hindlimb of SHR and WKY. Dose-response curves were constructed in the presence of 10 μM dl-propranolol plus 0.1 μM yohimbine. Basal values of perfusion pressure and VO₂ are shown in table 1. A and B represent WKY and C and D represent SHR. Control (circles with dash lines), 3.3 nM 5 MU (hexagons with solid lines), 1 nM KMD (squares with solid lines). Data are means ± S.E. (n = 4). A, P > .05 for 5 MU and P < .01 for KMD; B, significance was only found for the descending side of KMD (P < .01); C, P < .01 for both 5 MU and KMD; D, P > .05 for the ascending sides and P < .01 for the descending side of both 5 MU and KMD. Analyses were performed using ANOVA (ts. control).
TABLE 3
Effect of α1-AR subtype antagonists on the pEC_{50} for perfusion pressure and the pEC_{25} and pIC_{75} for VO_{2} produced by NE in the perfused hindlimb of SHR and WKY

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>5MU</th>
<th>KMD</th>
<th>BMY</th>
<th>SK&amp;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure pEC_{50}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>6.17 ± 0.06</td>
<td>6.05 ± 0.06</td>
<td>5.96 ± 0.03(^a)</td>
<td>6.14 ± 0.06</td>
<td>6.09 ± 0.05</td>
</tr>
<tr>
<td>SHR</td>
<td>6.62 ± 0.06(^b)</td>
<td>6.03 ± 0.03(^c)</td>
<td>6.24 ± 0.06(^bd)</td>
<td>6.09 ± 0.03(^d)</td>
<td>6.17 ± 0.08(^d)</td>
</tr>
<tr>
<td>VO_{2} pEC_{25}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>7.44 ± 0.06</td>
<td>7.70 ± 0.11</td>
<td>7.54 ± 0.103</td>
<td>7.05 ± 0.04(^d)</td>
<td>7.14 ± 0.06(^e)</td>
</tr>
<tr>
<td>SHR</td>
<td>7.98 ± 0.09(^b)</td>
<td>7.83 ± 0.10</td>
<td>8.02 ± 0.01(^b)</td>
<td>7.45 ± 0.15(^e)</td>
<td>7.16 ± 0.02(^e)</td>
</tr>
<tr>
<td>VO_{2} pIC_{75}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>5.45 ± 0.06</td>
<td>5.28 ± 0.02(^c)</td>
<td>4.60 ± 0.15(^c)</td>
<td>5.43 ± 0.05</td>
<td>5.43 ± 0.05</td>
</tr>
<tr>
<td>SHR</td>
<td>5.61 ± 0.02(^c)</td>
<td>4.91 ± 0.17(^d)</td>
<td>4.41 ± 0.10(^d)</td>
<td>5.19 ± 0.05(^e)</td>
<td>5.24 ± 0.02(^e)</td>
</tr>
</tbody>
</table>

5MU, 3.3 nM 5-methylurapidil, KMD, 1 nM KMD-3213, BMY: 0.1 μM BMY 7378, SK&F, 2 μM SK&F 105854. Data are means ± S.E. Four experiments were performed in each group in the presence of 10 μM propranolol plus 0.1 μM yohimbine.

\(^a\) P < .05 vs. control.

\(^b\) P < .01 vs. control.

\(^c\) P < .001 vs. control.

\(^d\) P < .01 vs. control.

\(^e\) P < .05 vs. control.

Fig. 6. Effect of α1D-antagonists on NE-induced perfusion pressure and VO_{2} in the perfused hindlimb of SHR and WKY. Dose-response curves were constructed in the presence of 10 μM dl-propranolol plus 0.1 μM yohimbine. Basal values of perfusion pressure and VO_{2} are shown in Table 1. A and B represent WKY and C and D represent SHR: control (circles with dash lines), 0.1 μM BMY (triangles with solid lines) and 2 μM SK&F (reversed triangles). Data are means ± S.E. (n = 4). A, P > .05 for BMY and SK&F; B, P < .05 for the ascending side of both BMY and SK&F only; C, P < .01 for both BMY and SK&F; D, P < .01 for the ascending side of both BMY and SK&F, P < .05 for the descending side of both BMY and SK&F. Analyses were performed using ANOVA (vs. control).

further support the argument that the α1D-subtype is probably mainly responsible for LNE-elicited increase in VO_{2} during vasoconstriction in SHR muscle vascular bed and the α1A-AR for HNE-elicited decreases in VO_{2}. Experiments in other laboratories have revealed that NE has much higher affinity for α1D-AR than for α1A-AR (Perez et al., 1994; Shibata et al., 1995). Thus, the proposal that LNE increases VO_{2} via the α1D-subtype while HNE decreases VO_{2} via the α1A-subtype would be also in agreement with those findings by Perez et al. (1994) and Shibata et al. (1995).

Mechanisms for α1-AR-mediated biphasic changes in VO_{2}. The mechanism for α1-AR mediated changes VO_{2} in muscle is not fully understood. As previously reviewed by us (Clark et al., 1995 and references therein), experiments using muscle preparations where nutrients are delivered through diffusion, α1-AR agonists are unable to show any marked stimulation of VO_{2}. However, α1-AR agonists cause remarkable changes in VO_{2} via α1-ARs in similar ways to other vasocostrictors in the perfused rat hindlimb where nutrients are delivered through the vascular system. These vasoconstrictor-controlled changes in VO_{2} seem to be determined by the ratio of nutritive to nonnutritive routes in the hindlimb presumably because of the heterogeneous distribution and affinities of different receptors or receptor subtypes in the vascular tree (Clark et al., 1995). For instance, heteroge-
neous distribution of α-1-AR subtypes has been shown in rat skeletal muscle bed (Leech and Faber, 1996). In the context of the present study, we speculate that the α-1D-AR subtype may be predominantly distributed on the capillary arterioles before the nonnutritive route, so that stimulation of this subtype may direct the flow from nonnutritive to nutritive routes, leading to rises in VO₂. In contrast, the α-1A-subtype may be predominantly located in arterioles controlling nutritive routes and stimulation of this subtype closes these nutritive routes, causing functional vascular shunting, however, further experiments are needed to reveal the distribution of α-1A- and α-1D-subtypes in relation to nutritive and nonnutritive routes in the microvasculature.

Conclusion

Two major findings have emerged from our study regarding alterations of ARs in the SHR muscle vascular bed. First, the role of α-2- and β-ARs in NE-elicited changes in vascular function and VO₂ in SHR muscle are impaired whereas the effects of α-1-ARs are markedly exacerbated. Second, both α-1A- and α-1D-subtypes are functionally up-regulated in SHR muscle vascular bed where increases in VO₂ seem to be predominantly mediated by the α-1D at a 100-fold lower concentration of NE than decreases in VO₂ which appear to be predominantly mediated by the α-1A-subtype. The results may provide some clue for the possible role of α-1-AR subtypes in the syndrome of hypertension and obesity. If similar findings also occur in vivo, the hypertension mediated by α-1A-AR subtypes might be more likely to be associated with obesity as they inhibit thermogenesis. Hence, highly selective α-1A-AR antagonists may offer better control of obesity than other α-1A-AR antagonists during the treatment of hypertension.

Acknowledgments

The authors thank Dr. K. A. Dora for her preliminary experiments in hooded Wistar rats which contributed to initiating this study, Dr. C. Han for the discussion on use of selective alpha-1 adrenoceptor subtype antagonists and Dr. S. Furler for performing ANOVA analysis.

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