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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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 whereas the VO₂ curve was shifted left- but downward, when compared with WKY. In the presence of 10 μM propranolol, prazosin (2.5 nM) shifted the pressure and VO₂ curves much more than yohimbine (0.1 μM) to the right in both strains and its effects were greater in SHR, suggesting that these effects were mediated largely by alpha-1 receptors, particularly in SHR. In the presence of propranolol plus yohimbine, the pressure curve was markedly shifted to the right by both the selective alpha-1-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 descending limb without affecting the ascending limb. Similar effects were also obtained with another alpha-1A antagonist 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 muscle vascular bed where the ascending limb of VO₂ is predominantly mediated by the alpha-1D at a much lower concentration for NE than the descending limb which is predominantly mediated by the alpha-1A subtype.

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-AR 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-1-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: α,1-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 α-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 α-1-AR-mediated vasoconstriction is also likely to be affected.

At least three α-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 α-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 α-1D-AR. The α-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 differently altered α-1-AR subtypes and these alterations may then lead to changes in NE-elicted thermogenesis in this tissue. Therefore, we compared the effects of seven selective α-1-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 2% protein, 4.6% lipid, 69% carbohydrate, 6% crude fiber with added 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) was measured in the descending limb of the VO$_2$ response and associated VO$_2$ by the perfused hindlimb 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 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. The rat 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 = 11). 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).

**Hindlimb perfusion.** The rats were anaesthetized 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 the abdominal cavity were ligated, where necessary, with sutures. Gut, seminal vesicles and the cut edges of the abdominal wall were ligated, where necessary, with sutures. Gut, seminal vesicles and the left inferior and superficial epigastric vessels, the inferior mesenteric and superior vesicle vessels, and the right hypogastric vessels, the left inferior and superficial epigastric vessels, the inferior mesenteric and superior vesicle vessels, and the iliolumbar and splanic 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 (Omaha, 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 L1-L3 vertebrate, 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 5.3 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 basal flow 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 (Bonen 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 with 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 cannula. 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. Theperfused muscle mass was 23.02 ± 0.56 and 22.18 ± 0.79 g for WKY and SHR, respectively (P > .05, n = 11). 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 β-ARs in NE-induced changes in vasoconstriction and associated VO$_2$ by comparing the effects of NE in the absence and presence of 10 μM propranolol. In set 2, experiments were divided to three groups and conducted in the presence of 10 μM propranolol to evaluate the role of α$_1$- and α$_2$-ARs in altered vasoconstriction and VO$_2$ induced by NE: control (from set 1), prazosin (2.5 nM) and yohimbine (0.1 μM). Assessments of the contribution of each α$_1$-AR subtype to the altered vasoconstriction and VO$_2$ were conducted in set 3 in the presence of propranolol (10 μM) plus yohimbine (0.1 μM). The experiments were assigned to the following groups: control (from set 2), 5 MU (3.3 nM), KMD (1 nM), CEC (10 μM), BMY (0.1 μM) and SK&F (2 μM). The doses of these α-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 μM) in SHR to further examine the role of α$_1$-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 α-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 constructed in a cumulative fashion. Each hindlimb was used for constructing the dose-response curves only once to ensure that the
metabolic characteristics of the preparations 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.** [D]-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 ((-)-R)-1-(3-hydroxypropyl)5-[2[(2-(2,2,2-trifluoroethoxy)phenoxyl)]ethyl]amino]propyl]indoline-7-carboxamide) was a gift from Dr. Y. Kurashita (Kissei Pharmaceutical Co., Matsumoto, Japan) and SK&F 105854 (furo-3-benzo[2-(2,2,2-trifluoroethoxy)phenoxyl]ethyl]amino]propyl]indoline-7-carboxamide) was 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. (Indianapols, 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 fit dose-response curves. 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). pK$_B$ values were calculated according to the following formula (Kenakin, 1993) using EC$_{50}$ of perfusion pressure: pK$_B$ = 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 vasodistortion, 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 vs. 4.40 ± 0.20 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 nM) 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**

Basal values of perfusion pressure and VO$_2$ in the constant-flow perfused hindlimb of SHR and WKY

<table>
<thead>
<tr>
<th></th>
<th>Prazosin</th>
<th>Yohimbine</th>
<th>5MU</th>
<th>KMD</th>
<th>CEC</th>
<th>BMY</th>
<th>SK&amp;F</th>
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<tr>
<td><strong>Pressure (mmHg)</strong></td>
<td></td>
<td></td>
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<tr>
<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>
<td>37.1 ± 1.1</td>
<td>37.0 ± 0.1</td>
</tr>
<tr>
<td>SHR</td>
<td>43.0 ± 1.2$^a$</td>
<td>45.7 ± 0.7$^a$</td>
<td>43.0 ± 1.8$^a$</td>
<td>43.5 ± 1.2$^a$</td>
<td>46.5 ± 2.2$^a$</td>
<td>46.8 ± 0.6$^a$</td>
<td>45.7 ± 1.8$^a$</td>
</tr>
<tr>
<td><strong>VO$_2$ (μmol g$^{-1}$ hr$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>WKY</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>
<td>6.9 ± 0.3</td>
</tr>
<tr>
<td>SHR</td>
<td>8.1 ± 0.2$^b$</td>
<td>8.0 ± 0.3$^b$</td>
<td>7.8 ± 1.3</td>
<td>8.0 ± 0.4</td>
<td>7.6 ± 0.2</td>
<td>7.9 ± 0.1$^b$</td>
<td>7.8 ± 0.2$^b$</td>
</tr>
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</table>

Prolipanol: 10 μM, prazosin: 2.5 nM, yohimbine: 0.1 μM, 5MU: 3.3 nM, 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.


Base perfusion pressure and VO$_2$. SHR have shown to have a higher vascular resistance in skeletal muscle in the absence (Cheng and Shibata, 1980) or presence (Adams et al., 1989) of a vasodilator presumably due to morphological changes of the resistance vessels in muscle vascular bed. The increased muscle vascular resistance in SHR was confirmed in the present experiment. Interestingly, the basal VO$_2$ 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$^+$-K$^+$-ATP pump and its overall compensatory activity to expel accumulated intracellular Na$^+$ 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 $\beta$-ARs in altered vasoconstriction and VO$_2$ induced by NE in SHR.** $\beta$-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 $\beta_1$/$\beta_2$-ARs by 1 $\mu$M 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 $\beta_1$/$\beta_2$-ARs by 10 $\mu$M propranolol in our study also enhanced NE-induced vasoconstriction in WKY with a small inhibition of VO$_2$ max. In comparison, propranolol did not show any significant effect on either vasoconstriction or VO$_2$ induced by NE in SHR, pointing to a reduced role of $\beta$-ARs in the muscle vascular bed. Coincidentally, a loss of $\beta$-AR-mediated vasodilation has been noted in portal veins (Doggrell and Surman, 1995) and mesenteric arteries (Blankesteijn et al., 1996) of SHR. Nonetheless, NE-induced bell-shaped VO$_2$ is not attributable to $\beta$-ARs because it was present in the presence of 10 $\mu$M propranolol.

**Roles of $\alpha_1$- and $\alpha_2$-ARs in altered vasoconstriction and VO$_2$ induced by NE in SHR.** Compared with $\beta$-ARs, $\alpha$-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$_2$ 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 $\alpha_1$-ARs in NE-induced vasoconstriction and associated bell-shaped changes of VO$_2$ in the perfused rat hindlimb is supported by a high $pK_b$ for prazosin (9.24) and low $pK_b$ for yohimbine (7.34) in WKY. These results are consistent with our recent findings that the $\alpha_1$-AR agonist phentolamine produces bell-shaped VO$_2$ with a strong vasoconstriction whereas the $\alpha_2$-AR agonist UK-14,304 elicits only a small and monophasic increase in VO$_2$ with a much weaker vasoconstriction in the perfused rat.
Roles of α1-AR subtypes in the altered vasoconstriction and associated VO₂ in SHR. The effects of α1A-AR subtype on NE-induced vasoconstriction and descending limb of the VO₂ 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 pKᵦ value of 8.94 is similar to those reported for its action on the α1A-AR subtype in mesenteric, carotid and caudal arteries of SHR (Villalobos-Molina and Ibarra, 1996). Intriguingly, HNE-elicited descending limb of the VO₂ 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 predominately mediated by the α1A-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 α1A-antagonists 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 α1A-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 α-1-AR subtypes with low affinity for prazosin (the α1-L and α1-N) and 5 MU is known to have low affinity for both α1-L and α1-N (Muramatsu et al., 1995). However, the effect of KMD, whose functionally high affinity for α1-AR (a putative α1-L-subtype) in human prostate has just been identified (Moriyama et al., 1997), on the α1-L and α1-N subtypes in muscle vascular bed is yet to be clarified. Further studies using other highly selective α1A-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).

Compared with the antagonistic effect of 5 MU, the most important difference is that LNE-elicited ascending limb of the VO2 dose-response curve was markedly decreased by BMY and SK&F. Because HNE-elicited descending limb of the VO2 curve in SHR hindlimb was similarly (although moderately) attenuated by BMY and SK&F as to that by 5 MU, we reduced the doses for these two α1D-subtype antagonists in the SHR hindlimb to 10 nM and 0.33 μM, respectively. At these reduced doses, the inhibitory effect of BMY and SK&F on LNE-elicited ascending limb of the VO2 curve still remained but the their attenuating effect on HNE-elicited descending limb of the VO2 curve was diminished. Meanwhile, the dose-response curve of perfusion pressure was slightly but significantly shifted to the right. These data

![Fig. 4. Comparison of NE-induced perfusion pressure and VO2 in the presence of prazosin or yohimbine.](Image)

![Fig. 5. Effect of α1A-antagonists on NE-induced perfusion pressure and VO2 in the perfused hindlimb of SHR and WKY.](Image)
Effect of α<sub>1</sub>-AR subtype antagonists on the pEC<sub>25</sub> for perfusion pressure and the pIC<sub>75</sub> and pIC<sub>25</sub> for VO<sub>2</sub> 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&lt;sub&gt;30&lt;/sub&gt;</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&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.14 ± 0.06</td>
<td>6.09 ± 0.05</td>
</tr>
<tr>
<td>SHR</td>
<td>6.62 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.03 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.24 ± 0.06&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>6.09 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.17 ± 0.08&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>VO&lt;sub&gt;2&lt;/sub&gt; pEC&lt;sub&gt;25&lt;/sub&gt;</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&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.14 ± 0.06&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>SHR</td>
<td>7.98 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.83 ± 0.10</td>
<td>8.02 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.45 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.16 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>VO&lt;sub&gt;2&lt;/sub&gt; pIC&lt;sub&gt;75&lt;/sub&gt;</td>
<td></td>
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<tr>
<td>WKY</td>
<td>5.45 ± 0.06</td>
<td>5.28 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.60 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.43 ± 0.05</td>
<td>5.43 ± 0.05</td>
</tr>
<tr>
<td>SHR</td>
<td>5.61 ± 0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.91 ± 0.17&lt;sup&gt;de&lt;/sup&gt;</td>
<td>4.41 ± 0.10&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.19 ± 0.05&lt;sup&gt;de&lt;/sup&gt;</td>
<td>5.24 ± 0.02&lt;sup&gt;de&lt;/sup&gt;</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.

<sup>a</sup> P < .05 vs. control.
<sup>b</sup> P < .01 vs. control.
<sup>c</sup> P < .001 vs. control.
<sup>d</sup> P < .01 vs. control.
<sup>e</sup> P < .05 vs. WKY.

Further support the argument that the α-1D-subtype is probably mainly responsible for LNE-elicited increase in VO<sub>2</sub> during vasoconstriction in SHR muscle vascular bed and the α-1A-AR for HNE-elicited decreases in VO<sub>2</sub>. Experiments in other laboratories have revealed that NE has much higher affinity for α-1D-AR than for α-1A-AR (Perez et al., 1994; Shibata et al., 1995). Thus, the proposal that LNE increases VO<sub>2</sub> via the α-1D-subtype while HNE decreases VO<sub>2</sub> via the α-1A-subtype would be also in agreement with those findings by Perez et al. (1994) and Shibata et al. (1995).

Mechanisms for α-1-AR-mediated biphasic changes in VO<sub>2</sub>. The mechanism for α-1-AR mediated changes VO<sub>2</sub> 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, α-1-AR agonists are unable to show any marked stimulation of VO<sub>2</sub>. However, α-1-AR agonists cause remarkable changes in VO<sub>2</sub> via α-1-ARs in similar ways to other vasoconstrictors in the perfused rat hindlimb where nutrients are delivered through the vascular system. These vasoconstrictor-controlled changes in VO<sub>2</sub> 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 precapillary 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 α1A- and α1D-subtypes are functionally up-regulated in SHR muscle vascular bed where increases in VO2 seem to be predominantly mediated by the α1D- at a 100-fold lower concentration of NE than decreases in VO2 which appear to be predominantly mediated by the α1A-subtype. The results may provide some clue for the possible role of α1-AR subtypes in the syndrome of hypertension and obesity. If similar changes also occur in vivo, the hypertension mediated by α1A-AR subtypes might be more likely to be associated with obesity as they inhibit thermogenesis. Hence, highly selective α1A-AR antagonists may offer better control of obesity than other α1-AR antagonists during the treatment of hypertension.

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References


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