Growth-dependent changes in endothelial factors regulating arteriolar tone

Julie Balch Samora, Jefferson C. Frisbee, and Matthew A. Boegehold

Department of Physiology and Pharmacology and Center for Interdisciplinary Research in Cardiovascular Sciences, West Virginia University School of Medicine, Morgantown, West Virginia

Submitted 26 June 2006; accepted in final form 18 August 2006

Balch Samora J, Frisbee JC, Boegehold MA. Growth-dependent changes in endothelial factors regulating arteriolar tone. Am J Physiol Heart Circ Physiol 292: H207–H214, 2007. First published August 25, 2006; doi:10.1152/ajpheart.00677.2006.—Previous studies from this laboratory suggest that during maturation, rapid microvascular growth is accompanied by changes in the mechanisms responsible for regulation of tissue blood flow. To further define these changes, we studied isolated gracilis muscle arterioles from weanling (~25 days) and juvenile (~44 days) Sprague-Dawley rats to test the hypothesis that endothelial mechanisms for the control of arteriolar tone are altered with growth. Responses to the endothelium-dependent dilator acetylcholine (ACH) were greater in weanling arterioles (WA) than in juvenile arterioles (JA), whereas there were no consistent differences between age groups in arteriolar responses to other endothelium-dependent agonists (A-23187, vascular endothelial growth factor, and simvastatin). Inhibition of nitric oxide synthase (NOS) with Nω-nitro-L-arginine methyl ester (L-NAME) attenuated ACh-induced dilation in JA but not in WA. In JA, combined inhibition of NOS and cyclooxygenase (with indomethacin) reduced the dilator responses to ACh and simvastatin by ~90% and ~70%, respectively, but had no effect in WA. Cytochrome P450 epoxygenase inhibition [with 2-propargyloxyphenyl] hexanoic acid] had no effect on responses to ACh or simvastatin in either age group. Inhibition of Ca2+-activated or ATP-dependent potassium channels (with tetracethylammonium or glibenclamide, respectively) reduced these arteriolar responses in JA but not those in WA. These findings suggest that in fully grown microvascular networks, endothelium-dependent arteriolar dilation is mediated by the combined release of endothelial nitric oxide and vasodilator prostanooids, and in part through activation of Ca2+-activated and ATP-dependent potassium channels. However, during earlier microvascular growth, this dilation is mediated by other factors yet to be identified. This may have significant implications for the regulation of tissue perfusion during microvascular development.

skeletal muscle microcirculation; endothelium; postnatal growth; nitric oxide; endothelium-dependent hyperpolarizing factor

There is mounting evidence to suggest that postnatal growth of the microvasculature is accompanied by progressive changes in a number of factors that can influence arteriolar tone and blood flow (22, 23, 29, 31, 45). During this rapid growth phase, the overall impact of any phenotypic changes in the endothelium or vascular smooth muscle could also be amplified or otherwise modulated by concomitant changes in arteriolar wall structure and/or local hemodynamic forces (23, 42, 46). Profound growth-related changes also occur at the capillary level. For example, in growing skeletal muscle, there is an increase in total tissue blood flow and in the absolute number of capillaries (1, 42) but a decrease in capillary density, capillary hematocrit, and individual capillary blood flow (33). Some of these changes may be necessary for the microvasculature to continually adapt to the changing metabolic demands associated with rapid tissue growth (2).

Growth-related changes in endothelial function were first documented in conduit arteries. Koga and colleagues (17) reported that in the rat aorta, endothelial P2 purinergic receptors coupled to the t-arginine/nitric oxide (NO) pathway are nonfunctional at 4–6 wk of age but become fully functional by 13 wk. This same group also found that rat aorta undergoes a progressive decline in its overall responsiveness to acetylcholine (ACh) from approximately 4 wk to 4 mo of age (18). Not surprisingly, such changes in responsiveness can begin soon after birth. For example, the endothelium-dependent relaxation of small porcine pulmonary arteries to ACh and bradykinin increases from 3 to 10 days of age (45). In some cases, these changes may also be biphasic; bradykinin causes endothelium-dependent contraction of small rat mesenteric arteries until 4 wk of age but causes endothelium-dependent relaxation of these vessels in older animals (6).

Little is known about growth-related changes in endothelial function at the microvascular level. Willis and Leffler (44) have reported that although pial arterioles in newborn and juvenile pigs show similar responsiveness to bradykinin, the predominant mediators of this dilation are endothelium-derived prostanoids in newborns and endothelium-derived NO in juveniles. Previous studies in our laboratory demonstrated that although arteriolar responsiveness to ACh in rat spinotrapezoid muscle is similar at 4 and 8 wk of age, these responses are more sensitive to Nω-nitro-L-arginine (L-NMMA) in the 4-wk-old animals, suggesting a greater contribution of NO to the overall dilation (22). We have also reported that vascular endothelial growth factor (VEGF) and simvastatin elicit a dose-dependent dilation of these arterioles at 8 wk of age but not at 4 wk of age (29). These two agonists stimulate endothelial NO release via activation of the phosphatidylinositil 3-kinase (PI3K)-Akt pathway, leading to direct phosphorylation and increased activity of endothelial NO synthase at low cytoplasmic Ca2+ levels (20). Therefore, Ca2+-independent signaling pathways for endothelial NO release may not yet be functional in arterioles of the younger animals.

We undertook the current study to gain further insight into these growth-related changes in the endothelium-dependent control of skeletal muscle arterioles. In this study, arterioles were isolated and studied in vitro to eliminate the possibility of any indirect and potentially complicating effects on arteriolar behavior due to circulating or tissue-derived factors. A clearer understanding of how the endothelium translates biochemical signals into vascular responses during blood vessel growth may

http://www.ajpheart.org

0363-6135/07 $8.00 Copyright © 2007 the American Physiological Society

H207

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
ultimately lead to the identification of new therapeutic targets for the endothelial dysfunction associated with cardiovascular diseases that can begin to develop well before adulthood.

**MATERIALS AND METHODS**

**Animals.** All surgical and experimental procedures were approved by the West Virginia University Animal Care and Use Committee. Experiments were performed on isolated gracilis muscle arterioles from male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) of two age groups: 3–4 wk (“weanlings”) and 6–7 wk (“juveniles”).

Rats were anesthetized by intraarterial injection of pentobarbital sodium (50 mg/kg body wt), with supplemental anesthetic (10% of original dose) administered if needed. The right carotid artery was cannulated with polyethylene tubing (PE-10 for weanlings, PE-50 for juveniles) for measurement of mean arterial pressure, which was assessed before removal of the gracilis arteriole.

**Preparation of isolated vessels.** An arteriolar branch of the femoral artery supplying the gracilis muscle was removed, handling only the surrounding connective tissue to minimize vessel stretching or damage. The rat was then euthanized immediately after vessel removal by intracardiac injection of pentobarbital sodium. The vessel was then placed in warmed physiological salt solution (PSS) equilibrated with 21% O2-5% CO2-74% N2 and having the following composition (mM): 119 NaCl, 4.7 KCl, 1.17 MgSO4, 1.6 CaCl2, 1.18 NaH2PO4, 24 NaHCO3, 0.026 EDTA, and neutralized to pH 7.4 with HCl. One-milliliter aliquots of this solution were then diluted to a volume of 35 ml with PBS to adjust the bath concentration of 10−6 M (7, 11, 24). The resulting solution was then diluted to a volume of 35 ml with PBS (7, 30). A 10−3 M bath concentration of TEA was added to the bath at a concentration of 10−3 M (25). This was used to block ATP-dependent (KATP) channels (36). Since endothelium-derived hyperpolarizing factors (EDHFs) modulate arteriolar tone through activation of potassium channels (4, 43), the contribution of EDHFs to arteriolar dilatation was assessed by using tetraethylammonium (TEA) (7, 11, 24) or the anti-diabetic sulfonylurea glibenclamide (24). We used a 10−3 M bath concentration of TEA to selectively block Ca2+−activated K+ (KCa) channels (28) and 10−6 M glibenclamide to selectively block ATP-dependent (KATP) channels (36).

**Inhibition of potassium channels.** Since endothelium-derived hyperpolarizing factors (EDHFs) modulate arteriolar tone through activation of potassium channels (4, 43), the contribution of EDHFs to arteriolar dilatation was assessed by using tetraethylammonium (TEA) (7, 11, 24) or the anti-diabetic sulfonylurea glibenclamide (24). We used a 10−3 M bath concentration of TEA to selectively block Ca2+−activated K+ (KCa) channels (28) and 10−6 M glibenclamide to selectively block ATP-dependent (KATP) channels (36).

**Data and statistical analyses.** To account for age-related differences in resting and passive arteriolar diameters (see Table 1), dilator responses were expressed as a percentage of maximum dilation ([diameter change]/Dmax – control diameter) × 100). All data are presented as means ± SE. For all analyses, a probability value of P < 0.05 was considered to be statistically significant.

**RESULTS**

General characteristics of all rats from which vessels were removed are reported in Table 1. Age, body weight, and mean arterial pressure were significantly greater in juvenile rats than in weanling rats. Table 1 also summarizes the general characteristics of all vessels studied. Resting and passive diameters of

To assess intrinsic vascular smooth muscle responsiveness to NO, the endothelium-independent vasodilator sodium nitroprusside (SNP, Sigma) was used at bath concentrations of 10−5 and 10−7 M.

**Endothelial denudation.** To determine the role of the endothelium in mediating arteriolar responses to ACh and simvastatin, the endothelium was removed by mechanical abrasion (38). The pipette tips on both sides of the vessel were gently advanced through the vessel lumen at least three times to ensure elimination of the endothelium. To ensure that the endothelial denudation did not adversely affect underlying arteriolar smooth muscle, vasoconstrictor responses to 10−5 M phenylephrine and vasodilator responses to 10−5 M SNP were assessed before and after the denudation procedure, and only those vessels with unchanged responses to both agonists were included in this study.

**Inhibition of NOS, cyclooxygenase, and cytochrome P450 enzymes.** To determine the contribution of endothelial NO production to arteriolar dilatation, the NOS inhibitor N4-nitro-L-arginine methyl ester (L-NAME) was added to the bath at a concentration of 10−5 M (12). To assess the contribution of vasodilator prostanooids, the cyclooxygenase inhibitor indomethacin was added to the bath at a concentration of 10−6 M (10). To assess the specific contribution of cytochrome P450 (CP450) epoxygenase-derived metabolites of arachidonic acid, the selective suicide substrate inhibitor 6-(2-propargyloxyphenyl)hexanoic acid (MS-PPOH) was added to the bath at a concentration of 10−6 M (41). All inhibitors were purchased from Sigma.

**Vessel diameter was measured using an onscreen video micrometer.** Any vessel that did not demonstrate endothelial viability, as judged by responsiveness to 10−7 M ACh, was not used in the study. Diameter measurements were made under static, zero-flow conditions after a 30-min equilibration period with continuous perfusion. Resting vascular tone was calculated as (ΔD/Dmax) × 100, where ΔD is the diameter increase from rest in response to Ca2+−free PSS (30–40 min equilibration with Ca2+−free bath solution and no luminal flow), and Dmax is the maximum diameter measured under these conditions.

**Ach.** All agonists were dissolved in PSS unless otherwise noted. ACh (Sigma Chemical, St. Louis, MO), at bath concentrations of 10−5, 10−6, or 10−7 M, and the Ca2+−ionophore A-23187 (Sigma), at a bath concentration of 10−6 M, were used to assess arteriolar capacity for Ca2+−dependent endothelial NO formation (40). To produce the desired concentration, 1 mg of A-23187 was first dissolved in 50 μl of dimethylsulfoxide (DMSO) and then diluted with Dulbecco’s phosphate-buffered saline (PBS) before addition to the bath.

VEGF (BD Biosciences, Lexington, KY) and simvastatin (Merck Research Laboratories, Rathway, NJ) were used to assess arteriolar capacity for Ca2+−independent endothelial NO formation (29). VEGF was dissolved in PBS at a concentration of 5 μg/ml and added directly to the bath. Simvastatin was activated by alkaline lysis (5.25 ml of 0.1 N NaOH per 140 mg, dissolved in 3.5 ml of ETOH) at 50°C for 2 h. The resulting solution was then diluted to a volume of 35 ml with PBS and neutralized to pH 7.4 with HCl. One-milliliter aliquots of this solution were then serially diluted with PBS, producing a final bath concentration of 10−4, 10−5, 10−6, or 10−7 M.
arterioles from juvenile rats were significantly greater than those of arterioles from weanling rats, whereas the level of spontaneous tone that developed with vessel pressurization was significantly less in juvenile arterioles than in weanling arterioles.

All agonists dilated both weanling and juvenile arterioles, with a slightly smaller dilation to ACh seen in juvenile arterioles (Fig. 1). However, there were no consistent differences between age groups in the magnitude of dilation to the other agonists, except for a moderately smaller response of juvenile arterioles to \(10^{-7}\) M simvastatin (Fig. 1). Neither L-NAME nor indomethacin treatment, alone or in combination, changed the resting diameters of either juvenile or weanling arterioles (data not shown). For juvenile arterioles, the mean response to a concentration of each agonist that initially caused close to half-maximal dilation was reduced by 60–90% in the presence of L-NAME (Fig. 2, top). Indomethacin significantly reduced only those responses to A-23187 and simvastatin (by 90% and 63%, respectively). In contrast, the responses of weanling arterioles to ACh and simvastatin by approximately the same amount as TEA alone. Glibenclamide alone had no significant effect on the dilation of juvenile arterioles to ACh but reduced the dilation of these vessels to simvastatin (Fig. 7, left). In contrast, TEA alone markedly reduced the responses of these vessels to both agonists (Fig. 7, center). Consistent with the lack of effect of glibenclamide on ACh responses, glibenclamide and TEA in combination reduced these responses by the same magnitude as TEA alone (Fig. 7, right). Glibenclamide + TEA also abolished juvenile arteriolar responses to simvastatin. Neither glibenclamide nor TEA, alone or in combination, reduced the dilation of weanling arterioles to either agonist (Fig. 7, bottom). Responses of weanling arterioles to ACh also remained

---

### Table 1. General characteristics of all rats and vessels used in this study

<table>
<thead>
<tr>
<th></th>
<th>Weanlings</th>
<th>Juveniles</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Animal characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N)</td>
<td>68</td>
<td>52</td>
</tr>
<tr>
<td>Age, days</td>
<td>25.3±0.3</td>
<td>42.0±0.7*</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>63.1±1.3</td>
<td>181.4±2.9*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>82.3±0.9</td>
<td>100.2±1.4*</td>
</tr>
<tr>
<td><strong>Vessel characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n)</td>
<td>68</td>
<td>52</td>
</tr>
<tr>
<td>Resting diameter, (\mu m)</td>
<td>44.1±1.5</td>
<td>61.6±2.1*</td>
</tr>
<tr>
<td>Passive diameter, (\mu m)</td>
<td>67.8±1.9</td>
<td>85.6±2.2*</td>
</tr>
<tr>
<td>Resting vascular tone, %</td>
<td>34.5±1.6</td>
<td>20.0±1.5*</td>
</tr>
</tbody>
</table>

Values are given as means ± SE; \(N\), number of rats; \(n\), number of vessels. MAP, mean arterial pressure. *\(P < 0.05\) vs. weanling group.

---

Glibenclamide had no effect on the resting diameters of either weanling or juvenile arterioles, whereas 1 mM TEA reduced the resting diameters of both juvenile and weanling arterioles by approximately the same amount (10–12%) (Table 2). The combination of glibenclamide + TEA significantly reduced the resting diameters of both juvenile and weanling arterioles by approximately the same amount as TEA alone. Glibenclamide alone had no significant effect on the dilation of juvenile arterioles to ACh but reduced the dilation of these vessels to simvastatin (Fig. 7, left). In contrast, TEA alone markedly reduced the responses of these vessels to both agonists (Fig. 7, center). Consistent with the lack of effect of glibenclamide on ACh responses, glibenclamide and TEA in combination reduced these responses by the same magnitude as TEA alone (Fig. 7, right). Glibenclamide + TEA also abolished juvenile arteriolar responses to simvastatin. Neither glibenclamide nor TEA, alone or in combination, reduced the dilation of weanling arterioles to either agonist (Fig. 7, bottom). Responses of weanling arterioles to ACh also remained

---

Fig. 1. Responses of isolated gracilis muscle arterioles from weanling and juvenile rats to acetylcholine (ACh), simvastatin, \(A-23187\), and vascular endothelial growth factor (VEGF). \(n\), Number of vessels. Values are given as means ± SE. *\(P < 0.05\) vs. weanlings at same agonist concentration.
unchanged when the bath TEA concentration was increased to 5 mM, although responses to $10^{-5}$ M simvastatin were reduced (by 44.5 ± 0.4%) under these conditions (data not shown).

Treatment with glibenclamide significantly attenuated responses to the K<sub>ATP</sub> channel opener pinacidil in both weanling and juvenile arterioles (Table 3). Although treatment with 1 mM TEA also attenuated the pinacidil responses of weanling arterioles, it had no effect on these responses in juvenile arterioles. Treatment with both glibenclamide and TEA attenuated pinacidil responses in both groups. Treatment with glibenclamide had no effect on arteriolar responses to the BK<sub>Ca</sub> channel opener A-23187.

Fig. 2. Responses of arterioles from juvenile and weanling rats to ACh, simvastatin, A-23187, and VEGF. Data are presented under control conditions and following inhibition of nitric oxide synthase (NOS) with $N^\text{G}$-nitro-L-arginine methyl ester (L-NAME, $10^{-4}$ M) and cyclooxygenase with indomethacin ($10^{-6}$ M). n, number of vessels. Values are given as means ± SE. *P < 0.05 vs. control.

Fig. 3. Effect of endothelial removal on responses of juvenile and weanling arterioles to ACh and simvastatin. n, number of vessels. Values are given as means ± SE. *P < 0.05 vs. vessel with intact endothelium.

Fig. 4. Effect of combined NOS and cyclooxygenase inhibition on responses of juvenile and weanling arterioles to ACh and simvastatin. n, number of vessels. Values are given as means ± SE. *P < 0.05 vs. control.

Fig. 5. Responses of arterioles from weanling and juvenile rats to sodium nitroprusside. n, number of vessels. Values are given as means ± SE.
although the clear role of potassium channels in these responses is consistent with the possibility that another type of EDHF is involved. In contrast, the endothelium-dependent dilation of weanling arterioles does not appear to rely on endothelial NO, prostanoids, CP450 epoxygenase products, or any other EDHF.

Bradykinin-induced dilation of newborn porcine pial arterioles relies on prostaglandins but becomes more reliant on NO release during juvenile growth (44). Consistent with these observations, cyclooxygenase expression and activity is similar in newborn and adult porcine cerebral microvessels, whereas the adult microvessels have significantly higher NOS expression and activity (31). From our current observations, it appears that NO, and in some cases cyclooxygenase products, contributes to the endothelium-dependent dilation of gracilis muscle arterioles from juvenile, but not weanling rats (Figs. 2 and 4). The absence of a role for NO in the endothelium-dependent dilation of weanling arterioles could theoretically be due to a lack of NO production, an accelerated breakdown of NO, or a reduced responsiveness of vascular smooth muscle to NO. The latter possibility appears unlikely, in light of the similar responsiveness to SNP in both age groups (Fig. 5). This has been a consistent finding in other studies as well (18, 22, 29, 45).

In adult animals, there is convincing evidence that endothelium-derived hyperpolarizing factors (EDHF) can contribute to endothelium-dependent arteriolar dilation (27, 32, 34). EDHF also appears to be an important mediator of vasodilation in the neonatal cerebral microcirculation (21). Although there is no single EDHF, metabolites of arachidonic acid by CP450 epoxygenase appear to play this role in rat cremaster muscle arterioles (26). To assess the potential role of CP450 4A-derived epoxyeicosatrienoic acids in the dilator responses we studied, the vessels were exposed to PPOH, a selective inhibitor of arachidonate epoxygenation reactions catalyzed by CP450 enzymes. PPOH had no effect on responses to ACh or simvastatin. Collectively, these observations indicate that CP450 epoxygenase metabolites do not contribute to the endothelium-dependent dilation of gracilis muscle arterioles from juvenile or weanling rats, which is consistent with previous findings in gracilis muscle arterioles of older (12 wk old) rats (39).

It is possible that an EDHF derived from some other enzymatic pathway could have contributed to endothelium-dependent dilation in either or both of the age groups we studied. EDHFs generally exert their effects through activation of vascular smooth muscle K⁺ channels (4, 43). Although some studies indicate that EDHFs only activate KCa channels (39), others have documented that KATP channels can also be acti-

channel opener NS-1619 in either group, whereas TEA abolished these responses in both groups (Table 4). As expected, the effect of glibenclamide + TEA on NS-1619-induced dilation (complete abolition) was the same as that of TEA alone in both groups.

**DISCUSSION**

Growth-related changes in the responsiveness of conduit arteries to endothelium-dependent agonists have been well documented (6, 17, 18, 45). Some investigators have reported varying degrees of such changes in endothelial function in the microcirculation (22, 29, 44), and the present study was designed to obtain further information on the endothelial factors that could control arteriolar tone at two different stages of juvenile growth. Our findings indicate that for juvenile arterioles, endothelium-dependent dilations to the agonists we used are mediated largely by NO, with vasodilator prostanoids also making an important contribution to these responses in some cases. However, metabolites of CP450 epoxygenase do not appear to contribute to any of the responses we investigated, although the clear role of potassium channels in these responses is consistent with the possibility that another type of EDHF is involved. In contrast, the endothelium-dependent dilation of weanling arterioles does not appear to rely on endothelial NO, prostanoids, CP450 epoxygenase products, or any other EDHF.

Bradykinin-induced dilation of newborn porcine pial arterioles relies on prostaglandins but becomes more reliant on NO release during juvenile growth (44). Consistent with these observations, cyclooxygenase expression and activity is similar in newborn and adult porcine cerebral microvessels, whereas the adult microvessels have significantly higher NOS expression and activity (31). From our current observations, it appears that NO, and in some cases cyclooxygenase products, contributes to the endothelium-dependent dilation of gracilis muscle arterioles from juvenile, but not weanling rats (Figs. 2 and 4). The absence of a role for NO in the endothelium-dependent dilation of weanling arterioles could theoretically be due to a lack of NO production, an accelerated breakdown of NO, or a reduced responsiveness of vascular smooth muscle to NO. The latter possibility appears unlikely, in light of the similar responsiveness to SNP in both age groups (Fig. 5). This has been a consistent finding in other studies as well (18, 22, 29, 45).

In adult animals, there is convincing evidence that endothelium-derived hyperpolarizing factors (EDHF) can contribute to endothelium-dependent arteriolar dilation (27, 32, 34). EDHF also appears to be an important mediator of vasodilation in the neonatal cerebral microcirculation (21). Although there is no single EDHF, metabolites of arachidonic acid by CP450 epoxygenase appear to play this role in rat cremaster muscle arterioles (26). To assess the potential role of CP450 4A-derived epoxyeicosatrienoic acids in the dilator responses we studied, the vessels were exposed to PPOH, a selective inhibitor of arachidonate epoxygenation reactions catalyzed by CP450 enzymes. PPOH had no effect on responses to ACh or simvastatin. Collectively, these observations indicate that CP450 epoxygenase metabolites do not contribute to the endothelium-dependent dilation of gracilis muscle arterioles from juvenile or weanling rats, which is consistent with previous findings in gracilis muscle arterioles of older (12 wk old) rats (39).

It is possible that an EDHF derived from some other enzymatic pathway could have contributed to endothelium-dependent dilation in either or both of the age groups we studied. EDHFs generally exert their effects through activation of vascular smooth muscle K⁺ channels (4, 43). Although some studies indicate that EDHFs only activate KCa channels (39), others have documented that KATP channels can also acti-

Table 2. Summary of vessel diameters under control conditions and after treatment with Glib and TEA separately and in combination

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 8)</th>
<th>Glib</th>
<th>Control (n = 5–6)</th>
<th>TEA</th>
<th>Control (n = 8)</th>
<th>Glib + TEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteriolar Diameter, µm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weanlings</td>
<td>39.8±4.0</td>
<td>39.6±3.8</td>
<td>42.3±2.5</td>
<td>38.3±2.6*</td>
<td>39.8±4.0</td>
<td>34.2±3.6*</td>
</tr>
<tr>
<td>Juveniles</td>
<td>55.3±3.7</td>
<td>55.2±3.9</td>
<td>56.7±3.7</td>
<td>49.8±4.5*</td>
<td>55.3±3.7</td>
<td>47.8±3.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of vessels. Glib, glibenclamide (10⁻⁶ M); TEA, tetraethylammonium (1 mM). *P < 0.05 vs. control.

* Downloaded from http://ajpheart.physiology.org by [Your Username] on [Date]
activated by EDHFs (3, 9). Therefore, we chose to target both of these channel types as potential mediators of dilation. The K_{ATP} channel inhibitor glibenclamide completely abolished simvastatin responses in juvenile arterioles but had no effect on these responses in weanling arterioles and had no effect on ACh responses in either juvenile or weanling arterioles (Fig. 7). Although inhibition of KCa channels with 1 mM TEA significantly reduced responses to both acetylcholine and simvastatin in juvenile arterioles (by 72–79%), it had no effect on these dilations in weanling arterioles. Inhibition of both K_{ATP} and KCa channels reduced responses to ACh by 60–70% and completely abolished responses to simvastatin in juvenile arterioles but had no effect on the responses of weanling arterioles. Also we used a high dose of TEA (5 mM) to block all KCa channels as well as other potential potassium channels in the weanling arterioles (19) but only achieved a slight attenuation of the response to 10^{-5} M simvastatin (data not shown). Therefore, although juvenile arterioles apparently rely on an EDHF to mediate dilations, we were not able to detect a contribution of EDHF to weanling arteriole dilations from our use of these potassium channel antagonists.

The K_{ATP} channel opener pinacidil dilated arterioles from rats of both age groups (Table 3), indicating the presence of recruitable K_{ATP} channels that are capable of modulating vascular smooth muscle tone. However, glibenclamide, at a concentration that inhibits most of this K_{ATP} channel activity, has no effect on the resting tone of arterioles from either group (Table 2). This suggests that if there is some level of K_{ATP} channel activity in these arterioles under our steady-state conditions, it is not sufficient to modulate smooth muscle contractile activity. In contrast, TEA, at a concentration that blocks the effect of a BK_{Ca} channel opener on arteriolar tone (Table 4), reduces the resting diameter of arterioles from both age groups by approximately the same amount (Table 2). This suggests that under resting conditions, there is ongoing arteriolar BK_{Ca} channel activity that is high enough to influence smooth muscle tone.

**Table 3. Responses of arterioles from weanling and juvenile rats to pinacidil before and after inhibition of K_{ATP} channels with Glib or inhibition of K_{Ca} channels with TEA, separately and in combination**

<table>
<thead>
<tr>
<th></th>
<th>Pinacidil (n = 5–6)</th>
<th>Pinacidil + Glib</th>
<th>Pinacidil (n = 4)</th>
<th>Pinacidil + TEA</th>
<th>Pinacidil (n = 5–6)</th>
<th>Pinacidil + Glib/TEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weanlings</td>
<td>56.7±9.3</td>
<td>9.4±3.0*</td>
<td>50.2±6.6</td>
<td>24.7±7.1*</td>
<td>56.7±9.3</td>
<td>13.0±5.0*</td>
</tr>
<tr>
<td>Juveniles</td>
<td>56.7±5.4</td>
<td>14.7±4.5*</td>
<td>43.6±3.5</td>
<td>33.7±6.0</td>
<td>56.7±5.4</td>
<td>11.5±1.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE (in % maximum dilation); n, number of arterioles. Pinacidil concentration was 10^{-5} M; Glib concentration was 10^{-6} M; TEA concentration was 1 mM. K_{ATP}, ATP-dependent K+ channel; K_{Ca}, Ca^{2+}-activated K+ channel. *P < 0.05 vs. preceding response to pinacidil.
contractile activity, and that the magnitude of this effect does not appreciably change during rapid juvenile growth. This apparent influence of BKCa channel activity on gracilis muscle arteriolar tone is consistent with previous findings for this vessel (39), but these observations do not extend to arterioles in all skeletal muscles. Other investigators have found that smooth muscle KCa channel activity is “silent” in arterioles under resting conditions in both hamstr and rat cremaster muscle (15). Our interpretations based on the effects of TEA should be made with some caution. Although TEA at a concentration of 1 mM will preferentially block KCa channels, the relatively broad specificity of this compound allows it to antagonize other types of potassium channels under some conditions (8). This may have occurred to some extent in the current study, as evidenced from our finding that 1 mM TEA did have some effect on the response of weanling arterioles to the KATP channel opener pinacidil (Table 3).

Consistent with the apparently similar influence of BKCa channels on resting tone in both age groups, the selective BKCa channel opener NS-1619 was as effective in dilating weanling arterioles as in diluting juvenile arterioles, with these responses being completely abolished by 1 mM TEA in both age groups (Table 4). This is not consistent with previous reports that there are changes in KCa channel activity and density with maturation and growth. Teng et al. (37) found that stretch-induced activation of KCa channels exert a greater effect on resting vascular tone in cerebral arteries from adult sheep than in those from fetal sheep. Other investigators, using whole cell patch-clamp recordings, determined that carotid body KCa channel expression increases with postnatal age in the rat (14). It is not clear why these differences exist, but perhaps some of the discrepancy can be explained by the use of different preparations and vessel size.

Direct microvascular pressure measurements indicate that in situ luminal pressure in the vessels we studied is equal to ~80% of mean arterial pressure (5). When equilibrated at this pressure in vitro, arterioles from weanling rats develop a significantly higher level of resting tone than arterioles from juvenile rats (Table 1), suggesting that arteriolar smooth muscle responsiveness to myogenic stimuli could be greater in the younger rats. However, a rigorous assessment of this possibility, which would require a determination of the different levels of myogenic tone developed over a wide range of intravascular pressures in each age group, is beyond the scope of this study. Nevertheless, it may be germane to point out that over a wide range of luminal pressures, the spontaneous tone developed by isolated small cerebral arteries from newborn mice is far greater than that developed by the same arteries from 6- to 8-wk-old mice (13). However, this is apparently not due to a greater myogenic responsiveness of smooth muscle in the younger vessels but rather to less moderation of this myogenic tone by endothelial factors in these vessels. It is possible that such a differential effect of the endothelium could also account for the difference in tone between the two age groups studied here, but this seems unlikely because we found no evidence for any endothelial modulation of resting tone, even in mature vessels (i.e., no effect of l-NAME, indomethacin, or PPOH on resting arteriolar diameters).

Using an in vivo exteriorized spinotrapezius muscle preparation, our laboratory (29) previously found that Ca2+-independent signaling pathways for endothelial NO release do not appear to be operational in arterioles of weanling rats. However, in the current study on isolated gracilis muscle arterioles, we found no systemic age-related differences in responsiveness to agonists that promote either Ca2+-dependent NO release (ACH, A-23187) or Ca2+- independent NO release (simvastatin, VEGF), with the exception of the slightly smaller dilation of juvenile arterioles to one concentration of acetylcholine (Fig. 1). This discrepancy may be due to the inherent heterogeneity between vascular beds.

As can be seen from Table 1, the period from 25 to 42 days of age is one of rapid juvenile growth in the rat, characterized almost a threefold increase in body mass. The phases of human growth that correspond to these ages are somewhat imprecise because they depend to some extent on the variables that are being compared. For example, if a comparison is made on the basis of projected weight gain, then our 25-day-old and 42-day-old rats with average weights that are respectively 11% and 33% of an expected 550-g adult weight (from comparing Table 1 with Harlan Sprague Dawley growth tables) would roughly correspond to boys at the ages of 2 and 8 yr (from Centers for Disease Control growth charts). However, if the comparison is made on the basis of current age relative to an average life expectancy of 728 days for the rat (Harlan Sprague Dawley) versus 71.5 yr for US men (centers for Disease Control), then these ages in the rat would roughly correspond to boys at 2 and 4 yr of age. Finally, if the comparison is made on the basis of current age in relation to mean age of sexual maturity (57 days for rats: 13 yr for men), then these ages in the rat would roughly correspond to boys at 6 and 10 yr of age.

In conclusion, at any given time, the tone of skeletal muscle arterioles does not reflect a single mechanism but rather an integrated effect of multiple mechanisms that, to some extent, may compensate for one another should one pathway be abolished. Endothelium-dependent arteriolar dilation in juvenile rats appears to be mediated by the combined release of endothelial NO and vasodilator prostanooids, with a non-CP450-derived hyperpolarizing factor possibly also contributing to the dilation. However, during earlier microvascular growth, this dilation is mediated by other factors yet to be identified.

Table 4. Responses of arterioles from weanling and juvenile rats to NS1619 before and after inhibition of KATP channels with Glib or inhibition of KCa channels with TEA, separately and in combination

<table>
<thead>
<tr>
<th></th>
<th>NS-1619 (n = 5)</th>
<th>NS-1619 + Glib (n = 4)</th>
<th>NS-1619 (n = 7)</th>
<th>NS-1619 + TEA (n = 5)</th>
<th>NS-1619 + Glib/TEA (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weanlings</td>
<td>30.6±4.4</td>
<td>32.6±6.9</td>
<td>58.0±22.1</td>
<td>3.7±6.4*</td>
<td>30.6±4.4</td>
</tr>
<tr>
<td>Juveniles</td>
<td>31.3±2.6</td>
<td>31.9±2.8</td>
<td>32.5±11.5</td>
<td>−15.0±4.7*</td>
<td>31.3±2.6</td>
</tr>
</tbody>
</table>

Values are means ± SE (in % maximum dilation); n, number of arterioles. NS-1619 concentration was 30 μM; Glib concentration was 10−5 M; TEA concentration was 1 mM. *P < 0.05 vs. preceding response to NS-1619.
REFERENCES


