Cerebral artery $K_{ATP}$ and $K_{Ca}$-channel activity and contractility: changes with development

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Long, Wen, Lubo Zhang, and Lawrence D. Longo. Cerebral artery $K_{ATP}$ and $K_{Ca}$-channel activity and contractility: changes with development. Am J Physiol Regulatory Integrative Comp Physiol 279: R2004–R2014, 2000.—The present study was designed to test the hypothesis that in cerebral arteries of the fetus, ATP-sensitive ($K_{ATP}$) and $Ca^{2+}$-activated $K^+$ channels ($K_{Ca}$) play an important role in the regulation of intracellular $Ca^{2+}$ concentration ([Ca$^{2+}$]) and that this differs significantly from that of the adult. In main branch middle cerebral arteries (MCA) from near-term fetal (~140 days) and nonpregnant adult sheep, simultaneously we measured norepinephrine (NE)-induced responses of vascular tension and [Ca$^{2+}$], in the absence and presence of selective $K^+$-channel openers/blockers. In fetal MCA, in a dose-dependent manner, both the $K_{ATP}$-channel opener pinacidil and the $K_{Ca}$-channel opener NS 1619 significantly inhibited NE-induced tension [negative logarithm of the half-maximal inhibitory concentration (pIC$_{50}$) = 5.0 ± 0.1 and 8.2 ± 0.1, respectively], with a modest decrease of [Ca$^{2+}$]. In the adult MCA, in contrast, both pinacidil and NS 1619 produced a significant tension decrease (pIC$_{50}$ = 5.1 ± 0.1 and 7.6 ± 0.1, respectively) with no change in [Ca$^{2+}$]. In addition, the $K_{Ca}$-channel blocker iberiotoxin (10$^{-7}$ to 10$^{-6}$ M) resulted in increased tension and [Ca$^{2+}$], in both adult and fetal MCA, although the $K_{ATP}$-channel blocker glibenclamide (10$^{-7}$ to 3 × 10$^{-5}$ M) failed to do so. Of interest, administration of 10$^{-7}$ M iberiotoxin totally eliminated vascular contraction and increase in [Ca$^{2+}$], seen in response to 10$^{-5}$ M ryanodine. In precontracted fetal cerebral arteries, activation of the $K_{ATP}$ and $K_{Ca}$ channels significantly decreased both tension and [Ca$^{2+}$], suggesting that both $K^+$ channels play an important role in regulating L-type channel $Ca^{2+}$ influx and therefore vascular tone in these vessels. In the adult, $K_{ATP}$ and the $K_{Ca}$ channels also appear to play an important role in this regard; however, in the adult vessel, activation of these channels with resultant vasorelaxation can occur with no significant change in [Ca$^{2+}$]. These channels show differing responses to inhibition, e.g., $K_{Ca}$-channel inhibition, resulting in increased tension and [Ca$^{2+}$], whereas $K_{ATP}$-channel inhibition showed no such effect. In addition, the $K_{Ca}$ channel appears to be coupled to the sarcoplasmic reticulum ryanodine receptor. Thus differences in plasma membrane $K^+$-channel activity may account, in part, for the differences in the regulation of contractility of fetal and adult cerebral arteries.

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PREVIOUSLY, WE AND OTHERS have reported significant differences in cerebral artery contractility with development from fetus to adult. Fetal arteries develop less tone but have greater aminergic activity than those of adult (33); they also show greater calcium sensitivity (26), rely less on Ins(1,4,5)P$_3$-mediated contractile mechanisms (27, 38), and have relatively small intracellular $Ca^{2+}$ stores compared with the adult (25). In cerebral (and other) arteries, $K^+$ channels play a major role in modulating membrane potential, voltage-gated $Ca^{2+}$-channel activity, intracellular $Ca^{2+}$ concentration ([Ca$^{2+}$]), and vascular tone. The activation of $K^+$ channels leads to $K^+$ efflux, resulting in hyperpolarization and decreased open probability of L-type $Ca^{2+}$ channels, thereby reducing $Ca^{2+}$ influx, [Ca$^{2+}$], and vascular tone. Conversely, norepinephrine (NE)-induced depolarization with inhibition of $K^+$ efflux leads to increased open probability of L-type $Ca^{2+}$ channels, increased influx of extracellular $Ca^{2+}$, increased [Ca$^{2+}$], and contraction. Because of the ionic properties of the cell membrane at rest, activity change of only a few $K^+$ channels is sufficient to alter membrane potential and thereby tone (30, 34). In general, the relationship of vessel diameter to membrane potential is linear, i.e., a decrease in membrane potential (less negative) is associated with increased tone and contraction (21).

A growing literature has considered the interactions of $K^+$ and $Ca^{2+}$ channels in several cell types, including the smooth muscle cells from main branch cerebral arteries (18, 24, 30). Four types of $K^+$ channels have been identified, and each is present in cerebral arteries: ATP-sensitive ($K_{ATP}$), $Ca^{2+}$-activated ($K_{Ca}$), voltage dependent ($K_v$), and inward rectifier ($K_{IR}$) (10, 11, 24, 30, 31). A wide variety of vasoactive stimuli have been shown to alter cerebral vessel $K^+$-channel activity, including NE, which acts to inhibit $K^+$ efflux (depolar-
ization) and thereby open Ca^{2+} channels (10, 11). Several studies have demonstrated the sensitivity of the main branch middle cerebral artery (MCA) to K^-channel activation in the adult (30, 34, 35). By comparison, few studies have examined responses to K^+-channel openers and blockers in cerebral arteries of the fetus or changes with development.

In previous studies, we have shown that, in contrast to the adult, fetal cerebral arteries are almost completely dependent on extracellular Ca^{2+} via L-type Ca^{2+} channels for contraction (25, 26). As noted above, in cerebral and other arteries, K^+ channels play a key role in regulating L-type Ca^{2+}-channel activity. Because of this dependence on L-type Ca^{2+} channels for Ca^{2+} flux and the fact that essentially nothing is known about K^+-channel function in developing cerebral vasculature, the present study was designed to test the hypothesis that in cerebral arteries of the fetus, the several K^-channels, particularly the K_{ATP} and K_{Ca} channels, play an important role in the regulation of [Ca^{2+}], and vascular tone and that this role differs significantly from that of the adult.

METHODS

Experimental animals and tissues. For these studies, we used main branch MCAs from near-term fetus (−140 days) and nonpregnant adult sheep (≥2 yr) obtained from Neubeck Ranch (Lancaster, CA), as we have previously described (27, 28). The ewes were anesthetized and killed with 100 mg/kg intravenous pentobarbital sodium, after which we obtained isolated cerebral artery segments. We have shown that this method of death has no significant effect on vessel reactivity, compared with use of other anesthetic agents (33). To avoid the complication of endothelial-mediated effects, we removed the endothelium by carefully inserting a small wire three times (28). Cerebral arteries (fetus 300–350 μM, adult 400–450 μM) were then used immediately for simultaneous measurements of the [Ca^{2+}], and tensions (26). To confirm endo-
thelium removal, we contracted the vessel with 10^{-5} M 5-hydroxytryptamine and, at the plateau, added 10^{-6} M ADP. Vessels that relaxed >20% after this treatment were rejected for further study. Unless otherwise noted, all chemical compounds were purchased from Sigma Chemical (St. Louis, MO).

Contraction and intracellular calcium measurements. We cut the MCAs into rings 2 mm in length and mounted them on two tungsten wires (0.13-mm diameter; A-M Systems, Carlsborg, WA). We attached one wire to an isometric force transducer (Kent Scientific, Litchfield, CT) and the other to a post attached to a micrometer used to vary resting tension in a 5-ml tissue bath mounted on Jasco CAF-110 intracellular Ca^{2+} analyzer (Jasco, Easton, MD) and measured vascular tension, as previously described (25, 26). This, with measurements of vessel inside diameter, wall thickness, length, and potassium-induced force, enabled calculation of force-per-unit cross-sectional area, as previously described (33). MCA rings were equilibrated under 0.3 g tension at 25°C for 40 min before loading with the acetoxymethyl ester of fura 2 (fura 2-AM; Molecular Probes, Eugene, OR), a fluorescent Ca^{2+} indicator that is a measure of mean cytoplasmic [Ca^{2+}], (14). Fura 2 fluorescence and force were measured simultane-
ously at 38°C, as previously described (26). As we have noted, although some investigators may prefer the transformation of fluorescence to [Ca^{2+}], in tissues such as cerebral arteries, the presentation of the ratio is less ambiguous (26).

During all contractility experiments, we continuously digi-
tized, normalized, and recorded contractile tensions and the fluorescence ratio (F_{340/380}) using an online computer. For all vessels, we evaluated the contractile response for tension and fluorescence ratio by measuring the maximum peak height and expressing it as percent K_{max} (a measure of “efficacy”) and calculated pD_{2} (the negative logarithm of the mean effective concentration at half-maximal response (EC_{50}) for NE and an index of tissue “sensitivity” or “potency”) (26). In the presence of fura 2, neither K^- nor NE-induced tensions were significantly different from those contractions in the absence of the dye (25).

Relative roles of the several K^-channels and their change with development. Because little is known of the role of the several types of K^-channels in modulating NE-induced changes in [Ca^{2+}], and vascular tension in the cerebral arteries and, particularly, in the fetus, we quantified these variables in the presence of selective K^-channel activators or blockers. For all studies, after initial K^- (120 mM) depolar-
ization) and thereby open Ca^{2+} efflux, hyperpolarize the vessel, and cause relaxation. From these data, we plotted the percent inhibition (e.g., %decrease of tension and [Ca^{2+}]), as a function of agonist dose. Then, in a related study, we gave 10^{-5} M pinacidil and, after 15 min, performed an NE dose-response study (10^{-9} to 10^{-4} M). From these latter data, we plotted the shift in the NE dose-response curve. To examine the effect of K_{ATP}-channel inhibition, we quantified NE-induced change in [Ca^{2+}], and tension after the K_{ATP}-channel blocker gliben-
clamide (3 × 10^{-5} M). In a related study, we first gave 3 × 10^{-7} M NE to achieve a 30% maximum response, then administered glibenclamide in increasing doses (10^{-7} to 3 × 10^{-5} M) to increase tension and [Ca^{2+}]. In addition, to examine the effect of glibenclamide alone, we administered 10^{-7} to 3 × 10^{-5} M of that compound.

To examine the role of activation of K_{ATP} on [Ca^{2+}], and tension, we first stimulated the vessel with 10^{-5} M NE. Then, on the plateau of the response curve, we added increasing concentrations of pinacidil (10^{-7} to 10^{-4} M) to stimulate K^- efflux, hyperpolarize the vessel, and cause relaxation. From these data, we plotted the percent inhibition (e.g., %decrease of tension and [Ca^{2+}]), as a function of agonist dose. Then, in a related study, we first stimulated the vessel with 10^{-5} M NE to achieve a 30% maximum response, then administered glibenclamide in increasing doses (10^{-7} to 3 × 10^{-5} M) to increase tension and [Ca^{2+}]. In addition, to examine the effect of glibenclamide alone, we administered 10^{-7} to 3 × 10^{-5} M of that compound.

To examine the role of activation of K_{Ca} on NE-induced [Ca^{2+}], and vascular tension, we quantified these after administration of 10^{-5} M NE. Then, on the plateau of the response, we administered increasing doses of the K_{Ca}-channel opener NS 1619 (10^{-9} to 10^{-6} M) to stimulate K^- efflux. In a related study, we first gave 3 × 10^{-7} M NE to achieve a 30% maximum response and then gave increasing doses of iberiotoxin (10^{-8} to 10^{-6} M). In addition, to examine the effect of iberiotoxin alone, we administered 10^{-9} to 10^{-6} M of that compound.

To examine the potential role of inhibition of K_{Ca} on Ca^{2+}-channel activity in fetal and adult cerebral arteries, we measured NE-induced [Ca^{2+}], and tension after administration of 4-aminoopyridine (4-AP; 10^{-4} M). Alternatively, we administered 3 × 10^{-7} M NE and then gave increasing doses of 4-AP (10^{-5} to 10^{-3} M). Finally, to determine the possible role of KIR in modulating Ca^{2+}-channel activity, we measured NE-induced [Ca^{2+}], and tension after addition of KCl (1.5 × 10^{-2} M) to activate the channel or barium chloride (10^{-5} M) to block it. In addition, we administered 3 × 10^{-7} M
Table 1. pIC_{50} values of K^+ -channel activators

<table>
<thead>
<tr>
<th></th>
<th>Fetus</th>
<th>Adult</th>
<th>(Ca^{2+})_i</th>
<th>[Ca^{2+}]_i</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinacidil (n = 4 each)</td>
<td>5.0 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>4.6 ± 0.1*</td>
<td></td>
</tr>
<tr>
<td>NS 1619 (n = 4 each)</td>
<td>8.2 ± 0.1*</td>
<td>7.6 ± 0.1</td>
<td>7.3 ± 0.1*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. pIC_{50}, negative logarithm of 50% inhibitory concentration. *P < 0.01 different from adult. [Ca^{2+}]_i, intracellular Ca^{2+} concentration.

Table 2. Peak responses of vascular tension and fluorescence ratio

<table>
<thead>
<tr>
<th></th>
<th>Adult</th>
<th>Fetus</th>
<th>(F340/380)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_{max} (122 mM)</td>
<td>1.81 ± 0.15 (12)</td>
<td>1.70 ± 0.14 (12)</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>Control 10^{-5} M NE</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.10 (12)</td>
</tr>
<tr>
<td>10^{-5} M NE</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>10^{-5} M Pin + 10^{-9} - 10^{-4} M NE</td>
<td>116 ± 14% (4)</td>
<td>103 ± 12%</td>
<td>92 ± 10%</td>
</tr>
<tr>
<td>Gli</td>
<td>68 ± 8%* (3)</td>
<td>4 ± 2%*† (3)</td>
<td>(3)</td>
</tr>
<tr>
<td>3 × 10^{-7} M Gli + 10^{-9} - 10^{-4} M NE</td>
<td>70 ± 8%* (3)</td>
<td>42 ± 7%*</td>
<td>42 ± 13%</td>
</tr>
<tr>
<td>10^{-7} M NS 1619 + 10^{-9} - 10^{-4} M NE</td>
<td>98 ± 6% (3)</td>
<td>98 ± 7%</td>
<td>98 ± 12%</td>
</tr>
<tr>
<td>Ib/Tx</td>
<td>95 ± 10% (3)</td>
<td>94 ± 13%</td>
<td>99 ± 14%</td>
</tr>
<tr>
<td>10^{-7} M Ib/Tx + 10^{-9} - 10^{-4} M NE</td>
<td>95 ± 4% (3)</td>
<td>90 ± 8%</td>
<td>99 ± 6% (3)</td>
</tr>
<tr>
<td>10^{-4} M 4-AP + 10^{-9} - 10^{-4} M NE</td>
<td>78 ± 5%* (2)</td>
<td>118 ± 9%</td>
<td>89 ± 8% (2)</td>
</tr>
<tr>
<td>10^{-5} M BaCl_2 + 10^{-9} - 10^{-4} M NE</td>
<td>91 ± 4% (2)</td>
<td>67 ± 8%*</td>
<td>56 ± 7% (2)</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in absolute terms as %maximal tension achieved to 120 mM K^+ (K_{max}; see METHODS for details) or as %norepinephrine (NE) maximum. Δ. Change in maximum tension and fluorescence ratio (F_{340/380}) from baseline; Pin, pinacidil; 4-AP, 4-aminopyridine; Gli, glibenclamide; Ib/Tx, iberiotoxin. Nos. in parentheses, no. of individual experiments for each protocol for adult and fetus. *P < 0.05, †P < 0.01 compared with control 10^{-5} M NE. See text for exact drug doses and timing.

RESULTS

Role of K ATP in vascular [Ca^{2+}]_i, and tension. To examine the role of ATP-sensitive K^+ -channel activation on tension and fluorescence ratio (R_{340/380}, an index of [Ca^{2+}]_i) in adult MCA, we first contracted the vessel with 10^{-5} M NE, then, on the plateau of the response, administered pinacidil in increasing half-log increments (10^{-7} to 10^{-4} M). As seen in Fig. 1A, at concentrations >10^{-6} M, pinacidil inhibited tension with a half-maximal inhibitory concentration (pIC_{50}) value of 5.1 ± 0.1. In contrast, fluorescence ratio showed essentially no change (Fig. 1B; n = 4). As also shown in Fig. 1, in contrast to the lack of pinacidil-induced decrease in [Ca^{2+}]_i, in the adult, the fetal MCA responded to increasing pinacidil concentrations with a decrease of both tension (Fig. 1A) and [Ca^{2+}]_i (Fig. 1B), with pIC_{50} values of 5.0 ± 0.1 and 4.6 ± 0.1, respectively (Table 1).

To determine further the role of K ATP channels in adult MCA NE-induced [Ca^{2+}]_i, and contraction responses, we quantified vascular tension and fluorescence ratio in response to increasing doses of NE (10^{-9} to 10^{-4} M). As shown in Fig. 2, in response to increasing NE dose, adult MCA showed typical increases in vascular tension (Fig. 2A) and [Ca^{2+}]_i (Fig. 2B). The mean maximal NE-induced tension and [Ca^{2+}]_i were 1.7 ± 0.1 g and 0.15 ± 0.01 units, respectively, values that were close to 100% K_{max} (n = 12). In other experiments, we first administered 10^{-5} M pinacidil, then, after 15 min, repeated the NE concentration response. As shown in Fig. 2A, after K ATP-channel activation by pinacidil, the NE-induced contractile response was attenuated to 1.0 ± 0.2 g (P < 0.05), whereas the fluorescence ratio was unchanged (Fig. 2B). There was no significant change in pD_{2} (n = 3).

For fetal MCA, Fig. 2 shows the NE dose-response increases in tension and [Ca^{2+}]_i, under control conditions. The mean maximal NE-induced tension and [Ca^{2+}]_i were 1.4 ± 0.1 g (Fig. 2C) and 0.14 ± 0.02 units (Fig. 2D), respectively (n = 12). When 10^{-5} M pinacidil was given followed, after 15 min, by NE in increasing half-log doses, neither the maximal responses of tension and [Ca^{2+}]_i, nor their pD_{2} values were significantly different from control values (n = 3; see Table 2 for values).

To examine to what extent K ATP-channel inhibition resulted in further contraction of adult and fetal cerebral arteries, we first contracted the vessel to ~30% maximum with 3 × 10^{-7} M NE and then administered glibenclamide in half-log doses (10^{-7} to 3 × 10^{-5} M). In neither adult nor fetal MCA did such glibenclamide treatment show a significant effect on either NE-induced tension or [Ca^{2+}]_i (n = 3 each; data not shown).

To examine the effect of K ATP-channel inhibition on NE-induced responses, we administered 3 × 10^{-7} M glibenclamide and then, after 15 min, determined the NE concentration-response curves. As seen in Fig. 3,
for adult MCA, glibenclamide had a modest effect in lowering maximum tension (Fig. 3A) and fluorescence ratio (Fig. 3B) 20–30%. Nonetheless, pD2 values were not significantly different from control. In a similar manner, in fetal MCA, 3 × 10−7 M glibenclamide slightly attenuated the NE-induced response with slight decreases in tension and [Ca2+]i (Fig. 3C and D). Again, pD2 values were not significantly different from NE controls. When given alone in half-log doses (10−9 to 10−6 M) to either adult or fetal MCA, glibenclamide showed no effect on tension or [Ca2+]i (n = 3 each; data not shown).

Role of Kca in vascular [Ca2+]i and tension. Figure 4 shows the effect of the Kca-channel opener NS 1619 (10−9 to 10−6 M) on adult and fetal MCA precontracted with 10−5 M NE. In the adult, at NS 1619 concentrations >3 × 10−8 M, vascular tension was modestly decreased with ~40% inhibition at 10−6 M (pIC50 = 7.6 ± 0.1; Fig. 4A). In contrast, NS 1619 did not affect the fluorescence ratio (Fig. 4B). Compared with the adult, Fig. 4 shows the profound effect of Kca-channel activation in decreasing fetal MCA tension (essentially 100% inhibition at 10−7 M NS 1619; pIC50 = 8.2 ± 0.1; Fig. 4A) and, to a lesser extent, fluorescence ratio (pIC50 = 7.3 ± 0.1; Fig. 4B; Table 1). In addition, because NS 1619 may also block Ca2+ channels, in both adult and fetal cerebral artery, we administered 10−7 M NS 1619, and, after 15 min, depolarized the cells with 120 mM K+. Under these conditions, the K+–induced increases in tension and fluorescence ratio were the same as control. Finally, we showed that in the presence of zero extracellular Ca2+ or after administration of 10−5 M nifedipine (L-type Ca2+ channel blocker), K+ failed to increase either tension or fluorescence ratio (data not shown).

To evaluate further Kca-channel activation in adult MCA, we administered 10−7 M NS 1619 and then, after 15 min, examined the NE dose response. As shown in Fig. 5, under these conditions, NE-induced tension (Fig. 5A) and fluorescence ratio (Fig. 5B) were minimally affected. Figure 5 also shows the fetal NE dose response after administration of 10−7 M NS 1619, with a 10% decrease in vascular tension (Fig. 5C) and a minimal decrease in fluorescence ratio (Fig. 5D). Again, however, there was no significant change in pD2.

To examine the effect of Kca-channel inhibition (and presumably increased Ca2+ flux through L-type Ca2+ channels), we administered iberiotoxin in half-log doses (10−8 to 10−6 M). As seen in Fig. 6, in adult MCA at 10−7 M iberiotoxin, both tension (Fig. 6A) and [Ca2+]i increased significantly (Fig. 6B; n = 3). In a similar manner, in the fetal artery, at 10−7 M iberiotoxin and above, tension (Fig. 6C) and [Ca2+]i (Fig. 6D) increased significantly. In another study, to determine to what extent this increase in tension and [Ca2+]i, were in fact due to increased Ca2+ flux through the L-type Ca2+ channels, we first administered nifedipine (10−5 M) and then, after 15 min, determined the iberiotoxin dose-response relationship (n = 3). Under these circumstances, neither adult nor fetal MCA showed a significant increase in either tension or [Ca2+]i.

Figure 7 shows further studies on the effect of inhibition of Kca channels on tension and fluorescence ratio in adult and fetal MCA. For the adult vessel, when the NE dose response was performed 15 min after administration of 10−7 M iberiotoxin, neither tension (Fig. 7A) nor fluorescence ratio (Fig. 7B) were significantly altered. pD2 values were the same as control (n = 3 each). Similarly, in fetal MCA, as shown in Fig. 7, C and D, for tension and fluorescence ratio, respectively, Kca-channel inhibition by 10−7 M iberiotoxin also had no significant effect (n = 3 each).

Role of K in [Ca2+]i and tension. We also examined the role of inhibition of K, on the tension and fluorescence ratio of adult MCA, measuring these variables after the administration of 10−4 M 4-AP followed by NE dose response. In the adult MCA, 4-AP was associated with a modest 10% decrease in tension with no significant change in fluorescence ratio. For fetal MCA, administration of 10−4 M 4-AP also had a negligible

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Fig. 1. A: % inhibition of norepinephrine (NE)-induced tension in adult (●) and fetal (▲) main branch middle cerebral artery (MCA) in response to the ATP-sensitive K+ (KATP)-channel activator pinacidil (n = 4 each). Vessels were first contracted with 10−6 M NE, then at the plateau of contraction, the channel agonist was given in half-log increments (see METHODS for details). For vascular tension, half-maximal inhibitory concentration (pIC50) values for adult and fetal MCA were 5.1 ± 0.1 and 5.0 ± 0.1, respectively. Points shown are means and SE of measurements. B: % inhibition by pinacidil of NE-induced fluorescence ratio (a measure of intracellular Ca2+ concentration) response in adult (●) and fetal (▲) MCA. For the fetal vessel, the pIC50 value was 4.6 ± 0.1, whereas that for the adult was indeterminable; P < 0.01.
effect on the NE dose response of tension and fluorescence ratio, with no change in pD2 values (n = 3 each; data not shown).

Role of Kir in [Ca2+]i and tension. To explore the possible role of Kir in modulating Ca2+-channel activity, we administered 10^{-5} M barium chloride to inhibit these channels, after which we performed an NE dose response. As seen in Fig. 8, in adult MCA, vascular tension (Fig. 8A) was not significantly altered. Fluorescence ratio (Fig. 8B) modestly increased, the pD2 values being 5.2 ± 0.2 and 4.9 ± 0.2, respectively (P < 0.05). In contrast, for fetal MCA, Fig. 8, C and D,
nescence ratio response in adult (\(F\)) of measurements. B: the agonist was given in half-log increments (see METHODS for details). For vascular tension, pIC\(_{50}\) values for adult and fetal MCA were

\[ \text{pIC}_{50} \]

with 10\(^{-2}\) M NE, then at the plateau of contraction, the channel agonist was given in half-log increments (see METHODS for details). For vascular tension, pIC\(_{50}\) values for adult and fetal MCA were 7.6 ± 0.1 and 8.2 ± 0.1, respectively. Points shown are means and SE of measurements. 

A: %inhibition of NE-induced tension in adult (○) and fetal (●) main branch MCA in response to the \(Ca^{2+}\)-activated K\(^+\) (K\(_{Ca}\))-channel activator NS 1619 (\(n = 4\) each). Vessels were first contracted with 10\(^{-5}\) M NE, then at the plateau of contraction, the channel agonist was given in half-log increments (see METHODS for details). For vascular tension, pIC\(_{50}\) values for adult and fetal MCA were 7.6 ± 0.1 and 8.2 ± 0.1, respectively. Points shown are means and SE of measurements. 

shows the profound effect of K\(_{IR}\) inhibition by 10\(^{-5}\) M BaCl\(_2\) both on tension and fluorescence ratio. The pD\(_2\) values were not significantly altered.

**DISCUSSION**

The present studies offer several important observations. First, activation of plasma membrane K\(_{ATP}\) channels by pinacidil, with presumed closure of L-type \(Ca^{2+}\) channels, resulted in significant inhibition (e.g., decrease) of NE-induced tension both in fetal and adult MCA (Fig. 1). In the fetal vessel, this occurred concurrently with a significant decrease in \([Ca^{2+}]_i\); however, \([Ca^{2+}]_i\) did not decrease significantly in the adult. This illustrates the greater dependence of fetal cerebral arteries on extracellular \(Ca^{2+}\) for maintenance of tone. In addition, it is an example of the much greater \(Ca^{2+}\) sensitivity (e.g., tension as a function of \([Ca^{2+}]_i\)) of the fetal cerebral artery compared with the adult. Second, in a somewhat similar manner, NS 1619 activation of K\(_{Ca}\) channels (again with presumed closure of L-type \(Ca^{2+}\) channels) resulted in a significant inhibition of tension in the fetal vessel with only a moderate inhibition in that of the adult (Fig. 4). This occurred with only slight decrease in \([Ca^{2+}]_i\) in the fetal vessel but no significant decrease in the adult. Again, this illustrates the extracellular \(Ca^{2+}\) requirement of the fetal cerebral artery and its great \(Ca^{2+}\) sensitivity. Third, in contrast to these findings, K\(_{ATP}\)-channel inhibition by glibenclamide (which inhibits the K\(_{ATP}\) channel and thereby activates or opens L-type \(Ca^{2+}\) channels) had essentially no effect on either vascular tension or \([Ca^{2+}]_i\). Nonetheless, K\(_{Ca}\)-channel inhibition by iberiotoxin resulted in a significant increase in both tension and \([Ca^{2+}]_i\) (Fig. 6), a response that was eliminated by the L-type \(Ca^{2+}\)-channel blocker nifedipine (10\(^{-5}\) M). In addition, whereas glibenclamide modestly attenuated NE dose response (Fig. 3), iberiotoxin failed to do so (Fig. 7). Fourth, inhibition of K\(_{V}\) channels also had little effect on NE-induced contraction. Fifth, BaCl\(_2\) inhibition of K\(_{IR}\) channels demonstrated only a small effect on \([Ca^{2+}]_i\), in adult MCA. In contrast, in the fetal artery, BaCl\(_2\) significantly inhibited both tension and \([Ca^{2+}]_i\), presumably as a result of blockade of L-type \(Ca^{2+}\) channels (Fig. 8).

**Role of K\(^+\) channels in cerebral artery smooth muscle.**

Smaller arteries, including those of the cerebral vasculature, exist in a partially contracted state and can constrict further or dilate depending on the tissue requirements for blood and/or oxygen. This basal tone is an important determinant of vascular resistance and blood pressure and, to a great extent, is regulated by the smooth muscle cell membrane potential, which, in turn, is regulated by the plasma membrane K\(^+\) channels (30). Increasing the open probability of K\(^+\) channels increases K\(^+\) efflux, resulting in membrane hyperpolarization as K\(^+\) approaches its equilibrium potential. This results in decreased open probability of L-type \(Ca^{2+}\) channels, decreasing \(Ca^{2+}\) influx, and vasodilatation. Conversely, inhibition of K\(^+\) channels results in membrane depolarization and vasoconstriction. As noted earlier, four distinct types of K\(^+\) channels have been identified, each of which serves a unique function in the regulation of smooth muscle membrane potential. Although these channel types are fairly well defined structurally and relatively selective pharmacological blockers exist for each, selective activators have been described for only the K\(_{ATP}\) and K\(_{Ca}\) channels (10).

**Role of K\(_{ATP}\) channels.** These channels respond to changes in cellular metabolic state, opening in response to a decrease in intracellular ATP concentration. Activation by pinacidil or lemakalim results in hyperpolarization and vasorelaxation, whereas inhibition by sulfonylurea drugs such as glibenclamide may result in vasoconstriction (5) and may disrupt cerebral autoregulation (17). Several endogenous substances produce hyperpolarization and relaxation of cerebral arteries that may be mediated by activation of K\(_{ATP}\) channels (10). For instance, the vasodilators adenosine and calcitonin gene-related peptide increased glibenclamide-sensitive currents, whereas vasoconstrictors serotonin and histamine inhibited pinacidil-induced potassium currents (20). Nonetheless, the overall reg-
Fig. 5. NE dose-response relationships for adult and fetal MCA in presence or absence of K_{Ca}-channel activator NS 1619. A: vascular tension (g) for adult MCA in response to NE under control conditions (•, solid line). Also shown is the NE dose response after administration of 10^{-7} M NS 1619 (n = 3; ▲, dashed line). Points shown are means and SE. B: fluorescence ratio for adult MCA in response to NE under control conditions and after administration of NS 1619. All symbols are the same as in A. C: vascular tension (g) for fetal MCA under control conditions (•, solid line). Also shown is the NE dose response after administration of 10^{-7} M NS 1619 (n = 3; ▲, dashed line). D: fluorescence ratio for fetal MCA under control conditions and after administration of NS 1619. All symbols are the same as in A.

Fig. 6. Typical iberiotoxin (IbTx) dose-response relationships for adult and fetal main branch MCAs under control conditions. Arterial segments were first contracted with 120 mM K^+ to obtain peak tension. After washing and reequilibration to baseline tension, cumulative doses of IbTx were added in half-log increments (see METHODS for details). A: vascular tension (g) for adult MCA showing a significant increase in response to 10^{-7} M IbTx. B: fluorescence ratio for adult MCA in response to IbTx (n = 3). C: vascular tensions for fetal MCA, showing a significant increase after 10^{-7} M IbTx. D: fluorescence ratio for fetal MCA in response to IbTx (n = 3).
ulation of these channels by physiological mediators is poorly understood (30).

As noted in Fig. 1, a key observation of the present studies is the inhibition (e.g., decrease) of vascular tension both in adult and fetal cerebral arteries after pinacidil-induced activation of K<sub>ATP</sub> channels (Fig. 1A). In fetal MCA, the fluorescence ratio decreased pari passu with the decrease in tension, suggesting relatively great Ca<sup>2+</sup> sensitivity (e.g., dependence of tension on [Ca<sup>2+</sup>]<sub>i</sub>) in the fetal artery. In contrast, in the adult vessel, this decrease in tension was accompanied by no significant change in [Ca<sup>2+</sup>]<sub>i</sub> (Fig. 1B). In view of the significant effect of pinacidil in decreasing tension and in fetal MCA fluorescence ratio (Fig. 1), it is somewhat surprising that opening the K<sub>ATP</sub> channel, with relative closure of the L-type Ca<sup>2+</sup> channel, had less...
effect on inhibiting the NE dose response (Fig. 2). This was particularly the case in the fetal vessel that showed such a great response to pinacidil.

In 5- to 7-day-old lambs, Pearce and Elliott (32) reported that the K<sub>ATP</sub>-channel agonist lemakalim resulted in relaxation of 10<sup>-6</sup> M serotonin-induced contraction in second and fourth branch MCAs with pIC<sub>50</sub> values of 5.5 ± 0.3 and 5.2 ± 0.2, respectively. In the adult sheep, the values were 7.2 ± 0.4 and 6.6 ± 0.4 in these vessels, respectively. In neither newborn nor adult were the differences between second and fourth branch vessels significant. These somewhat different results from those of the present study might be anticipated as a consequence of different K<sub>ATP</sub>-channel agonists used (pinacidil vs. lemakalim), different contractile agent (NE vs. serotonin), different developmental age (fetus vs. newborn), or the different manner in which the studies were conducted (cumulative half-log dose on plateau of contraction vs. repeated contraction of vessel with administration of lemakalim at each contraction).

Also, inhibition of the K<sub>ATP</sub>-channel by glibenclamide itself (10<sup>−9</sup> to 10<sup>−6</sup> M; with presumed opening of L-type Ca<sup>2+</sup> channels) failed to increase tension or [Ca<sup>2+</sup>]<sub>i</sub> in either adult or fetal MCA. In regard to the responses seen in Fig. 3, one might argue that inhibition of the K<sub>ATP</sub>-channel with opening of the L-type Ca<sup>2+</sup> channel should result in no further augmentation of NE-induced opening of Ca<sup>2+</sup> channels (with increases in [Ca<sup>2+</sup>]<sub>i</sub> and tension). Nonetheless, the decrease in [Ca<sup>2+</sup>]<sub>i</sub> and tension seen at 3 × 10<sup>−6</sup> M NE and above may have been a nonspecific effect.

Role of K<sub>Ca</sub> channels. Large-conductance K<sup>+</sup> channels activated by membrane depolarization and an increase in [Ca<sup>2+</sup>]<sub>i</sub> are located in essentially all vascular and other smooth muscle cells (29). Activated by NS 1619 and inhibited by iberiotoxin, charybdotoxin, and related peptides (10), the role of physiological regulators in mediating K<sub>Ca</sub>-channel activity is largely unknown (30). In ovine pulmonary arteries, Reeve et al. (36) demonstrated a significant maturational change with development from fetus to adult, with a change from K<sub>Ca</sub> to K<sub>V</sub> channels regulating resting membrane potential. Although several studies have examined K<sub>Ca</sub>-channel activity in cerebral (4, 6, 8, 12, 16, 23) and pial (2, 3, 13) arteries, none of these examined the interrelations of tension and [Ca<sup>2+</sup>]<sub>i</sub> or changes with development.

As shown in Fig. 4A, another critical observation of the present study is that NS 1619 activation of the K<sub>Ca</sub>-channel profoundly inhibited vascular tension in fetal MCA but decreased tension to a lesser extent in the adult vessel. In the fetal vessel, this K<sub>Ca</sub>-channel activation was associated with a modest inhibition of fluorescence ratio (in contrast to the case for K<sub>ATP</sub>-channel activation). By comparison, in adult MCA, this was accompanied by no significant decrease in [Ca<sup>2+</sup>]<sub>i</sub> (as was also the case with K<sub>ATP</sub>-channel activation). Nonetheless, these results again suggest a greater dependence of tension on [Ca<sup>2+</sup>]<sub>i</sub> (e.g., sensitivity) of fetal cerebral arteries compared with those of the adult.

Again, it may be surprising that opening of K<sub>Ca</sub>-channels with resultant blockade of the Ca<sup>2+</sup>-channel did not show more effect on NE-induced contraction and increase in [Ca<sup>2+</sup>]<sub>i</sub> (Fig. 5). A related issue is to what extent NS 1619 blocks plasma membrane Ca<sup>2+</sup>-channels in addition to activating the K<sub>Ca</sub>-channel. Such an effect has been reported in rat cerebral arteries (16). As noted above, both in adult and fetal MCA, tension and fluorescence ratio depolarization responses to 120 mM K<sup>+</sup>, after administration of 10<sup>−7</sup> M NS 1619, were not significantly different from control. This suggests that, indeed, the decreased tension and fluorescence ratio in response to NS 1619 (Fig. 4) was the result of activation of the K<sub>Ca</sub>-channel rather than to block the L-type Ca<sup>2+</sup>-channel.

In addition, as shown in Fig. 6, iberiotoxin inhibition of the K<sub>Ca</sub>-channel (with opening of the L-type Ca<sup>2+</sup>-channel) resulted in significant increases in tension and [Ca<sup>2+</sup>]<sub>i</sub>, in cerebral arteries of both age groups. This is in marked contrast to the lack of such an effect by K<sub>ATP</sub>-channel blockade, suggesting that they exert their effect on Ca<sup>2+</sup>-channels by different mechanisms. Importantly, iberiotoxin-induced contraction was totally blocked by administration of the L-type Ca<sup>2+</sup>-channel blocker nifedipine (10<sup>−5</sup> M). Also, in view of iberiotoxin’s profound effect in increasing tension and fluorescence ratio, it is somewhat surprising that it had such a minimal effect on the NE dose-response relationship (Fig. 7). A related phenomenon of interest is the coupling of the K<sub>Ca</sub>-channel to the sarcoplasmic reticulum ryanodine receptor in cerebral arteries that we have recently reported (25) and that may be more common than is generally appreciated (18). In both fetal and adult MCA, administration of 10<sup>−7</sup> M iberiotoxin followed after 15 min by 10<sup>−5</sup> M ryanodine totally blocked the increase in tension and fluorescence ratio seen after ryanodine alone (25).

Role of K<sub>V</sub> channels. When the plasma membrane is depolarized, these channels (also called delayed rectifier channels) open to allow K<sup>+</sup> efflux and membrane repolarization (15); thus they play a key role in the regulation of membrane potential and vascular tone (21, 22). Although there exist several K<sub>V</sub>-channel subtypes, in vascular smooth muscle, these all are inhibited by 4-AP. Nonetheless, in the present study, 4-AP failed to demonstrate significant effects on either tension or [Ca<sup>2+</sup>]<sub>i</sub> when given alone or in concert with an NE dose-response curve.

Role of K<sub>IR</sub> channels. In contrast to K<sub>Ca</sub> and K<sub>V</sub> channels, which are activated by membrane depolarization, the K<sub>IR</sub>-channels (named for greater inward than outward K<sup>+</sup> movement when voltage clamped) are activated by membrane hyperpolarization. As such, they may play a role in maintaining resting membrane potential and K<sup>+</sup>-induced vasodilatation, although this regulation is poorly understood (10, 30). Several groups have examined the role of these channels in determining resting tone in cerebral arteries (9, 19), membrane potential (34), and in the metabolic regulation of cerebral blood flow in response to changes in K<sup>+</sup> concentration (24). We know of no studies on the
possible changing role of these channels with development. As noted above, the BaCl2 inhibition of tension and Ca2+ in the fetal MCA was probably secondary to Ba2+ blockade of L-type Ca2+ channels rather than to inhibition of KIR channels per se.

Perspectives

The role of the several plasma membrane K+ channels and their sensitivity to various agents may differ greatly as a function of vessel type, species, and developmental age. The marked dependence of fetal cerebral arteries on external [Ca2+] following activation of KATP or KCa channels fits with previous studies from our group (25, 26) and others on developmental differences in cerebral artery contractility. Fetal cerebral arteries develop less tone but have greater aminergic activity than those of adult (33); the newborn MCA requires more transmembrane calcium uptake than the adult (39); fetal arteries show greater calcium sensitivity (1, 26); and fetal arteries rely less on Ins(1,4,5)P3-mediated contractile mechanisms (27, 38). In addition, the presence of relatively small intracellular stores in immature vessels (7, 25, 37) further emphasizes the dependence of fetal cerebral arteries on extracellular Ca2+ compared with the adult.

By quantifying simultaneously [Ca2+],i and tension, the present studies are the first to demonstrate the role of the several K+ channels in the NE-induced contractile response of fetal and adult cerebral arteries. As we have shown previously, for their contraction, fetal cerebral arteries demonstrate considerable dependence on extracellular Ca2+ and Ca2+ flux via L-type Ca2+ channels (26). This dependence is associated with the fetal vessels being exquisitely sensitive to KATP and KCa-channel activation (with presumed blockade of L-type Ca2+ channels), compared with the adult. Overall, contractility of fetal cerebral arteries is highly dependent on Ca2+ flux through L-type Ca2+ channels, with a less important role for SR Ca2+ store-dependent mechanisms (25, 26). With maturation to the adult, SR Ca2+ store-dependent mechanisms increase in importance (25). On the basis of data of the present study, KATP and KCa channels each play an important role in the regulation of vascular tone in fetal and adult cerebral arteries. These roles appear to differ, however, as evidenced by their somewhat different responses to inhibition, e.g., KCa-channel blockade resulting in increased tension and [Ca2+],i, whereas KATP-channel blockade shows no such effect. Importantly, the KCa channel appears to be coupled to the SR ryanodine receptor (17, 25).

The obvious question arises as to when during the course of development from fetus to newborn to adult the role of plasma membrane K+ -channel activity and interactions with L-type Ca2+ channels alter and cerebral vessels become less dependent on extracellular [Ca2+]. Additional questions relate to the role of developmental changes in the density, affinity, and isoforms of the several K+ channels and their relative roles in maintaining membrane potential. These areas are the subject of current studies in the elucidation of the mechanisms of cerebral vascular reactivity and their change with development.

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