Ionic mechanisms mediating the myogenic response in newborn porcine cerebral arteries

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Ahmed, Abu, Christopher M. Waters, Charles W. Leffler, and Jonathan H. Jaggar. Ionic mechanisms mediating the myogenic response in newborn porcine cerebral arteries. *Am J Physiol Heart Circ Physiol* 287: H2061–H2069, 2004. First published July 29, 2004; doi:10.1152/ajpheart.00660.2004.—Mechanisms that underlie autoregulation in the newborn vasculature are unclear. Here we tested the hypothesis that in newborn porcine cerebral arteries intravascular pressure elevates wall tension, leading to an increase in intracellular calcium concentration ([Ca²⁺]) and a constriction that is opposed by pressure-induced K⁺ channel activation. Incremental step (20 mmHg) elevations in intravascular pressure between 10 and 90 mmHg induced an immediate transient elevation in arterial wall [Ca²⁺], and a short-lived constriction that was followed by a smaller steady-state [Ca²⁺] elevation and sustained constriction. Pressures between 10 and 90 mmHg increased steady-state arterial wall [Ca²⁺], between 12142 and 299 nM and myogenic (defined as passive-active) tension between 25 and 437 dyn/cm. The relationship between pressure and myogenic tension was strongly Ca²⁺ dependent until forced dilation. At low pressure, 60 mM K⁺ induced a steady-state elevation in arterial wall [Ca²⁺], and a constriction. Nimodipine, a voltage-dependent Ca²⁺ channel blocker, and removal of extracellular Ca²⁺ similarly dilated arteries at low or high pressures. 4-Aminopyridine, a voltage-dependent K⁺ channel blocker, induced significantly larger constrictions at high pressure, when compared with those at low pressure. Although selective Ca²⁺-activated K⁺ (KCa) channel blockers and intracellular Ca²⁺ release inhibitors induced only small constrictions at low and high pressures, a low concentration of caffeine (1 μM), a ryanodine-sensitive Ca²⁺ release (RyR) channel activator, increased KCa channel activity and induced dilation. These data suggest that in newborn cerebral arteries, intravascular pressure elevates wall tension, leading to voltage-dependent Ca²⁺ channel activation, an increase in wall [Ca²⁺], and Ca²⁺-dependent constriction. In addition, pressure strongly activates KCa channels that opposes constriction but only weakly activates KCa channels.

Wall tension; intravascular pressure; intracellular calcium; voltage-dependent potassium channel; calcium-activated potassium channel

It has been known for more than 100 years that stretch induces vasoconstriction in a reaction referred to as the “myogenic response” (5, for review, see Ref. 12). In small-resistance size arteries, the myogenic response functions primarily to maintain constant blood flow during changes in intravascular pressure and to provide a baseline diameter that can be subsequently modulated by vasoconstrictors or vasodilators (12).

Mechanisms that mediate the myogenic response have been studied primarily in adult arterial preparations. In small adult arteries, an elevation in intravascular pressure leads to membrane depolarization and the activation of smooth muscle cell voltage-dependent Ca²⁺ channels (12, 19, 30). The resulting elevation in intracellular Ca²⁺ concentration ([Ca²⁺]) leads to contraction due to the activation of Ca²⁺/calmodulin-dependent myosin light chain kinase (12, 22). Constriction in response to pressure occurs in the absence of an intact endothelium or neural system, indicating that the myogenic response is inherent to smooth muscle cells (12). Myogenic constriction may also occur due to an increase in the Ca²⁺ sensitivity of the contractile apparatus, particularly at high intravascular pressures (12, 13, 40, 54).

In adults, intravascular pressure activates Ca²⁺-activated K⁺ (KCa) channels and voltage-dependent K⁺ (Kv) channels, inducing negative-feedback pathways that limit depolarization and constriction (8, 12, 29). In arterial smooth muscle cells, KCa channel activity is regulated by localized [Ca²⁺], transients, termed “Ca²⁺ sparks,” that occur due to Ca²⁺ release by several ryanodine-sensitive Ca²⁺ release (RyR) channels on the sarcoplasmic reticulum (SR) (26). In pressurized adult cerebral arteries, Ca²⁺ spark and KCa channel blockers constrain similarly, indicating that Ca²⁺ sparks and KCa channels signal as a functional negative-feedback unit (27, 31).

In contrast to our knowledge of mechanisms that underlie and modulate the myogenic response in adults, little is known of these mechanisms in newborns. Understanding mechanisms that mediate the myogenic response in newborns is important. Disorders of the newborn cerebral circulation, in particular, are a predominant cause of mortality and can result in life-long disabilities in survivors. Recent studies have indicated that the newborn and adult vasculature show important physiological differences, including those relating to resting membrane potential (14), voltage-dependent Ca²⁺ channels (7), intracellular Ca²⁺ dynamics (35), localized intracellular Ca²⁺ release events (17), and cGMP sensitivity (37). A functional myogenic response is known to be present at the newborn stage of development and appears to involve the activation of voltage-dependent Ca²⁺ channels (17). Similarly, in newborn rodent cerebral arteries, a step increase in intravascular pressure elevates [Ca²⁺], and induces constriction (16, 17, 34). Nisoldipine, an L-type Ca²⁺ channel blocker, reduces arterial wall [Ca²⁺], and dilates pressurized newborn rat cerebral arteries (17), suggesting that the pressure-induced [Ca²⁺], elevation occurs due to voltage-dependent Ca²⁺ channel activation. Negative-feedback mechanisms that limit constriction also appear to be present in the newborn, although modified when compared with the adult. Constrictions induced by 4-aminopyridine (4-AP), a voltage-dependent K⁺ channel blocker, are attenuated in the newborn, when compared with adult, ovine cerebral artery rings (53). Similarly, Ca²⁺ spark and KCa channel blockers do not alter the diameter of newborn porcine pial arterioles in vivo or in isolated pressurized newborn rat...
cerebral arteries (17, 24, 32). Although studies have examined responses to pressure in newborn arteries, the pressure and Ca\(^{2+}\) dependence of myogenic constriction in the newborn is unclear. Similarly, in newborns the pressure dependence of negative-feedback mechanisms mediated via K\(^{+}\) channel activation are also relatively uncharacterized.

The goal of this study was to test the hypothesis that in newborn porcine cerebral arteries intravascular pressure elevates wall tension, leading to an increase in [Ca\(^{2+}\)], and a constriction that is opposed by pressure-induced K\(^{+}\) channel activation. Data demonstrate that intravascular pressure between 10 and 90 mmHg elevates arterial wall [Ca\(^{2+}\)], and constricts newborn cerebral arteries via a dihydropyridine-sensitive and strongly Ca\(^{2+}\)-dependent mechanism. In addition, pressure activates K\(_c\) channels that oppose constriction but only weakly activates K\(_ca\) channels. Nevertheless, at low or high pressures, opening RyR channels activates K\(_ca\) channels, leading to vasodilation.

**MATERIALS AND METHODS**

**Tissue preparation.** The animal protocols used were reviewed and approved by the Animal Care and Use Committee of the University of Tennessee Health Science Center. Newborn pigs (1–5 days old) were purchased by the Department of Comparative Medicine at the University of Tennessee from a commercial breeder in the Memphis, Tennessee area. Piglets of either sex were anesthetized with ketamine hydrochloride (33 mg/kg im) and acepromazine (3.3 mg/kg im). The brain was removed and placed into cold (4°C) physiological saline solution (PSS) of the following composition (in mM): 132 NaCl, 3 KCl, 24.6 NaHCO\(_3\), 1.8 CaCl\(_2\), 1.5 MgCl\(_2\), and 10 glucose, which was bubbled with 21% O\(_2\)-5% CO\(_2\)-74% N\(_2\), to pH 7.4. Arteriolar branches of the middle cerebral artery (100–200 \(\mu\)m in diameter) were dissected from the brain and cleaned of basolateral connective tissue.

**Pressurized artery diameter and wall tension measurements.** An arterial segment 1–2 mm in length was cannulated at each end in a temperature-controlled perfusion chamber (Living Systems Instrumentation: Burlington, VT). The chamber was continuously perfused with 3–6 ml/min of PSS equilibrated with a mixture of 21% O\(_2\)-5% CO\(_2\)-74% N\(_2\), to pH 7.4, and maintained at 37°C. Arteries were observed with a charge-coupled device (CCD) camera attached to an inverted microscope (Nikon TS 100-F). External arterial wall diameter was measured by using the automatic edge-detection function of IonWizard software (Ionoptix, Milton, MA) and digitized at 1 Hz using a personal computer. Steady-state changes in intravascular pressure were achieved by elevating an attached reservoir and monitored using a pressure transducer (Living Systems Instrumentation). Intravascular pressure was increased at a rate of ~2 mmHg/s. No luminal flow was present during the experiments. Tested compounds were applied via chamber perfusion. A 60 mM K\(^{+}\)-free PSS solution was formulated by omitting 57 mM Na\(^{+}\) from the PSS and supplementing with 57 mM K\(^{+}\).

With the assumption that wall thickness was much less than vessel diameter, we can estimate the wall tension according to the Laplace equation:

\[
T = \Delta P \times r
\]

where \(T\) is wall tension (in dyn/cm), \(\Delta P\) is the transmural pressure (in dyn/cm\(^2\)), and \(r\) is the arterial radius (in cm). Throughout the range of intravascular pressures that were applied, passive wall thickness was <3.3% of arterial diameter (see RESULTS). Thus wall tension was approximated based on the outer radius, which was monitored continuously during the experiment.

**Simultaneous arterial wall intracellular [Ca\(^{2+}\)], and diameter measurements.** Before cannulation, artery segments were incubated in PSS containing fura-2 AM (3 \(\mu\)M) and pluronic F-127 (0.06%) for 45 min at room temperature. After being washed with PSS, arteries were cannulated and allowed to deesterify fura-2 AM for a further 15 min before experimentation. Fura-2 was alternately excited at 340 or 380 nm using a PC-driven hyperswitch (Ionoptix). Arteries were also illuminated with 665-nm incident light to obtain images of the arterial wall using a CCD camera (Ionoptix). A 585LP dichroic mirror split fura-2 emission to a photomultiplier tube (PMT) and incident red light to the CCD camera (Ionoptix). Arterial diameter was measured as described above using IonWizard edge-detection software. Background-corrected fura-2 ratios were collected every 1 s at 510 nm using the PMT. Arterial wall [Ca\(^{2+}\)], concentrations were calculated using the following equation (18):

\[
[Ca^{2+}] = \frac{K_D}{(R - R_{min}) (S_2)} = \frac{R_{max} - R}{R_{max} - R_{min}}
\]

where \(R\) is the 340/380 nm ratio, \(R_{min}\) and \(R_{max}\) are the minimum and maximum ratios determined in Ca\(^{2+}\)-free and saturating Ca\(^{2+}\) solutions, respectively, \(S_2/S_0\) is the ratio of Ca\(^{2+}\) free to Ca\(^{2+}\) replete emissions at 380 nm excitation, and \(K_D\) is the dissociation constant for fura-2 (282 nM, previously estimated by others in rat cerebral arteries, see Ref. 30). \(R_{min}\), \(R_{max}\), \(S_2\), and \(S_0\) were determined at the end of experiments and in separate experiments by increasing Ca\(^{2+}\) permeability with ionomycin (10 \(\mu\)M) and perfusing cells with high Ca\(^{2+}\) (10 mM) or Ca\(^{2+}\)-free (no added Ca\(^{2+}\), 10 mM EGTA) PSS (18).

**Arterial wall thickness.** After cannulation, arteries were pressurized to either 10 or 90 mmHg in Ca\(^{2+}\)-free PSS. Arteries were fixed in the chamber by perfusion with Ca\(^{2+}\)-free PSS containing 2.5% glutaraldehyde for 30 min. Arteries were embedded in Spurr’s resin after routine fixation, dehydration, and infiltration. Transverse sections (1 \(\mu\)m thick) were cut using an Reichert-Jung ultracut microtome E and mounted on glass slides. Arterial sections were stained with 0.1% toluidine blue in 1% boric acid. Arterial wall thickness was measured using a Sanyo CCD camera attached to a Nikon TS100 and Ionwizard edge-detection software (Ionoptix).

**Statistical analysis.** Values are presented as means ± SE. Student’s \(t\)-tests were used for comparing paired or unpaired data, and ANOVA and the Student-Newman-Keuls test were used for comparing multiple data sets. \(P < 0.05\) was considered significantly different.

**Chemicals.** Unless otherwise stated, all chemicals were purchased from Sigma Chemical (St. Louis, MO). Fura-2 and pluronic F-127 were purchased from Molecular Probes (Eugene, OR). Iberitoxin was purchased from Alomone Labs (Jerusalem, Israel).

**RESULTS**

**Intravascular pressure elevates arterial wall [Ca\(^{2+}\)], and constricts newborn cerebral arteries.** At rest, 60–80 mmHg is a typical mean arterial pressure for a newborn pig (9), resulting in an first-order pial arteriolar pressure of ~30–35 mmHg. Conceivably, under certain physiological or pathological circumstances, cerebral arteries in the newborn could experience pressures between one-third and three times those observed at rest. Therefore, in the present study intravascular pressure regulation of newborn cerebral artery wall [Ca\(^{2+}\)], and diameter was studied by raising the pressure between 10 and 90 mmHg in 20-mmHg step increments.

Between 10 and 90 mmHg, a step elevation in intravascular pressure initially induced an [Ca\(^{2+}\)] transient that subsequently declined to a lower sustained [Ca\(^{2+}\)] elevation (Figs. 1 and 2). Pressure steps also induced an initial transient constriction that subsequently declined to a smaller steady-state constriction (Figs. 1 and 2). During both the transient and
sustained responses to an elevation in pressure, arterial wall [Ca\(^{2+}\)], and diameter were inversely correlated (Figs. 1 and 2). The amplitude of pressure-induced [Ca\(^{2+}\)] transients and transient constrictions decreased as steady-state pressure increased (Figs. 1 and 2). For example, a pressure elevation from 10 to 30 mmHg transiently increased mean arterial wall [Ca\(^{2+}\)] by \(61 \text{ nM}\) and constricted by \(20\%\) of passive diameter, when compared with the steady-state level achieved at 30 mmHg. However, elevating pressure from 50 to 70 mmHg transiently increased mean [Ca\(^{2+}\)], by \(18 \text{ nM}\) and constricted by \(4\%\) of passive diameter, when compared with sustained responses at 50 mmHg. Intravascular pressures between 10 and 90 mmHg elevated steady-state mean arterial wall [Ca\(^{2+}\)], between \(-142\) and \(299 \text{ nM}\) and induced sustained constrictions between \(-80\) and \(68\%\) of passive arterial diameter (Fig. 2, A and B).

In one of six arteries, forced dilation occurred \(-5\) min after an elevation in pressure to 70 mmHg, and in two arteries forced dilation occurred at 90 mmHg. Figure 1 illustrates an artery in which forced dilation occurred \(-5\) min after an increase in pressure to 90 mmHg. In these three arteries, forced dilation resulted in a rapid increase in mean arterial wall [Ca\(^{2+}\)], to \(-413 \pm 76 \text{ nM}\) and an attenuated myogenic constriction that was \(94 \pm 0.9\%\) of passive diameter (values provided at 90 mmHg).

In the absence of extracellular Ca\(^{2+}\), an elevation in intravascular pressure did not induce a transient or steady-state
increase in arterial wall [Ca$^{2+}$], (Fig. 2A). These data suggest that in newborn pig cerebral arteries intravascular pressure activates Ca$^{2+}$ influx, leading to an elevation in arterial wall [Ca$^{2+}$]$_i$, that stimulates contraction. At intravascular pressures $\geq 70$ mmHg, forced dilation can occur resulting in a further elevation in arterial wall [Ca$^{2+}$]$_i$.

**Intravascular pressure elevates active, passive, and myogenic tension.** We sought to investigate the relationship among intravascular pressure, arterial wall [Ca$^{2+}$]$_i$, and wall tension. Passive arterial wall thickness, as determined in transverse fixed sections, was 5.7 $\pm$ 0.2 and 3.7 $\pm$ 0.3 $\mu$m, respectively, at 10 and 90 mmHg ($n = 8$ for each). Therefore, throughout the full range of intravascular pressures that were applied, wall thickness was at least 1.9% and at most 3.3% of arterial diameter. These thin-walled arteries permit the use of the Laplace equation to calculate wall tension.

Definition of the terms “wall tension” and “myogenic tension” is appropriate. Wall tension produces arterial expansion, whereas myogenic tension acts in the opposite direction to compress (i.e., constrict) the artery. One problem encountered when calculating biomechanical properties of smooth muscle is the lack of a uniform definition for active stress (48). Passive and active arterial diameter is often determined in the absence and presence of external Ca$^{2+}$, respectively (e.g., see Ref. 30). Thus we estimated passive and active wall tension. In Ca$^{2+}$-free conditions, intravascular pressure and calculated passive wall tension exhibited a linear relationship (Fig. 3A). Thus between 10 and 90 mmHg, arterial wall elasticity essentially determines passive diameter. In the presence of extracellular Ca$^{2+}$, the myogenic response of smooth muscle works in opposition to the distending force of the pressure such that the active wall tension is reduced. Figure 3A illustrates these differences. We define the difference between the two curves as the myogenic tension (i.e., the reduction in wall tension due to smooth muscle contraction): Myogenic tension = passive wall tension (0 Ca$^{2+}$) minus active wall tension (Ca$^{2+}$).

We can then plot the myogenic tension as a function of arterial wall [Ca$^{2+}$]$_i$, as shown in Fig. 3B. Intravascular pressures between 10 and 90 mmHg increased myogenic tension between 25 and 437 dyn/cm (Fig. 3A). When the relationship between myogenic tension and arterial wall [Ca$^{2+}$]$_i$ was examined, it was found to be strongly Ca$^{2+}$ dependent (Fig. 3B). The relationship between myogenic tension and arterial wall [Ca$^{2+}$]$_i$ was nonlinear, with tension rising as arterial wall [Ca$^{2+}$]$_i$ increased (Fig. 3B). In contrast, forced dilation resulted in a large increase in mean active wall tension to 1,282 $\pm$ 103 dyn/cm and a decrease in myogenic tension to $\sim 78$ $\pm$ 9 dyn/cm (values provided at 90 mmHg).

Collectively, these data suggest that in newborn porcine cerebral arteries an elevation in intravascular pressure to between 10 and 90 mmHg raises wall tension, activates Ca$^{2+}$ influx, elevates arterial wall [Ca$^{2+}$]$_i$, and stimulates contraction. At intravascular pressures $\geq 70$ mmHg, the relationship between arterial wall [Ca$^{2+}$]$_i$ and myogenic tension can be overcome, leading to forced dilation.

**Membrane depolarization elevates arterial wall [Ca$^{2+}$]$_i$ and constricts.** In adult cerebral arteries, intravascular pressure activates voltage-dependent Ca$^{2+}$ channels, leading to an elevation in arterial wall [Ca$^{2+}$]$_i$, and contraction (12, 23, 30). In arteries from some vascular beds, including mesenteric and skeletal muscle, pressure may also activate Ca$^{2+}$ influx via additional pathways, including store-operated or capacitative Ca$^{2+}$ entry (45, 59). To investigate mechanisms by which pressure elevates arterial wall [Ca$^{2+}$]$_i$, and stimulates contraction in newborn pig cerebral arteries, we induced membrane depolarization at low pressure by elevating extracellular K$^+$ from 3 to 60 mM K$^+$. At a steady intravascular pressure of 10 mmHg, 60 mM K$^+$ increased mean arterial wall [Ca$^{2+}$]$_i$ from $\sim 131$ to $305$ nM and constricted arteries from $\sim 82$% to 43% of passive diameter (Fig. 2). These data indicate that membrane depolarization elevates arterial wall [Ca$^{2+}$]$_i$, and induces constriction in newborn porcine cerebral arteries.

**Nimodipine and extracellular Ca$^{2+}$ removal similarly dilates pressurized arteries.** To investigate whether intravascular pressure induces contraction due to voltage-dependent Ca$^{2+}$ channel activation, arterial diameter regulation by nimodipine (a voltage-dependent Ca$^{2+}$ channel blocker) was studied at low and high pressures in the presence and absence of extracellular Ca$^{2+}$. At 10 and 50 mmHg, nimodipine (500 nM) increased...
mean arterial diameter by \(~41\) and \(62\ \mu m\) or by \(19\) and \(25\%\), respectively (Fig. 4). In the presence of nimodipine, removal of extracellular \(Ca^{2+}\) did not cause any further change in diameter at either \(10\) or \(50\ mm\)Hg, suggesting that myogenic constriction occurs due to \(Ca^{2+}\) entry via voltage-dependent \(Ca^{2+}\) channels (Fig. 4). These data support the notion that voltage-dependent \(Ca^{2+}\) channels are the major \(Ca^{2+}\) entry pathway activated by intravascular pressure in newborn cerebral arteries.

Regulation of arterial diameter at low and high pressures by inhibitors of \(K_{Ca}\) channels, \(K_v\) channels, RyR channels, and the SR \(Ca^{2+}\)-ATPase. In adult cerebral arteries, an elevation in intravascular pressure activates \(K_{Ca}\) and \(K_v\) channels, which opposites pressure-induced depolarization and constriction (8, 23, 26, 29, 38). Mechanisms that oppose vasoconstriction in newborn cerebral arteries are unclear. Data in the present study suggest that newborn arteries are constricted even at low pressures. Conceivably this may be due to the absence of negative-feedback mechanisms in the newborn. Therefore, to examine newborn arterial diameter regulation by \(K_{Ca}\) and/or \(K_v\) channels, effects of inhibitors were investigated at low and high pressures.

At \(10\) and \(50\ mm\)Hg, iberiotoxin (100 nM) or papilline (100 nM), selective \(K_{Ca}\) channel blockers (see Refs. 15 and 33), slightly reduced arterial diameter (maximum effect, \(4\ \mu m\) constriction with papilline at \(50\ mm\)Hg, Fig. 5). In contrast, tetraethylammonium (TEA\(^+\)) (1 mM), a less-selective \(K_{Ca}\) channel blocker, constricted arteries by \(\sim 8\) and \(17\ \mu m\) at \(10\) and \(50\ mm\)Hg, respectively (Fig. 5). In addition to blocking \(K_{Ca}\) channels, TEA\(^+\) also inhibits \(K_v\) channels. To examine whether TEA\(^+\) constricted newborn cerebral arteries by blocking \(K_v\) rather than \(K_{Ca}\) channels, we investigated the regulation of arterial diameter by 4-AP, a \(K_v\) channel blocker. At \(10\) and \(50\ mm\)Hg, 4-AP (100 \(\mu m\)) constricted arteries by \(\sim 8\) and \(42\ \mu m\), respectively (Fig. 5). These data suggest that in newborn cerebral arteries, pressure-induced vasoconstriction is attenuated due to \(K_v\) channel activation. In contrast, pressure only induces a small increase in \(K_{Ca}\) channel activity.

In pressurized adult rat cerebral arteries, \(K_{Ca}\) channel activity is regulated by \(Ca^{2+}\) released from the SR due to the opening of RyR channels (26). However, in adult rabbit cerebral arteries, the SR also regulates contractility via \(K_{Ca}\) channel-independent mechanisms (50). We next examined whether \(Ca^{2+}\) release modulates newborn pig cerebral artery diameter at low and high pressures. At \(10\) and \(50\ mm\)Hg, thapsigargin (100 nM), a RyR channel blocker, only slightly reduced arterial diameter (maximum effect, \(\sim 2\ \mu m\) constriction at \(50\ mm\)Hg, Fig. 5). Thapsigargin (100 nM), a SR \(Ca^{2+}\)-ATPase inhibitor, constricted arteries by \(\sim 3\) and \(8\ \mu m\) at \(10\) and \(50\ mm\)Hg, respectively. These data suggest that pressure-induced SR \(Ca^{2+}\) release and \(K_{Ca}\) channel activation only slightly opposes constrictions in newborn cerebral arteries.

Caffeine dilates newborn cerebral arteries. Several mediators, including carbon monoxide, opioids, hypoxia, \(N\)-methyl-D-aspartate, and hypotension dilate newborn cerebral arteries via \(K_{Ca}\) channel activation, indicating functional importance (2–4, 32, 44). Although intravascular pressure weakly activated \(K_{Ca}\) channels, we sought to determine whether an increase in SR \(Ca^{2+}\) release would dilate newborn cerebral arteries by increasing \(K_{Ca}\) channel activity and whether pressure modulates this potential vasoregulatory mechanism. To examine these hypotheses, the regulation of arterial diameter by a low concentration of caffeine (1 \(\mu m\)), a RyR channel activator, was examined at intravascular pressures of 10 and 50

![Fig. 4. Nimodipine and removal of extracellular Ca\(^{2+}\) similarly dilate arteries pressurized to 10 and 50 mmHg (\(n = 6\) for each). *\(P < 0.05\) when compared with control at the same pressure. NS, nonsignificant difference.](image-url)
mmHg. Although caffeine has typically been used at millimolar concentrations to simultaneously activate large numbers of RyR channels to elicit a global cytosolic \([Ca^{2+}]_i\), transient (e.g., see Ref. 10), low micromolar caffeine concentrations stimulate \(Ca^{2+}\) sparks and \(Ca^{2+}\) spark-induced transient \(K_{Ca}\) currents in smooth muscle cells (25, 41, 47, 55).

Caffeine (1 \(\mu\)M) dilated newborn cerebral arteries pressurized to 10 or 50 mmHg (Fig. 6). Caffeine induced a larger vasodilation at 50 mmHg, when compared with that which occurred at 10 mmHg (Fig. 6). Paxilline (100 nM) abolished caffeine-induced vasodilation at both low and high pressures (Fig. 6). These data suggest that RyR channel stimulation dilates newborn cerebral arteries by increasing sarcolemmal \(K_{Ca}\) channel activity. Data also suggest that an elevation in intravascular pressure augments caffeine-induced dilations.

**DISCUSSION**

Novel data are presented that show graded elevations in intravascular pressure increase arterial wall tension and \([Ca^{2+}]_i\), and constrict newborn porcine cerebral arteries. Data also suggest that pressure elevates arterial wall \([Ca^{2+}]_i\), via voltage-dependent \(Ca^{2+}\) channel activation and induces constriction via a strongly \(Ca^{2+}\)-dependent mechanism. Intravascular pressure activates \(K_\text{v}\) channels, which limits pressure-induced constriction. Although, pressure only weakly activates \(K_{Ca}\) channels, RyR channel activation elevates \(K_{Ca}\) channel activity, leading to vasodilation at either low or high pressures.

**Pressure-induced constriction occurs via voltage-dependent \(Ca^{2+}\) channel activation.** Mechanisms that lead to the development and modulation of myogenic tone have been studied primarily using adult arterial preparations. In adults, it is generally well accepted that an elevation in intravascular pressure induces membrane depolarization, leading to an elevation in arterial wall \([Ca^{2+}]_i\), activation of \(Ca^{2+}\)/calmodulin-dependent myosin light chain kinase, and constriction (12). Several mechanisms have been proposed to mediate pressure-induced arterial wall depolarization, including the activation of smooth muscle cell cation (56, 57) or \(Cl^-\) (39) channels. In the present study, the regulation of \([Ca^{2+}]_i\), and diameter was investigated in arteries with an endothelium. No attempt was made to restrict fura-2 loading to the smooth muscle cell layer, which in these thin-walled arteries is only 1–2 cells thick. Therefore, arterial wall \([Ca^{2+}]_i\), measurements in this study represent the average of both smooth and endothelial cell \([Ca^{2+}]_i\). It is generally well accepted that the endothelium does not contribute to \([Ca^{2+}]_i\), and diameter responses that occur due to pressure changes in cerebral arteries (49). In addition, cerebral artery endothelial cells do not express voltage-dependent \(Ca^{2+}\) channels (28). Data here show that extracellular \(Ca^{2+}\) removal and nimodipine fully dilated arteries at low and high pressures, and \(Ca^{2+}\) removal decreased arterial wall \([Ca^{2+}]_i\), to approximately the same level at all pressures. Thus measurements of arterial wall \([Ca^{2+}]_i\), in this study will primarily reflect changes in smooth muscle cell \([Ca^{2+}]_i\), with a negligible contribution from endothelial cells.

Intravascular pressure may activate voltage-dependent \(Ca^{2+}\) channels in smooth muscle cells by inducing membrane depolarization (23, 30). Another potential mechanism is that integrins, a family of membrane-spanning glycoproteins, mechanotransduce changes in intravascular pressure to voltage-dependent \(Ca^{2+}\) channel activity (21, 36). In addition, intravascular pressure may also activate \(Ca^{2+}\) influx via alternative pathways, including store-operated or capacitative \(Ca^{2+}\)-entry (45, 59). Our data suggest that in newborn cerebral arteries, pressure induces constriction via the following sequence of events: intravascular pressure elevates arterial wall tension leading to voltage-dependent \(Ca^{2+}\) channel activation, an elevation in arterial wall \([Ca^{2+}]_i\), and constriction. Although membrane potential was not measured directly, findings suggest that an elevation in pressure leads to arterial wall depolarization, including that: 1) \(60 \text{ mM K}^{+}\), which would depolarize arteries to approximately \(-20 \text{ mV}\) (calculated using Nernst equation), elevated arterial wall \([Ca^{2+}]_i\), and constricted arteries at low pressure; and 2) voltage-dependent \(K^+\) channel activity increased with pressure. Thus \(60 \text{ mM K}^{+}\)-induced membrane depolarization elevates \([Ca^{2+}]_i\), and constricts, and pressure activates voltage-dependent \(K^+\) channels that reduce depolarization and thus constriction. Because removal of extracellular \(Ca^{2+}\) did not induce any further dilation in the presence of nimodipine, our data suggest that the major pressure-induced \(Ca^{2+}\) entry pathway in newborn porcine cerebral artery smooth muscle cells is via voltage-dependent \(Ca^{2+}\) channels. Similarly, in arteries at low- and high-pressure thapsigargin and ryanodine induced constriction rather than dilation. These data indicate that the pressure-induced arterial wall \([Ca^{2+}]_i\) elevation and constriction is not due to, but instead opposed by, SR \(Ca^{2+}\) release. Consistent with this notion is evidence that in ovine fetal cerebral arteries norepinephrine-induced contractions occur primarily due to \(Ca^{2+}\) influx, whereas in adults \(Ca^{2+}\) released from intracellular stores also contributes (35). Whether, in addition to depolarization, mechanosensitive elements directly signal pressure-induced voltage-dependent \(Ca^{2+}\) channel activation in the newborn cerebrovasculature cannot be concluded from our study (21, 36).

**Fig. 6.** Caffeine (1 \(\mu\)M) induces paxilline-sensitive dilations at 10 and 50 mmHg (\(n = 5\) for each). *\(P < 0.05\) compared with control. †\(P < 0.05\) compared with caffeine. ‡\(P < 0.05\) when compared with caffeine dilatation at 10 mmHg.

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In newborn cerebral arteries, pressure-induced constriction is Ca$^{2+}$ dependent. In adult rat cerebral arteries, Osol and co-authors (40) recently proposed a three-phase model to explain arterial responses to various levels of intravascular pressure. In adult rat posterior cerebral arteries, the three phases occur at ~40–60, 60–140, and >140 mmHg, respectively (40). Phase 1 is an initial development of myogenic tone that is accompanied by large elevations in arterial wall [Ca$^{2+}$]. Phase 2, termed “myogenic reactivity,” is defined as an increase in tone with relatively small changes in [Ca$^{2+}$]. Phase 3, termed “forced dilation,” is essentially the absence of force development accompanied by a large elevation in [Ca$^{2+}$]. (40).

Data from the present study suggest that when compared with adult rat cerebral arteries, newborn porcine cerebral arteries exhibit a different pattern of responses to intravascular pressure. One principal difference is that newborn porcine cerebral arteries exhibit significant myogenic tone (~19% of passive diameter) at 10 mmHg, consistent with previous findings in neonatal mouse middle cerebral arteries and newborn porcine mesenteric arteries (16, 46). Enhanced sensitivity to stretch has also been reported in the newborn when compared with adult guinea pig large pulmonary arteries (6). Another major difference is that myogenic tension development in the newborn is entirely Ca$^{2+}$-dependent until a critical pressure is achieved, whereby forced dilation occurs. Our data did not detect a Ca$^{2+}$-independent myogenic constriction in newborn pig cerebral arteries. Thus a two-phase model occurs in newborn porcine cerebral arteries: phase 1 is activated over the entire pressure range between 10 and 90 mmHg and is composed of Ca$^{2+}$-dependent constriction, whereas phase 2 occurs once a critical wall tension is exceeded, leading to forced dilation. The absence of Ca$^{2+}$-independent constriction in the newborn would eliminate potential modulation of this mechanism by other positive- and negative-feedback pathways that regulate arterial diameter. Conceivably, the absence of this contractile mechanism would also lead to less myogenic tone development, particularly at higher pressures. However, as stated above, our data suggest that newborn porcine cerebral arteries are more sensitive to intravascular pressure than adult cerebral arteries.

Mechanisms that lead to the enhanced contractile sensitivity to intravascular pressure in newborns are unclear. Several possibilities exist, including hyperresponsiveness to wall tension that may lead to greater membrane depolarization and/or increased Ca$^{2+}$ entry. Certainly, the absence of a pressure-induced negative-feedback pathway mediated via K$_{Ca}$ channel activation would lead to enhanced membrane depolarization and constriction. However, elevated arterial wall [Ca$^{2+}$] does not appear to fully explain enhanced sensitivity to pressure. At an intravascular pressure of 10 mmHg, steady-state arterial wall Ca$^{2+}$ was similar to that measured in adult arterial preparations (~100 nM; Refs. 29 and 40). Furthermore, between 10 and 90 mmHg, arterial wall [Ca$^{2+}$] ranged between ~100 and 300 nM, which is similar to that observed in adult preparations (12). An additional explanation is that myofilament Ca$^{2+}$ sensitivity may be higher in newborn cerebral arteries when compared with adults, as has been proposed previously for fetal, when compared with adult ovine cerebral arteries (1). Furthermore, regardless of age, cerebral arteries from different species may exhibit diverse patterns of responses to intravascular pressure. Comparing myogenic responses and arterial wall [Ca$^{2+}$] in newborn and adult cerebral arteries of the same species would help to address these possibilities.

As in adult rat cerebral arteries, forced dilation in newborn pigs was accompanied by a large elevation in arterial wall [Ca$^{2+}$], and a loss of myogenic tension development (40). In vivo, forced dilation leads to disruption of the blood-brain barrier, an event that can contribute to hypertensive encephalopathy (51). Although mechanisms are unclear, one hypothesis is that forced dilation occurs due to G-actin depletion (40). After forced dilation, myogenic tone can be recovered by reducing intravascular pressure, indicating that within certain limits cellular damage does not appear to explain or to result from this response (11).

Step elevations in intravascular pressure induced a biphasic arterial wall [Ca$^{2+}$], and diameter response. A step increase in pressure initially induced a transient arterial wall [Ca$^{2+}$], elevation and constriction that was followed by a smaller sustained [Ca$^{2+}$] elevation and constriction. [Ca$^{2+}$] transients induced by step elevations in intravascular pressure have been observed in a variety of arterial preparations, including rat cremaster muscle (20), rat mesenteric (54), and ferret coronary arterioles (13). These [Ca$^{2+}$] transients have been accompanied by vessel distension and have been proposed to not contribute directly to diameter regulation (20). However, in the present study, [Ca$^{2+}$] transients were clearly associated with transient constrictions (see Fig. 1). Because [Ca$^{2+}$] transients and constrictions were not observed in the absence of extracellular Ca$^{2+}$, these responses appear to occur due to Ca$^{2+}$ influx. In vivo, Ca$^{2+}$-dependent transient contractions may function to prevent large, rapid pressure fluctuations from damaging smaller downstream arterioles and capillaries.

Myogenic constriction is strongly opposed by K$_{Ca}$, but weakly opposed by K$_{Ca}$, channel activation. In adult arteries, an elevation in intravascular pressure activates K$_{Ca}$ and K$_{Ca}$ channels (8, 29). These negative-feedback pathways attenuate pressure-induced depolarization and limit constriction. In adult arterial smooth muscle cells, K$_{Ca}$ channel activity is regulated by Ca$^{2+}$ sparks (26). Ca$^{2+}$ sparks also occur in newborn piglet cerebral artery smooth muscle cells, but frequency is lower and effective coupling to K$_{Ca}$ channels is weaker when compared with those properties in adult rats (10, 24). In the present study, selective K$_{Ca}$ channel blockers only slightly reduced arterial diameter at low and high pressure. In contrast, 4-AP induced a large contraction at 50 mmHg, when compared with that at 10 mmHg. These data suggest that in newborn cerebral arteries, intravascular pressure strongly activates 4-AP-sensitive K$_{Ca}$ channels but only slightly activates K$_{Ca}$ channels. Newborn porcine cerebral artery smooth muscle cells express relatively Ca$^{2+}$-insensitive K$_{Ca}$ channels, when compared with K$_{Ca}$ channels in adult arterial smooth muscle cells (42, 52, 58). The Ca$^{2+}$ sensitivity of K$_{Ca}$ channels in human coronary artery smooth muscle cells is 4 μM at 0 mV (52), and in adult rat arterial smooth muscle cells is 19 μM at ~40 mV (42). In comparison, the K$_{d}$ for Ca$^{2+}$ of newborn porcine cerebral arteriole smooth muscle cell K$_{Ca}$ channels is 31 μM at 0 mV (58). At ~40 mV, ~60% of Ca$^{2+}$ sparks activate K$_{Ca}$ channels in newborn piglet cerebral artery smooth muscle cells, compared with a coupling ratio of >95% in adult mouse and rat cells from the same arteries (10, 24, 43). One explanation for the reduced coupling in the newborn may be the low K$_{Ca}$.
channel Ca\(^{2+}\) sensitivity (58). Ca\(^{2+}\) spark frequency is also approximately two- to threefold lower in newborn porcine cerebral arterial smooth muscle cells than in adult rodents when measured under similar experimental conditions (24, 26). In combination, the lower Ca\(^{2+}\) spark frequency and effective coupling to \(K_{Ca}\) channels would explain the ~10-fold lower transient \(K_{Ca}\) current frequency in newborn porcine arterial smooth muscle cells, when compared with adult rodent arterial smooth muscle cells at the same voltage (24–26). These findings may explain why \(K_{Ca}\) channels in newborns are less sensitive to intravascular pressure, when compared with adult rodent cerebral arteries.

A low concentration of caffeine elevates Ca\(^{2+}\) spark and transient \(K_{Ca}\) current frequency and amplitude in smooth muscle cells (25, 41, 47, 55). Our data show that at both low and high pressures, caffeine (1 \(\mu\)M) induces paxilline-sensitive dilations in newborn cerebral arteries. Thus at low and high pressures RyR channel activation dilates newborn cerebral arterioles. Hypotension dilates pial arteries by \(K_{ATP}\) and \(K_{Ca}\) channel activation, an elevation in intracellular Ca\(^{2+}\) concentration, and a reduction in arterial wall [Ca\(^{2+}\)].

In summary, data suggest that in newborn cerebral arteries, intravascular pressure elevates arterial wall tension, leading to voltage-dependent Ca\(^{2+}\) channel activation, an elevation in arterial wall [Ca\(^{2+}\)], and a Ca\(^{2+}\)-dependent compression. Pressure also activates \(K_{Ca}\) channels, which oppose constriction. Although \(K_{Ca}\) channel activity was not greatly increased by an elevation in pressure, RyR channel stimulation activates \(K_{Ca}\) channels, leading to vasodilation.

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REFERENCES

NEWBORN ARTERIAL [CA^{2+}]}, AND DIAMETER


