Ionic mechanism for contractile response to hyposmotic challenge in canine basilar arteries

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Yano, Shunsuke, Tomohisa Ishikawa, Hidetaka Tsuda, Kazuo Obara, and Koichi Nakayama. Ionic mechanism for contractile response to hyposmotic challenge in canine basilar arteries. Am J Physiol Cell Physiol 288: C702–C709, 2005. First published November 3, 2004; doi:10.1152/ajpcell.00367.2003.—A hyposmotic challenge elicited contraction of isolated canine basilar arteries. The contractile response was nearly abolished by the removal of extracellular Ca2+ and by the voltage-dependent Ca2+ channel (VDCC) blocker nicardipine, but it was unaffected by thapsigargin, which depletes intracellular Ca2+ stores. The contraction was also inhibited by Gd3+ and ruthenium red, cation channel blockers, and Cl− channel blockers DIDS and niflumic acid. The reduction of extracellular Cl− concentrations enhanced the hypertonically induced contraction. Patch-clamp analysis showed that a hyposmotic challenge activated outwardly rectifying whole cell currents in isolated canine basilar artery myocytes. The reversal potential of the current was shifted toward negative potentials by reductions in intracellular Cl− concentration, indicating that the currents were carried by Cl−/H1001. Moreover, the currents were abolished by 10 mM BAPTA in the pipette solution and by the removal of extracellular Ca2+. Taken together, these results suggest that a hyposmotic challenge activates cation channels, which presumably cause Ca2+ influx, thereby activating Ca2+-activated Cl− channels. The subsequent membrane depolarization is likely to increase Ca2+ influx through VDCC and elicit contraction.

BLOOD VESSELS ARE ALWAYS SUBJECTED TO HEMODYNAMIC STRESSES SUCH AS THOSE DUE TO BLOOD PRESSURE AND BLOOD FLOW. IT IS WELL ESTABLISHED THAT THE ARTERIES CONTRACT IN RESPONSE TO SUCH MECHANICAL STRESSES (9). CEREBRAL ARTERIES ARE PARTICULARLY SENSITIVE TO MECHANICAL STRESSES, WHICH INITIATE A MYOGENIC CONTRACTION THAT PLAYS A PIVOTAL ROLE IN MAINTAINING CONSTANT CEREBROVASCULAR CIRCULATION (5, 12, 18, 21). MYOGENIC CONTRACTION IS CONSIDERED TO BE PRODUCED, AT LEAST IN PART, BY THE ACTIVATION OF VOLTAGE-DEPENDENT CA2+ CHANNELS (VDCC) AFTER MEMBRANE DEPOLARIZATION, BECAUSE MYOGENIC CONTRACTION IS SENSITIVE TO VDCC BLOCKERS (9). HOWEVER, THE IONIC MECHANISM UNDERLYING THE MYOGENIC DEPOLARIZATION REMAINS TO BE ELUCIDATED COMPLETELY.

SEVERAL TYPES OF MECHANOSENSITIVE CHANNELS IN VASCULAR SMOOTH MUSCLE HAVE BEEN DESCRIBED, INCLUDING Ca2+-activated K+ channels (KCa), volume-sensitive Cl− channels, and nonselective cation channels (9). IN MOST OF THESE STUDIES, MECHANOSENSITIVE CHANNELS WERE ACTIVATED BY APPLICATION OF NEGATIVE OR POSITIVE PRESSURE TO THE PATCHED MEMBRANE OR BY APPLICATION OF A HYPOSMOTIC CHALLENGE TO INDUCE A VOLUME INCREASE IN ISOLATED SMOOTH MUSCLE CELLS. IT IS therefore UNCERTAIN WHETHER THESE MECHANICAL STRESSES MIMIC MECHANICAL STIMULI UNDER PHYSIOLOGICAL CONDITIONS. TO FURTHER ELUCIDATE THE CONTRIBUTION OF MECHANOSENSITIVE CHANNELS TO MYOGENIC CONTRACTION, IT IS NECESSARY TO CHARACTERIZE THE TYPE OF CHANNELS ACTIVATED BY THESE MECHANICAL STRESSES IN INTACT TISSUES.

IN THIS STUDY, A HYPOSMOTIC CHALLENGE WAS APPLIED TO BOTH RING SEGMENTS AND ISOLATED CELLS OF CANINE BASILAR ARTERIES TO DETERMINE THE TYPES OF ION CHANNELS CONTRIBUTING TO HYPTONOTICALLY INDUCED RESPONSES. WE DEMONSTRATE THAT THE CONTRACTILE RESPONSE TO A HYPOSMOTIC CHALLENGE IS INHIBITED BY THE VDCC BLOCKER NICARDIPINE, THE CATION CHANNEL BLOCKERS Gd3+ AND RUTHENIUM RED, AND THE Cl− CHANNEL BLOCKERS DIDS AND NIFLUMIC ACID. THE ION CHANNELS ACTIVATED BY A HYPOSMOTIC CHALLENGE WERE FURTHER INVESTIGATED USING PATCH-CLAMP ANALYSIS. WE HAVE SHOWN THAT A HYPOSMOTIC CHALLENGE INDIRECTLY ACTIVATES Ca2+-activated Cl− (ClCa) CHANNELS BY ELEVATING INTRACELLULAR Ca2+ CONCENTRATION ([Ca2+]i). COLLECTIVELY, OUR FINDINGS SUGGEST THAT A HYPOSMOTIC CHALLENGE FIRST ACTIVATES Gd3+-SENSITIVE CATION CHANNELS, WHICH IN TURN ACTIVATE ClCa CHANNELS BY ELEVATING [Ca2+]i, AND THAT THE ACTIVATION OF BOTH CHANNELS RESULTS IN MEMBRANE DEPOLARIZATION, THEREBY ACTIVATING VDCC AND CAUSING CONTRACTION.

METHODS

Preparations. Healthy mongrel dogs of either sex weighing 8–20 kg were housed in separate cages in a temperature-controlled room and treated according to guidelines set by the Institutional Animal Care and Use Committee of the University of Shizuoka as well as the Guidelines for Animal Experiments established by the Japanese Pharmacological Society. The dogs were anesthetized with pentobarbital sodium (30 mg/kg intravenously) and exsanguinated by bleeding from the carotid arteries. The basilar artery was dissected and placed in cold Krebs-Henseleit solution containing (in mM) 113 NaCl, 4.7 KCl, 2.6 CaCl2, 1.2 MgSO4, 25 NaHCO3, 1.2 KH2PO4, and 11.1 glucose, aerated with 95% O2:5% CO2.

Tension measurements. Isometric tension was measured as previously described (24, 25). Briefly, a cylindrical segment of the basilar artery, 20 mm long, was isolated and cut into ring segments ~2–3 mm in width. Endothelial cells were removed from all arteries by rotating a stainless steel rod along the luminal surface; successful removal was confirmed by the absence of acetylcholine (30 nM)-induced relaxation of the artery precontracted with U46619 (100 nM). The ring segments were mounted on two tungsten wires (0.1 mm diameter), with one attached to a force transducer (T7-8-240; Orientec, Tokyo, Japan) and the other to a micromanipulator, under 0.3-g resting tension in isolated...
organ baths comprising the isotonic bath solution containing (in mM) 65 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES, and 160 mannitol (pH 7.4; 300 mosM). The hypotonic bath solution containing (in mM) 65 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES, and 20 mannitol (pH 7.4; 170 mosM).

Current measurements. Single arterial smooth muscle cells were enzymatically isolated from canine basilar arteries as described previously (16). Briefly, the dissected basilar arteries were cut into small segments and placed in Ca²⁺-free Hanks’ solution containing (in mM) 125 NaCl, 5.4 KCl, 15.5 NaHCO₃, 0.44 KH₂PO₄, 0.34 Na₂HPO₄, 10 glucose, and 2.9 sucrose, aerated with 95% O₂-5% CO₂ for 90 min at 37°C. Every 15 min, Ca²⁺-free Hanks’ solution was replaced with fresh solution. The segments were then transferred to the Ca²⁺-free Hanks’ solution containing 1.5 mg/ml collagenase (type IA; Sigma, St. Louis, MO), 0.1 mg/ml protease (type XIV; Sigma), 0.2 mg/ml bovine serum albumin, and incubated for 15 min at 37°C with gentle agitation. After digestion, the supernatant was discarded and the remaining segments were rinsed and further incubated in Kraftbrühe solution containing (in mM) 110 KOH, 70 glutamic acid, 10 taurine, 25 KCl, 10 KH₂PO₄, 5 HEPES, 0.5 EGTA, and 11 glucose (pH 7.4) for 15 min. Single cells were dispersed by gentle agitation with a wide-pore glass pipette. Isolated cells were kept in Kraftbrühe solution containing 1 mg/ml bovine serum albumin and incubated for ~15 min at 37°C with gentle agitation. The osmolarity of the superfusing solution was reduced by the removal of mannitol without an alteration of the ionic strength, and the isotonic bath solution was equilibrated for 40 min before each experiment. The osmolarity of the superfusing solution was reduced by the removal of mannitol without an alteration of the ionic strength.

Drugs. The following drugs were used. Gadolinium chloride, ibotenate, nifedipine, and niflu-mic acid were obtained from Sigma; 4,4’-disothiocyanostilbene-2,2’-disulfonic acid (DIDS), ryanodine, ruthenium red, and thapsigargin were obtained from Wako (Osaka, Japan); and fura-2 acetoxymethyl ester (fura-2 AM) was purchased from Dojindo Laboratories (Kumamoto, Japan).

DIDS, fura-2 AM, niflumic acid, and thapsigargin were dissolved in dimethyl sulfoxide. We confirmed that the final solvent concentration (0.1–1.0%) had no effect on contractile responses or membrane currents. Other drugs were dissolved in water.

Statistical analysis. Data are expressed as means ± SE, and n indicates the number of experiments performed. The effects of treatment were analyzed by the paired or unpaired Student’s t-test or analysis of variance, followed by Bonferroni’s post hoc test. P < 0.05 was accepted as the level of statistical significance.

RESULTS

Hypotonically induced contraction of canine basilar arteries. As shown in Fig. 1, a 45% hyposmotic challenge (from 310 to 170 mosM) elicited contraction of canine basilar arteries. The hypotonically induced contraction was nearly abolished by extracellular Ca²⁺ removal containing 2 mM EGTA (Fig. 1, A and D) and by nifedipine (1 μM), a VDCC blocker (Fig. 1, B and D). In contrast, thapsigargin (1 μM), an inhibitor of sarcoplasmic reticulum (SR) Ca²⁺-ATPase that depletes SR Ca²⁺ stores, did not affect the hypotonically induced contraction (Fig. 1, C and D). We confirmed that the contraction induced by caffeine (10 mM) was abolished under these conditions (data not shown).

Gd³⁺, a cation channel blocker, inhibited the hypotonically induced contraction in a concentration-dependent manner and...
at 100 μM decreased it by ~80% (Fig. 2, A and B). The same concentration of Gd<sup>3+</sup> (100 μM) had no inhibitory effect on the contraction induced by 20, 40, or 80 mM K<sup>+</sup> (Fig. 2, C and D). Another cation channel blocker, ruthenium red (100 μM), also suppressed the hypotonically induced contraction by ~70% (Fig. 3). The contraction was also inhibited by DIDS (100 μM) and niflumic acid (100 μM), Cl<sup>-</sup> channel blockers, by ~50 and 80%, respectively (Fig. 3).

The contribution of Cl<sup>-</sup> and cation channels to the hypotonically induced contraction was investigated further. We reasoned that if Cl<sup>-</sup> efflux or Na<sup>+</sup> influx were involved in the contraction by depolarizing the membrane, then reducing extracellular Cl<sup>-</sup> concentration ([Cl<sup>-</sup>]<sub>o</sub>) or extracellular Na<sup>+</sup> concentration ([Na<sup>+</sup>]<sub>o</sub>) would enhance or suppress contraction. When [Cl<sup>-</sup>]<sub>o</sub> was reduced from 76 to 11 mM by the substitution of gluconate for Cl<sup>-</sup>, the hypotonically induced contraction was enhanced by ~40% (Fig. 4, A and C). In contrast, when extracellular Na<sup>+</sup> was replaced with NMDG, the hypotonically induced contraction was not significantly changed (Fig. 4, B and C).

To test whether large-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> (BKCa) channels contributed to the contraction induced by a hypomotic challenge, an effect of iberiotoxin, a specific BKCa channel blocker, on the contraction was investigated. As shown in Fig. 5, iberiotoxin (100 nM) did not cause any changes in the resting tension but significantly increased the hypotonically induced contraction.

Whole cell current activated by hypomolarity in canine basilar artery myocytes. The whole cell currents activated by a hypomotic challenge were investigated using the patch-clamp technique. Under conditions in which K<sup>+</sup> currents were eliminated by extracellular 1 mM Ba<sup>2+</sup>, intracellular 10 mM TEA, and the substitution of intracellular K<sup>+</sup> with Cs<sup>+</sup>, whole cell currents were recorded by the application of voltage-ramp pulses from a holding potential of −70 to +100 mV. In isotonic extracellular solution, a small outwardly rectifying current was observed (Fig. 6A). A 50% hypomotic challenge (from 300 to 150 mosM) increased both inward and outward currents. When exposed to the hypotonic solution, the cells became rounded and shortened in the longitudinal direction. Figure 6B represents the difference current obtained by subtraction of whole cell currents in the isotonic solution from those in the hypotonic solution. The difference current showed outward rectification, and the reversal potential of the current was 8.5 ± 2.2 mV (n = 5). This hypotonically induced current was still observed in the presence of nicardipine (1 μM; data not shown). When Cl<sup>-</sup> concentrations in the pipette solution were reduced from 130 to 30 mM by substitution of Cl<sup>-</sup> with aspartate, which shifts the calculated Cl<sup>-</sup> equilibrium potential from +15 to −22 mV, the reversal potential of the hypotonically activated current was shifted toward a more negative potential, i.e., −23.0 ± 1.4 mV (n = 4; Fig. 6C). In contrast, the reversal potential for swelling-activated whole cell currents did not shift drastically when Na<sup>+</sup> in the bath solution was replaced with NMDG (data not shown; n = 3).

Not only swelling-activated Cl<sup>-</sup> channels but also Cl<sub>Ca</sub> channels are present in vascular smooth muscle cells. We therefore examined the contribution of Ca<sup>2+</sup>- to hypotonically activated currents. As shown in Fig. 7, a hypomotic challenge
did not activate any currents when the pipette solution included 10 mM BAPTA (Fig. 7A) or when the extracellular Ca²⁺ was removed (Fig. 7B), suggesting that hypotonically activated currents are Ca²⁺ sensitive.

**DISCUSSION**

We investigated the properties of the contraction induced by a hyposmotic challenge in canine basilar arteries. The results of this study suggest that osmotic cell swelling activates Ca²⁺-permeable cation channels; ClCa channels, which are secondarily activated by Ca²⁺ influx through cation channels; and VDCC. Thus activation of VDCC by membrane depolarization resulting from activation of cation and ClCa channels results in contraction. Our results suggest that mechanosensitive cation channels play a pivotal role in both hypotonically induced contraction and possibly myogenic contraction.

**Fig. 4.** Effect of low-Cl⁻ or Na⁺-free extracellular solution on hypotonically induced contractions in ring segments of canine basilar arteries. A and B: typical traces of contraction induced by hyposmotic challenge (Hypo). C: summary of the responses. Ring segments were first exposed to a 45% hypotonic solution. Subsequently, the segments were again exposed to a 45% hypotonic solution whose Cl⁻ concentration was lowered to 11 mM (Low [Cl⁻]; A) or whose Na⁺ was substituted with N-methyl-D-glucamine ([Na⁺]-free; B). Contractions are expressed as a percentage of the contraction induced by the first exposure to the hypotonic solution. Each bar represents the mean ± SE of 11–13 experiments. *P < 0.05 vs. control.

**Fig. 5.** Effect of iberiotoxin on hypotonically induced contraction in ring segments of canine basilar arteries. A: typical traces of contraction induced by a hyposmotic challenge (Hypo). B: summary of the responses. Ring segments were exposed to a 45% hypotonic solution. The hyposmotic challenge was again applied in the absence (Control) and presence of iberiotoxin (100 nM). Contractions are expressed as a percentage of the contraction induced by the first exposure to the hypotonic solution. Each bar represents the mean ± SE of 8 experiments. **P < 0.01 vs. control.

**Fig. 6.** Effect of hyposmotic challenge on whole cell currents in canine basilar artery myocytes. A: typical current-voltage (I-V) relationship of whole cell currents recorded in isotonic and hypotonic solutions. Currents were elicited by voltage-ramp pulses from −70 to +100 mV with change in volume over time (dV/dt) of 0.17 mV/ms with a holding potential of −70 mV under the condition in which K⁺ currents were eliminated. Hyposmotic challenge caused an increase in both inward and outward currents. B: the difference current obtained by subtraction of whole cell currents in the isotonic solution from those in the hypotonic solution shown in A. C: difference current recorded with intracellular Cl⁻ concentrations of 30 mM. The difference currents were obtained using the method described in B.
Our study confirms earlier observations demonstrating contractions in response to hyposmotic challenge in rat cerebral arteries (6, 20, 33), portal veins (6, 20, 33), and guinea pig aortae (6, 20, 33). In agreement with these studies, we have demonstrated that hypotonically induced contractions of canine basilar arteries were nearly abolished by the VDCC blocker nicardipine. These findings suggest that hypotonically induced contraction is produced by Ca\(^{2+}\) influx through VDCC.

The activation of VDCC could be caused by membrane depolarization. The electrophysiological experiments with a glass microelectrode have demonstrated that hyposmotic challenge causes membrane depolarization sensitive to Gd\(^{3+}\) in rat cerebral arteries (33). In line with this finding, we have shown that hypotonically induced contractions were inhibited by Gd\(^{3+}\). Although Gd\(^{3+}\) does directly inhibit VDCC in other cell types (3, 11, 19), we have confirmed that even at a high concentration of 100 \(\mu\)M, Gd\(^{3+}\) had no inhibitory effects on high-K\(^{+}\)-induced contraction in canine basilar arteries. These results suggest that osmotic cell swelling opens Gd\(^{3+}\)-sensitive cation channels, thereby causing membrane depolarization and VDCC activation. The data with ruthenium red also support the involvement of cation channels in the response. Our findings are in agreement with earlier observations that cell swelling activates Gd\(^{3+}\)-sensitive cation currents in isolated smooth muscle cells of rat mesenteric arterioles (27) and cerebral arteries (33).

Nevertheless, the substitution of NMDG for Na\(^{+}\) in the bath solution did not significantly change the hypotonically induced contraction. This implies that Na\(^{+}\) influx through cation channels is not essential for hypotonically induced membrane depolarization. However, these data do not necessarily rule out the possible contribution of cation channels to hypotonically induced contraction. It is noteworthy that Na\(^{+}\)-free extracellular solution augments contraction by inhibiting the Ca\(^{2+}\) efflux via a Na\(^{+}\)/Ca\(^{2+}\) exchanger (4). Possibly, this augmentation may have somewhat counteracted the inhibitory effect of the Na\(^{+}\) substitution on hypotonically induced membrane depolarization. Another possibility is that Ca\(^{2+}\) influx through cation channels primarily contributes to hypotonically induced membrane depolarization.

Nelson et al. (23) showed that the Cl\(^{-}\) channel blockers IAA-94 and DIDS caused hyperpolarization and dilatation of pressurized rat cerebral arteries, suggesting the contribution of Cl\(^{-}\) channels to myogenic depolarization in rat cerebral arteries. In contrast, a recent report by investigators at the same laboratory (33) identified a swelling-activated cation conductance that was sensitive to Cl\(^{-}\) channel blockers such as DIDS and tamoxifen in rat cerebral artery myocytes. In light of these findings, they concluded that a cation channel sensitive to Cl\(^{-}\) channel blockers is essential for swelling- and pressure-induced depolarization in rat cerebral arteries. In our experiments, the hypotonically induced contraction of canine basilar arteries was inhibited not only by Gd\(^{3+}\) but also by DIDS and niflumic acid. These results might support the presence of a swelling-activated cation channel sensitive to Cl\(^{-}\) channel blockers. However, our patch-clamp experiments have indicated that the outwardly rectifying currents activated by hyposmotic challenge are Cl\(^{-}\) currents because the reversal potential for the swelling-activated whole cell currents did not change when [Na\(^{+}\])\(_{o}\) was reduced but did shift accordingly when pipette Cl\(^{-}\) concentrations were reduced. Moreover, the hypotonically induced currents that we recorded were abolished by 10 mM BAPTA in the pipette solution and by the removal of extracellular Ca\(^{2+}\). These results suggest that the swelling-activated, outwardly rectifying currents are Cl\(_{Ca}\) currents that are secondarily activated by an elevation of [Ca\(^{2+}\]). Furthermore, the hypotonically induced contraction was enhanced by the reduction of the extracellular Cl\(^{-}\) concentrations, which suggests the contribution of Cl\(^{-}\) efflux to the contraction. It is thus likely that [Ca\(^{2+}\)]\(_{i}\) elevation induced by a hyposmotic challenge activates Cl\(_{Ca}\) channels, which in turn cause membrane depolarization (Fig. 8).

The hypotonically activated outwardly rectifying currents observed in this study are suggested to be Cl\(_{Ca}\) currents. It is unlikely that the currents were activated by Ca\(^{2+}\) influx through VDCC, because they were observed under voltage-clamp conditions and in the presence of VDCC channel blocker nicardipine. The results of the tension measurements, which showed that the hypotonically induced contraction was inhibited by Gd\(^{3+}\) and ruthenium red, but not by thapsigargin, seem to be consistent with the hypothesis that the Cl\(_{Ca}\) currents are activated by Ca\(^{2+}\) influx through cation channels, most likely stretch-activated cation (SAC) channels. SAC channels in smooth muscle cells are known to be Ca\(^{2+}\)-permeable (8, 17, 31). In intact tissues, it is possible that Ca\(^{2+}\) influx through VDCC in addition to SAC channels activates Cl\(_{Ca}\) channels, which further leads to membrane depolarization and VDCC activation. The enhancement of VDCC currents by hypotonic-
The molecular identity of SAC channels in vascular smooth muscle cells has not been resolved. It has recently been shown, however, that a mammalian transient receptor potential (TRP) homolog of the canonical subfamily (TRPC), TRPC6, a Ca$^{2+}$-permeable cation channel, is involved in the pressure-induced depolarization of rat cerebral arteries (32). Another TRP homolog cation channel of the vanilloid subfamily (TRPV), TRPV2, also has been shown to be activated by a hyposmotic challenge in mouse aortic myocytes (22). It is possible, therefore, that TRP homologs comprise the arterial SAC channel. The present study also has shown that the hyptonically induced contraction was inhibited by ruthenium red, a specific blocker of TRPV channels, suggesting that the cation channel activated by hyponicity in canine basilar artery myocytes may be a TRPV homolog. Because we could not detect cation currents activated by hyponicity in the patch-clamp experiments, the identity of the channels remains to be determined. Although we do not have a clear explanation why the cation currents were not detected, it is possible that the currents may have been too small to be detected under our experimental conditions.

Alternatively, the possibility still remains that Gd$^{3+}$ might directly inhibit the Cl$^{-}$ channels activated by cell swelling. Although Gd$^{3+}$ does not appear to block swelling-activated Cl$^{-}$ channels in vascular smooth muscle cells (35), Gd$^{3+}$ has been shown to inhibit Cl$_{\text{Ca}}$ channels (28), Ca$^{2+}$-inactivated Cl$^{-}$ channels (2), and hyponicity-activated Cl$^{-}$ channels (1) in Xenopus oocytes. The results of the present study, however, suggest that the Ca$^{2+}$ influx through cation channels is an obligatory step in the hyponicity-activated Cl$_{\text{Ca}}$ channels. It is more likely, therefore, that Gd$^{3+}$ inhibits cation channels, thereby secondarily inhibiting Cl$_{\text{Ca}}$ channels (Fig. 8).

Interestingly, both Cl$_{\text{Ca}}$ channels and BK$_{\text{Ca}}$ channels can be activated by [Ca$^{2+}$]$_{i}$ elevation, with the activation of these channels leading to depolarization and hyperpolarization, respectively, in vascular smooth muscles. In canine basilar arteries, a Cl$_{\text{Ca}}$ channel-mediated depolarization seems to be more prominent than a BK$_{\text{Ca}}$ channel-mediated hyperpolarization in response to hyposmotic challenge. This difference may be attributable to the voltage dependence and Ca$^{2+}$ sensitivity of BK$_{\text{Ca}}$ channels; at resting membrane potential, BK$_{\text{Ca}}$ channels may not be sufficiently activated by increases in [Ca$^{2+}$]$_{i}$ to activate Cl$_{\text{Ca}}$ channels. It has been shown that BK$_{\text{Ca}}$ channels are activated by [Ca$^{2+}$]$_{i}$ elevations, with $K_{d}$ of 10 $\mu$M at $-83$ mV and 1.8 $\mu$M at $-11$ mV (7), while the $K_{d}$ for Cl$_{\text{Ca}}$ channels is 0.37 $\mu$M (26). Therefore, Cl$_{\text{Ca}}$ channels may first be activated by an increase in [Ca$^{2+}$]$_{i}$ induced by Ca$^{2+}$ influx through cation channels, which leads to membrane depolarization and further increases in [Ca$^{2+}$]$_{i}$, sufficient to activate BK$_{\text{Ca}}$ channels.

It has been hypothesized that stretch-induced depolarization cannot be maintained unless an endogenous inhibitor of BK$_{\text{Ca}}$ channels is produced and that 20-hydroxyecosatetraenoic acid (20-HETE) may be one such substance (13). Our previous studies of the ring strips of canine basilar arteries, in which we found that the contraction induced by mechanical stretch was potentiated by BK$_{\text{Ca}}$ channel blockers such as iberiotoxin, charybdotoxin, and TEA (25) or 20-HETE (24), support these views. The idea that BK$_{\text{Ca}}$ channels counteract stretch-induced depolarization is also supported by the present study, which has shown that the selective BK$_{\text{Ca}}$ channel blocker iberiotoxin drastically increased the hyposmotically induced contraction. Because the resting tension was not affected by iberiotoxin, BK$_{\text{Ca}}$ channels were unlikely to have been activated in the resting state. These results are in agreement with earlier studies showing that iberiotoxin increases the magnitude of longitudinal, stretch-induced, cation channel-mediated membrane depolarization in isolated coronary myocytes (34). Although we have not examined the mechanism for the activation of BK$_{\text{Ca}}$ channels by a hyposmotic challenge, it is likely that BK$_{\text{Ca}}$ channels are secondarily activated by an elevation of [Ca$^{2+}$]$_{i}$.

It is unclear whether mechanical stimulation of cell swelling and stretch activates the same signal transduction pathways. The present study has demonstrated that the contraction induced by hyposmotic challenge shares several features of the stretch-induced myogenic response. First, hyptonically induced contraction was inhibited by a VDCC blocker. Myogenic tone is also attenuated by VDCC blockers in most smooth muscle preparations (9). Second, hyptonically induced contraction was inhibited by Gd$^{3+}$. Because the high-K$^{+}$-induced contraction of canine basilar arteries was not affected by Gd$^{3+}$ at the concentration we used (100 $\mu$M), the inhibition of hyptonically induced contraction is likely to result from the inhibition of Gd$^{3+}$-sensitive cation channels and not from the direct inhibition of VDCC by Gd$^{3+}$. Our previous study showed that Gd$^{3+}$ inhibits myogenic response induced by mechanical stretch in the ring segments of canine basilar arteries (25). Gd$^{3+}$ also reverses membrane depolarization induced by intraluminal pressure in rat cerebral arteries (33). Moreover, Gd$^{3+}$-sensitive cation currents are activated by whole cell stretch of isolated vascular smooth muscle cells (8). Thus the activation of Gd$^{3+}$-sensitive cation channels may be an obligatory pathway in both myogenic response and hyptonically induced contraction. However, the role of Gd$^{3+}$-sensitive cation channels seems to be somewhat different because the activation of Gd$^{3+}$-sensitive cation channels is the main cause of depolarization associated with myogenic contraction, whereas it is a trigger event for hyptonically induced contraction.
It is of interest that 100 μM niflumic acid, an effective blocker of ClCa channels, does not affect myogenic tone in pressurized rat cerebral arteries (23). In the present study, the same concentration of niflumic acid nearly abolished hypotonically induced contraction. Because niflumic acid is known to block some types of nonselective cation channels (10, 29) and VDCC (the present study), part of the effect of niflumic acid may be due to a direct inhibition of cation channels and/or VDCC. However, the apparent difference in the sensitivity to niflumic acid suggests that ClCa channels contribute to hypotonically induced contraction, but not to myogenic contraction.

Depletion of the SR Ca2+ store with ryanodine or thapsigargin increases myogenic tone in pressurized skeletal arterioles (30) and cerebral arteries (15). These drugs also abolish Ca2+ sparks, which are highly localized elevations of [Ca2+]i, occurring in close proximity to the plasma membrane in pressurized cerebral arteries (15). At physiological potentials, Ca2+ sparks are proposed to stimulate BKCa channels, thereby causing membrane hyperpolarization. In this study, treatment with thapsigargin did not cause significant changes in contraction induced by hyposmotic challenge, suggesting a minimal role for Ca2+ release from the SR in hypotonically induced contraction. However, these results do not necessarily rule out the possible contribution of the Ca2+ release to the contraction. The present study has shown that not only BKCa channels but also ClCa channels are activated during hypotonically induced contraction. Because both channels could be activated by Ca2+ release from the SR, they may effectively oppose each other’s effects on contraction.

In this study, a hypotonic solution was prepared by removing mannitol from an isotonic solution containing mannitol. If hyposmotic stimulation was achieved by reducing extracellular NaCl concentration, then the concentration gradients of Na+ and Cl− would be decreased, which would lead to a decrease in inward Na+ currents and an increase in inward Cl− currents. Therefore, reducing extracellular NaCl concentration was unsuitable for the purposes of the present study. Because Na+ was isosmotically replaced by mannitol, the isotonic solution in our study contained only 65 or 70 mM Na+. Such low [Na+]o is not physiological and may have affected the reactivity. Partial isosmotic replacement of NaCl with mannitol has been shown to lead to a transient depolarization, followed by a partial recovery, in vascular smooth muscle cells (20). Therefore, the amplitude of the hypotonically induced contraction observed in the present study may have been overestimated because of membrane depolarization induced by low [Na+]o. It also has been shown that stretch-induced myogenic tone, but not histamine-induced tone, is increased by reducing [Na+]o with ion substitution in rabbit facial veins and cerebral arteries (14). In this case, the mechanism seems to be independent of membrane depolarization because the enhancement is not inhibited by a Ca2+ channel blocker. This enhancement mechanism is unlikely to be involved in the hypotonically induced contraction in canine basilar arteries, because the contraction was nearly abolished by nicardipine.

In summary, a hyposmotnic challenge evoked contraction of canine basilar arteries. The contraction was inhibited by nicardipine (a VDCC blocker), by Gd3+ (a cation channel blocker), and by DIDS and niflumic acid (Cl− channel blockers) and was enhanced by reducing extracellular Cl− concentration. Our patch-clamp experiments also provide evidence that a hyposmotnic challenge activates ClCa channels by elevating [Ca2+]i, in canine basilar artery myocytes. On the basis of our present findings, we propose the following mechanism for hypotonically induced contraction (Fig. 8). Osmotic cell swelling first activates Ca2+-permeable, Gd3+-sensitive cation channels, most likely SAC channels, through which Ca2+ influx activates ClCa channels. The Cl− efflux through ClCa channels results in membrane depolarization, thereby activating VDCC and causing contraction.

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