KCa channel blockers reveal hyperpolarization and relaxation to K+ in rat isolated mesenteric artery

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Received 21 November 2001; accepted in final form 12 March 2002

Dora, Kim A., Nicola T. Ings, and Christopher J. Garland. KCa channel blockers reveal hyperpolarization and relaxation to K+ in rat isolated mesenteric artery. Am J Physiol Heart Circ Physiol 283:H606–H614, 2002.First published March 21, 2002; 10.1152/ajpheart.01016.2001.—Raising extracellular K+ concentration ([K+]o) around mesenteric resistance arteries reverses depolarization and contraction to phenylephrine. As smooth muscle depolarizes and intracellular Ca2+ and tension increase, this effect of K+ is suppressed, whereas efflux of cellular K+ through Ca2+-activated K+ (KCa) channels is increased. We investigated whether K+ efflux through KCa suppresses the action of exogenous K+ and whether it prestimulates smooth muscle Na+-K+-ATPase. Under isometric conditions, 10.8 mM [K+]o had no effect on arteries contracted >10 mN, unless 100 nM iberiotoxin (IbTX), 100 nM charybdoxin (ChTX), and/or 50 nM apamin were present. Simultaneous measurements of membrane potential and tension showed that phenylephrine depolarized and contracted arteries to 52 ± 2.3 mV and 13.8 ± 1.6 mN (n = 5) after blockade of KCa, but 10.8 mM K+ reversed fully the responses (107.6 ± 8.6 and 98.8 ± 0.6%, respectively). Under isobaric conditions and preconstriction with phenylephrine, 10.7 mM [K+]o reversed contraction at both 50 mmHg (77.0 ± 8.5%, n = 9) and 80 mmHg (83.7 ± 5.5%, n = 5). However, in four additional vessels at 80 mmHg, raising K+ failed to reverse contraction unless ChTX was present. Increases in isometric and decreases in isobaric tension with phenylephrine were augmented by either ChTX or ouabain (100 μM), whereas neither inhibitor altered tension under resting conditions. Inhibition of cellular K+ efflux facilitates hyperpolarization and relaxation to exogenous K+, possibly by indirectly reducing the background activation of Na+-K+-ATPase.

smooth muscle; membrane potential; Na+-K+-ATPase; contraction; dilatation

IN MANY small resistance arteries, increasing extracellular K+ concentration ([K+]o) up to 15–20 mM stimulates both smooth muscle cell hyperpolarization and relaxation. Increases in [K+]o follow increased cellular K+ and whether it prestimulates smooth muscle Na+-K+-ATPase. Under isometric conditions, 10.8 mM [K+]o had no effect on arteries contracted >10 mN, unless 100 nM iberiotoxin (IbTX), 100 nM charybdoxin (ChTX), and/or 50 nM apamin were present. Simultaneous measurements of membrane potential and tension showed that phenylephrine depolarized and contracted arteries to 52 ± 2.3 mV and 13.8 ± 1.6 mN (n = 5) after blockade of KCa, but 10.8 mM K+ reversed fully the responses (107.6 ± 8.6 and 98.8 ± 0.6%, respectively). Under isobaric conditions and preconstriction with phenylephrine, 10.7 mM [K+]o reversed contraction at both 50 mmHg (77.0 ± 8.5%, n = 9) and 80 mmHg (83.7 ± 5.5%, n = 5). However, in four additional vessels at 80 mmHg, raising K+ failed to reverse contraction unless ChTX was present. Increases in isometric and decreases in isobaric tension with phenylephrine were augmented by either ChTX or ouabain (100 μM), whereas neither inhibitor altered tension under resting conditions. Inhibition of cellular K+ efflux facilitates hyperpolarization and relaxation to exogenous K+, possibly by indirectly reducing the background activation of Na+-K+-ATPase.

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teries, respectively, failed to obtain reproducible and convincing relaxation to K+ (10, 20). As such, this work clearly questions the role of K+ as an EDHF, per se.

Relaxation in rat mesenteric arteries tensioned under either isometric or isobaric conditions is usually observed against agonist-stimulated contraction. By comparing experimental records, it appears that there is a relationship between levels of isometric tension and the ability of K+ to evoke relaxation. The relaxation responses to K+ reported by Lacy et al. (20) were obtained against contraction to a high (10 μM) concentration of either norepinephrine or phenylephrine. The subsequent failure to obtain robust and sustained relaxation to K+ may therefore be explained by physiological or functional antagonism as a result of the high level of stimulation. Simultaneous measurements of smooth muscle membrane potential and tension change in the mesenteric artery reveal that raising [K+]o (from 4.8 to 19.8 mM) does evoke clear, sustained, and correlated hyperpolarization and relaxation (8). However, particularly at the lower end of the concentration-response range (up to ~11 mM), these effects of K+ are reduced if the smooth muscle cells are contracted and depolarized with phenylephrine above ~10 mN and ~40 mV, respectively (8). The mechanism responsible for suppressing these responses to K+ is not clear.

One possibility is that antagonism results from an increasing efflux of K+ from both the smooth muscle and endothelial cells, as the intensity of stimulation with phenylephrine increases. Stimulation of both cell types is known to be associated with a significant efflux of K+ (2, 6, 12, 16). KCa provide a predominant route for the efflux, and not surprisingly these channels have very important functional consequences. In cerebral arteries, Ca2+ sparks appear to regulate myogenic tone solely through the activation of KCa (3, 18), and, during stimulation with phenylephrine in the mesenteric artery, K+ efflux through BKCa channels is sufficient to suppress contraction by 75% (9).

In the rat small mesenteric artery, functional KIR channels are confined to the endothelium, and relaxation to exogenous K+ in endothelium-denuded arteries is blocked with ouabain (8). Therefore, it appears that the predominant pathway for K+-mediated hyperpolarization and relaxation of smooth muscle cells is through the activation of Na+-K+-ATPase. As a result of these observations, we suggested that extracellular K+ accumulation during stimulation with phenylephrine might maximally activate the Na+-K+-ATPase and thus prevent additional stimulation with exogenous K+ (8). We therefore examined whether cellular K+ efflux through KCa influences smooth muscle responses to exogenous K+ in both wire-mounted and pressurized mesenteric arteries.

METHODS

Male Wistar rats (200–250 g) were euthanized by cervical dislocation and exsanguination following procedures required by the Animals Scientific Procedure Act of the United Kingdom (Section 1, Revised 1986) and monitored by the Home Office. The mesentery was removed and placed in Krebs buffer. A 3- to 5-mm-long segment (third-order branch of the superior mesenteric artery) was cleared of adherent connective tissue.

Isometric Tension and Microelectrode Experiments

A 2-mm-long artery segment was cannulated with gold-plated tungsten wire (25 μm diameter, Goodfellow) and mounted in a Mulvany-Halpern myograph (model 400A or 610M, Danish MyoTechnology). The segment was set to a resting tension equivalent to that generated at 0.9 times the diameter of the vessel at 100 mmHg. Endothelial cell viability in the presence of 100 μM Nω-nitro-L-arginine methyl ester (l-NAME) was assessed as sustained maximal relaxation (>95%) to 1 μM acetylcholine in arteries constricted with submaximal concentrations of phenylephrine (1–3 μM). On the basis of this control response, there was no requirement to discard any arteries. In some experiments, endothelial cells were removed by rubbing the lumen of the artery with a human hair. Endothelium-denuded arteries were used if phenylephrine (1–3 μM) was able to evoke stable contractions and acetylcholine (1 μM) was unable to relax the arteries by >10%.

Smooth muscle membrane potential and tension were simultaneously recorded (10 Hz; MacLab version 4.0.1, ADInstruments), as previously described (15). The artery was superfused with oxygenated, heated Krebs buffer at 3–4 ml/min. Individual smooth muscle cells were impaled with glass electrodes (2 M KCl, tip resistances ~100 MΩ). Inhibitors of KCa were added to the bath after cessation of superfusion and then mixed by gassing. Increasing concentrations of phenylephrine were added to depolarize and contract beyond ~40 mV and 10 mN. Repolarization and relaxation were then assessed to a single concentration of exogenous K+ (10.8 mM final bath concentration).

Isobaric Diameter Experiments

Arteries were isolated (4°C) and transferred to a pressure myograph (model 120CP, Danish MyoTechnology) containing 3-(N-morpholino)propanesulfonic acid and bovine serum albumin (MOPS-BSA) at room temperature. Each artery was cannulated at both ends, pressurized to 50 mmHg, and perfused with MOPS-BSA to remove residual blood components. Only arteries without visible side branches were used. Arteries were superfused with MOPS-buffered saline at a rate of 3–4 ml/min, and luminal flow ceased for the remainder of the experiment. The artery was warmed to 37°C (~0.5°C) and longitudinally stretched, and the position was set with a micrometer during maximal inflation (at 80 mmHg). The myograph chamber was positioned on an inverted microscope (model IX70, Olympus) and the artery visualized (~10 objective, Olympus) with a charge-coupled device camera (model KPM1, Hitachi) to give ×300 magnification. Internal diameter was measured using video calipers (resolution ±1 μm; Microcirculation Research Institute). Diameter, inflow and outflow pressure, and temperature were continuously recorded at 2 Hz (MacLab version 4.0.1, ADInstruments). A viable endothelium was accepted as sustained maximal dilation (>95%) to 1 μM acetylcholine in phenylephrine (4 μM)-contracted arteries in the presence of l-NAME (100 μM). On the basis of this control response, there was no requirement to discard any arteries. None of the arteries developed myogenic tone.

K+ Concentration-Response Curves

For concentration-response curves, K+ was added cumulatively to phenylephrine-contracted arteries in a static
Data are expressed as means ± SE of n experiments. Values were analyzed nonparametrically, with P < 0.05 considered as statistically significant.

RESULTS

Effects of Exogenous K\textsuperscript{+} on Wire-Mounted Mesenteric Arteries

Effect of apamin and ChTX in combination. The smooth muscle membrane potential in endothelium-intact mesenteric arteries was $-52.5 ± 2.3$ mV ($n = 5$). Increasing concentrations of phenylephrine were titrated to depolarize and contract mesenteric arteries sufficiently to block the hyperpolarization and relaxation to 10.8 mM K\textsuperscript{+} (8). As K\textsuperscript{+} channel blockers augment the action of phenylephrine, relatively low concentrations of the agonist were required. Whereas the combined application of apamin (to block SK\textsubscript{Ca}) and ChTX (to block IK\textsubscript{Ca} and BK\textsubscript{Ca}) did not affect either the resting membrane potential ($-51.9 ± 2.4$ mV, $n = 5$) or tension, a 10 times lower concentration of phenylephrine (0.26 ± 0.09 $\mu$M) was sufficient to develop an equivalent arterial contraction to vessels without the toxins present (13.8 ± 1.6 mN, $n = 5$) and depolarized the arteries to $-32.2 ± 2.3$ mV ($n = 5$). The action of phenylephrine was associated with small oscillations in membrane potential and tension, with the change in potential always immediately preceding tension change. Often, membrane potential changes appeared to summate and initiate an action potential-like, rapid depolarization to near zero mV. Each event was followed by rapid repolarization, an overshooting slower hyperpolarization, and then depolarization before the cycle recommenced (Fig. 1). Under these conditions, with apamin and ChTX present, raising [K\textsuperscript{+}]\textsubscript{o} to 10.8 mM evoked a clear smooth muscle repolarization (107.6 ±...
8.6%, \( n = 5 \) and relaxation \( 98.8 \pm 0.6\% \), \( n = 5 \) (Fig. 1). This contrasted markedly to similar levels of stimulation in the absence of these toxins, when 10.8 mM \( [K^+]_o \) had little effect (Fig. 1).

**Effect of apamin, ChTX, or IbTX on relaxation to \( K^+ \).** To assess the relative contribution made by different \( K_{Ca} \) channels, separate concentration-relaxation curves were obtained to \( K^+ \) with either apamin, ChTX, or IbTX (the latter a specific blocker of \( BK_{Ca} \)). In each case, phenylephrine concentrations were selected to raise tension >10 mN and to a similar level in each experiment (overall mean 11.8 ± 0.4 mN, representing 63 ± 4% maximum contraction, \( n = 21 \)). The mean concentration of phenylephrine required before addition of toxins was 3.1 ± 0.3 \( \mu M \), and in each individual experimental series, this was reduced to 1.5 ± 0.4 \( \mu M \) with apamin, 0.4 ± 0.1 \( \mu M \) with ChTX, 0.3 ± 0.1 \( \mu M \) with apamin and ChTX, and 0.5 ± 0.1 \( \mu M \) with IbTX (\( n = 7 \)).

After the endothelium was removed, the tension evoked with phenylephrine was slightly lower than in intact arteries, 8.1 ± 0.5 mN (developed with 1.2 ± 0.2 \( \mu M \) phenylephrine and representing 71 ± 5% maximal contraction, \( n = 15 \)). In endothelium-denuded arteries, apamin now did not reduce the concentration of phenylephrine required to stimulate an equivalent contraction (1.6 ± 1.0 \( \mu M \), \( n = 5 \)), whereas lower concentrations of phenylephrine were required with either ChTX or IbTX (0.11 ± 0.7 and 0.26 ± 0.08 \( \mu M \), respectively, \( n = 5 \)). In these experiments, there was no significant difference in the level of contraction between the different groups.

In both endothelium-intact and -denuded arteries, either 100 nM ChTX or IbTX markedly facilitated the ability of \( K^+ \) to stimulate relaxation (Fig. 2). In endothelium-intact arteries, the relaxation to \( K^+ \) in the presence of ChTX was further augmented with 50 nM apamin. With apamin alone, the ability of \( K^+ \) to stimulate relaxation was only facilitated in endothelium-intact arteries (Fig. 2).

The ability of \( K^+ \) channel blockers to reveal relaxation to exogenous \( K^+ \) did not appear to reflect the fact that lower concentrations of phenylephrine were used in the presence of the blockers. In a separate series of experiments, complete relaxation in response to exogenous \( K^+ \) was obtained in endothelium-denuded arteries, in which the concentration of phenylephrine applied in the presence of IbTX was higher than in the same arteries that had previously failed to relax to \( K^+ \) (0.7 ± 0.3 and 0.3 ± 0.1 \( \mu M \) phenylephrine, respectively, Fig. 3; \( n = 5 \)).

**Phenylephrine-Induced Activation of \( Na^+-K^+-ATPase \)**

Inhibition of the arterial \( Na^+-K^+-ATPase \) with ouabain (100 \( \mu M \)) did not significantly alter tension in

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**Fig. 2.** \( Ca^{2+} \)-activated \( K^+ \) channel (\( K_{Ca} \)) antagonists reverse inhibition of \( K^+ \) relaxation at high tension. Summary data showing %relaxation against PE-contracted arteries in (A) endothelium-intact arteries (\( n = 7 \)) and (B) endothelium-denuded arteries (\( n = 5 \)) are shown. Control responses with \( Na^+-K^+-ATPase \) ouabain (100 \( \mu M \)), iberiotoxin (IbTX) (100 \( nM \)), or apamin. The concentration of PE was varied to contract the arteries to approximately the same level (−12 mN in endothelium-intact arteries and −8 mN in endothelium-denuded arteries, see RESULTS). The \( K^+ \) concentration-response curve under control conditions and after the addition of an inhibitor of \( K_{Ca} \) was always compared within a given preparation, so the effect of toxins to unmask the ability of \( K^+ \) to evoke robust relaxation could be more directly assessed. Values are means ± SE of \( n \) arteries.
unstimulated mesenteric arteries. However, in the presence of variable but submaximal concentrations of phenylephrine (41 ± 10% maximum contraction), ouabain rapidly increased tension and by a mean of 4.8 ± 1.2 mN (to 75 ± 8% maximum contraction, n = 5). The time course of contraction was similar to that during stimulation with phenylephrine alone (Fig. 1).

**Vasodilatation to K**⁺ in Pressurized Mesenteric Arteries

At 50 mmHg, the resting diameter of mesenteric arteries was 303 ± 12 μm (n = 13) and significantly increased to 323 ± 13 μm when the pressure was elevated to 80 mmHg (n = 13). There was no significant difference between the diameter at 70 mmHg (321 ± 13 μm) and 80 mmHg. Between 20 mmHg (230 ± 10 μm diameter) and 70 mmHg, each 10-mmHg increment did significantly increase the diameter (P < 0.005, paired nonparametric test, n = 13). Thus the artery was maximally inflated at 80 mmHg. Pressure (50 vs. 80 mmHg) had no influence on the ability of acetylcholine to reverse contraction to phenylephrine. No myogenic tone was observed in these arteries. This may reflect the size of artery used, as it appears that myogenic tone is only observed in smaller-diameter rat mesenteric arteries and arterioles (32).

At 50 mmHg, increasing the concentration of [K⁺]o from 4.7 mM (to between 10.7 and 22.7 mM) always stimulated dilatation (n = 9, Fig. 4), an action that was not modified by the extent of stimulation with phenylephrine. Phenylephrine reduced diameter by 63 ± 5% to 112 ± 16 μm (range 78–196 μm; n = 9) and decreased tension from 1,015 ± 26 to 378 ± 55 dyn/cm (n = 9). Under isobaric conditions, contraction is associated with a decrease in tension (Laplace theory: tension = pressure × radius). Both upstream and downstream pressure remained constant after the addition of phenylephrine. Under these conditions, increasing [K⁺]o to 10.7 mM stimulated 77.0 ± 8.5% dilatation (n = 9, Fig. 4).

**Effect of pressure on vasodilatation to K⁺**. Increasing transmural pressure to 80 mmHg reduced the ability of K⁺ to cause dilatation, but only in some of the arteries studied. This effect was all or none. In five of nine arteries, phenylephrine stimulated contraction to 136 ± 17 μm (range 80–171 μm) and reduced tension from 1,815 ± 75 to 731 ± 93 dyn/cm. [K⁺]o (10.7 mM) almost completely reversed the effect of phenylephrine (83.7 ± 5.5%; Fig. 4). In the remaining four arteries, phenylephrine stimulated similar levels of contraction (to 121 ± 12 μm, range 90–141 μm) and reduced tension from 1,833 ± 85 to 650 ± 63 dyn/cm. [K⁺]o (10.7 mM) either had no effect or caused a transient response (Fig. 5), so that overall it caused a small mean constriction (−3.4 ± 2.8% dilatation). In the presence of phenylephrine (39 ± 6% reduction in diameter), the addition of ChTX (100 nM) caused a further decrease in diameter, but by >2 times (to 52 ± 5% total reduction in diameter, n = 4). However, in the presence of ChTX, 10.7 mM [K⁺]o now stimulated dilatation of the same order as the response obtained in the arteries without toxin present (85.7 ± 9.7%, n = 4).

**Phenylephrine-induced activation of Na⁺-K⁺-ATPase in pressurized arteries.** As with isometric conditions, inhibition of Na⁺-K⁺-ATPase with ouabain (100 μM) did not significantly alter tension in unstimulated, pressurized mesenteric arteries. However, in the presence of variable but submaximal concentrations of phenylephrine (48 ± 2% reduction in diameter),
ouabain now rapidly reduced diameter by 49 ± 14 μm (to 62 ± 4% reduction in diameter, n = 4).

DISCUSSION
These data exemplify the importance of KCa channels in modulating responses to exogenous K⁺ in the rat small mesenteric artery. As such, they support our (9) previous observations indicating the functional importance of these channels, which serve to reduce the magnitude of contraction to the α₁-adrenergic agonist phenylephrine in this artery. The main observations in the present study show that blocking smooth muscle and endothelial cell KCa channels can reveal both smooth muscle hyperpolarization and relaxation to exogenous K⁺ in isolated arteries. The effect was most prominent in arteries mounted for isometric recording in a wire myograph and presumably reflects a reduction in the amount of inherent stimulation of the smooth muscle Na⁺-K⁺-ATPase, enabling stimulation of the pump with exogenous K⁺, and thus effecting relaxation.

In the rat small mesenteric artery, raising the [K⁺]₀ from 4.8 mM (in the Krebs buffer) evokes smooth muscle hyperpolarization and relaxation until concentrations of ~20 mM when depolarization and contraction predominate (8). At the lower end of the concentration range, up to ~12 mM, the ability of K⁺ to evoke hyperpolarization and relaxation is dramatically influenced by the intensity of ongoing smooth muscle stimulation. Once depolarization and contraction to the α₁-adrenergic agonist phenylephrine takes place, phenylephrine exceeds around ~40 mV and 10 mN, respectively, the inhibitory effects of K⁺ are reduced (8). The initial stimulation with phenylephrine in the present study (11.8 ± 0.4 mN, n = 21) was greater than in our previous study (8), and, as expected, was associated with lower maximum relaxations to K⁺ (only 25–50% compared with 90–100%), stressing the importance of contraction intensity in determining the extent of the subsequent relaxation. However, despite these relatively small control relaxations, either ChTX or IbTX dramatically increased K⁺-evoked relaxation to levels approaching 100%.

Arterial tissue appears to contain BKCa, IKCa, and SKCa, each of which could contribute to K⁺ efflux. The available evidence indicates that BKCa channels are present on the vascular smooth muscle cells, whereas IKCa and SKCa channels appear to be restricted to the endothelial cells (12, 14, 21, 22, 34). BKCa channels have a considerable influence on vascular function. By generating a tonic hyperpolarization, they oppose depolarization stimulated by pressure and contribute to the regulation of myogenic tone. This regulation appears to occur through the release of Ca²⁺ from ryanodine-sensitive stores acting locally to activate BKCa, preventing global increases in Ca²⁺ and thus contraction (3, 18). Removal of this control mechanism results in elevated blood pressure in mice, in which the β₁-subunit of BKCa has been disrupted (28). BKCa channels also modulate the action of constrictor agonists such as phenylephrine on vascular smooth muscle, both directly and indirectly. Blockade of BKCa markedly increases contraction to phenylephrine in both endothelium-intact and -denuded mesenteric arteries (9). In addition, an indirect influence is mediated through the endothelium. Smooth muscle stimulation with phenylephrine raises Ca²⁺ and directly activates BKCa, but in addition, endothelial cell SKCa (and most likely IKCa) are also activated, as well as nitric oxide synthesis, which together attenuate the smooth muscle contraction (7, 9).

Because stimulation of smooth muscle cells with constrictor agonists is associated with the activation of KCa channels, increasing stimulation will be associated with an increase in the efflux of K⁺ through these channels. Evidence that phenylephrine can stimulate K⁺ efflux came originally from monitoring ⁸⁶Rb efflux (6). In the present study, the degree of activation of the KCa is indicated by the effect on the contraction to phenylephrine. Under isometric conditions, blockade of KCa augmented the contraction to phenylephrine by ~10-fold (3.1 ± 0.3 and 0.27 ± 0.10 μM, before and
after addition of apamin and ChTX, respectively). The predominant $K_{\text{Ca}}$ activated was BKCa, as shown by the augmentation by IbTX alone, both in endothelium-intact and -denuded arteries. As previously reported (9), the involvement of SKCa appeared confined to the endothelium, because apamin had no effect on the concentration of phenylephrine required to evoke contraction in denuded arteries. In the presence of $K^+$ channel blockers, one consequence of the augmented contraction to phenylephrine was that lower concentrations were used to evoke contractions similar to control. Because $\alpha$-agonists can stimulate the $Na^+-K^+$-ATPase (26), any reduction in this effect may facilitate a subsequent stimulation with exogenous $K^+$. This would mean that relaxation to $K^+$ was in fact not a result of the presence of $K^+$ channel blockers per se. However, the fact that IbTX enabled relaxation to $K^+$ when the concentration of phenylephrine was increased rather than decreased, and in paired experiments, indicates this mechanism is unlikely.

The implication of this massive augmentation in contraction is that under control conditions, the levels of $K^+$ efflux are sufficient to almost fully relax the artery. This clearly demonstrates the extent of the functional influence of $K^+$ efflux from the artery. By comparison with a $K^+$ concentration-response curve, this equates to concentrations approaching 16 mM $K^+$. The large conductance of these channels raises the possibility that their activation is sufficient to raise the local $[K^+]_o$ to an extent that can then block the subsequent action of exogenous $K^+$. In pressurized arteries, the ability of $K^+$ to evoke relaxation appeared to be less influenced by phenylephrine contraction. Raising $[K^+]_o$ caused dilatation in all vessels at a transmural pressure of 50 mmHg and just over one-half of the arteries at 80 mmHg, regardless of the concentration of phenylephrine. However, four of the nine vessels studied at 80 mmHg did not respond robustly to added $K^+$, consistent with the observations of Doughty et al. (10). This tendency toward a lack of response with $K^+$ may reflect a greater stimulation of BKCa at the higher pressure in these arteries. Our finding that the addition of ChTX was able to unmask the ability of 10.7 mM $K^+$ to relax all these arteries supports this suggestion. The relationship between the effects of pressure, wall tension, and $K^+$ conductance is complex (35). However, pressure elevation does depolarize mesenteric arteries (31). This will open voltage-gated Ca$^{2+}$ channels, raise smooth muscle intracellular [Ca$^{2+}$] as in cerebral arteries (17), and stimulate BKCa. Part of the depolarization caused by pressures above -20 mmHg appears due to a block of $K_{Ca}$ channels (35), so perhaps by limiting $K^+$ efflux this facilitates the dilatation readily obtained at 50 mmHg, whereas at 80 mmHg, overall efflux becomes a more dominant influence. It is clear that $K_{Ca}$ channels are activated at high pressures in this study because ChTX was able to evoke contraction in the presence of phenylephrine. However, the activation was considerably less than under isometric conditions. The differences do not appear to be explained by the wall tension because this was similar in the $K^+$ responsive and unresponsive groups at 80 mmHg.

Smooth muscle hyperpolarization and relaxation in response to increases in $[K^+]_o$ reflect the activation of $Na^+-K^+$-ATPase and $K_{R}$ (12). In the rat mesenteric artery, $K_R$ appears to be restricted to the endothelium, so direct smooth muscle stimulation with $K^+$ to evoke hyperpolarization and relaxation occurs by stimulating the $Na^+-K^+$-ATPase (8). Mesenteric artery smooth muscle cells contain both the $\alpha_2$ and $\alpha_3$ isoforms of $Na^+-K^+$-ATPase, which have apparent affinities ($K_0$,5) for activation with $K^+$ in the range of 3.6–6.2 mM ($\alpha_2$$\beta_1$, $\alpha_2$$\beta_2$, $\alpha_3$$\beta_1$, and $\alpha_3$$\beta_2$) (1, 5). The fact that $K^+$ is applied against a background depolarization to phenylephrine will effectively increase the $Na^+-K^+$-ATPase current amplitude, at least initially, because the membrane potential is then further from the pump reversal potential (23). The fact that ouabain increased phenylephrine contraction in both wire-mounted and pressurized arteries, but not in unstimulated arteries, clearly indicates an activation of the $Na^+-K^+$-ATPase when phenylephrine is present. It is interesting that the contraction to ouabain was greater in the wire-mounted arteries, where the responses to $K^+$ were more prone to block during contraction. In addition, the extent of depolarization to $\alpha_1$-adrenoceptor agonists appears less under isobaric conditions compared with isometric conditions (31), again supporting the idea that $K_{Ca}$ and the $Na^+-K^+$-ATPase are stimulated to a greater extent by phenylephrine during isometric contraction. To our knowledge, there is no report directly comparing the levels of smooth muscle intracellular [Ca$^{2+}$] stimulated by phenylephrine under isometric versus isobaric conditions in the rat mesenteric artery. However, because the arteries are more depolarized under isometric conditions (31), it appears likely that Ca$^{2+}$ influx through voltage-gated Ca$^{2+}$ channels is also greater. Because BKCa is both voltage and Ca$^{2+}$ sensitive, activation would be greater under isometric conditions, causing more $K^+$ efflux.

Interestingly, blockade of endothelial cell SKCa and IKCa (but not BKCa) inhibits the EDHF response to agonists such as acetylcholine (11, 27, 33). Consequently, whereas acetylcholine releases $K^+$ through these channels, they must be functional for $K^+$ to act as an EDHF. The current observations qualify the conditions required for $K^+$ to act as an EDHF in the rat small mesenteric artery. As the intensity of stimulation with $\alpha$-adrenoceptor agonists increases, the contribution that $K^+$ makes to EDHF-mediated relaxation would be predicted to decrease. So it is likely that $K^+$ acts as an EDHF in this artery only when the level of background stimulation of smooth muscle BKCa and the $Na^+-K^+$-ATPase is low. However, it is also clear that another pathway for smooth muscle hyperpolarization in response to acetylcholine exists in this artery (12). Because there is evidence for myoendothelial gap junctions in this artery (30), these connections could potentially enable the spread of hyperpolarizing current from the activated endothelium to the adjacent smooth muscle cells (10, 12, 13). This pathway would
act in parallel to K⁺, ensuring that maximal EDHF relaxation was still possible under conditions where K⁺ was unable to contribute to relaxation (Fig. 5) (8, 10, 20).

Our data explain the inconsistent ability of others to obtain relaxation to K⁺ in isometrically mounted mesenteric arteries (10, 20) and vasodilatation to K⁺ in pressurized mesenteric arteries (10). Central to this explanation is the fact that responses to K⁺ become more variable as the perfusion pressure increases (in isobaric experiments) or as phenylephrine concentrations increase (in isometric preparations). In wire-mounted mesenteric arteries, raising extracellular K⁺ to ~11 mM either failed to cause relaxation (10) or gave a relatively small, transient relaxation (20). In those studies, 10 μM phenylephrine or norepinephrine were used to stimulate the background contraction necessary for relaxation. It now seems clear that the failure to observe robust responses to K⁺ is related to the intensity of this contraction, and the consequent activation of KCa. In the presence of the KCa inhibitors, variability was very low, with every artery (both under isometric and isobaric conditions) maximally relaxing to applied K⁺. Endothelium-independent smooth muscle hyperpolarization and relaxation can be reproducibly stimulated with 10.8 mM K⁺, but only with lower concentrations of phenylephrine (8), or after blockade of KCa. This explanation is supported by a recent microelectrode study with pinned-out mesenteric arteries (29).

In the study by Richards et al. (29), increasing K⁺ to ~10 mM directly stimulated smooth muscle hyperpolarization in endothelium-denuded arteries, an effect that was then inhibited in the presence of 10 μM phenylephrine. Consistent with our present observations, the repolarizing action of K⁺ could be completely restored in the presence of K⁺ channel blockers (and the continued presence of 10 μM phenylephrine). Importantly, although input resistance was reduced by phenylephrine, this change was not significantly altered by the K⁺ channel blockers. This indicates that a decrease in resistance was not responsible for the loss of hyperpolarization to exogenous K⁺. Interestingly, in endothelium-intact mesenteric arteries, Richards et al. (29) did observe robust hyperpolarization to K⁺ in the presence of phenylephrine, in contrast to the observations of Dora and Garland (8). This may simply reflect the different experimental setups employed (pinned-out superfused artery exposed to a bolus injection of K⁺ versus wire-mounted arteries and steady-state concentrations of K⁺) and the fact that endothelium-intact preparations are more responsive to K⁺ so that the inhibitory influence of background contraction is less obvious as K⁺ concentrations increase. For example, responses to 10.8 mM K⁺ were more sensitive to the extent of background contraction than responses to 14 mM (8). Nevertheless, both studies show that exogenous K⁺ can act directly on mesenteric artery smooth muscle cells and that this effect can be suppressed in the presence of phenylephrine and recovered in the additional presence of K⁺ channel blockers.

The physiological consequences of these data relate to both the activation of the endothelium and the smooth muscle cells. The importance of endothelium-dependent relaxation due to hyperpolarization increases with decreases in artery size. This, at least in part, is presumably due to resistance arteries, including the rat mesenteric artery, depending largely on changes in membrane potential to alter Ca²⁺ influx through voltage-gated Ca²⁺ channels and hence control the level of contraction. Thus the mechanisms for smooth muscle hyperpolarization are of direct physiological relevance. In terms of smooth muscle activation, for example, by sympathetic nerves, as the intensity of stimulation increases, the efflux of K⁺ through KCa will modulate the contraction, and in turn will influence the ability of released K⁺ (from the parenchyma or endothelium) to evoke vasodilatation. In many small resistance arteries, increasing the [K⁺]o up to 15–20 mM stimulates both smooth muscle cell hyperpolarization and relaxation. Increases in [K⁺]o of this magnitude are known to follow increased cellular metabolic activity (25) and play an important part in the local increases in blood flow that follow raised metabolic activity in, for example, the cerebral and coronary vascular beds (4, 19).

In summary, we have shown that at ~11 mM, K⁺ was able to evoke dilatation in all arteries studied, with the caveat that it was necessary to inhibit KCa under some conditions. This was the case with mesenteric arteries mounted under either isometric or isobaric conditions. We speculate that the efflux of K⁺ through BKCa inhibits Na⁺-K⁺-ATPase, which provides the predominant mechanism for K⁺-mediated relaxation of smooth muscle cells in this artery. These data provide an explanation for the inability of some studies to record robust and reproducible relaxation with modest increases in extracellular K⁺. They provide further evidence that is consistent with a role for K⁺ as an EDHF in this artery.

This work was supported by the Wellcome Trust of the United Kingdom.

REFERENCES

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