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DPO-1-sensitive K^+ channels contribute to the vascular tone and reactivity of resistance arteries from brain and skeletal muscle

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Abstract

Objective—Many types of vascular smooth muscle cells exhibit prominent delayed rectifier K^+ (K_{DR}) currents. These K_{DR} currents may be mediated, at least in part, by $K_V1.5$ channels, which are sensitive to inhibition by diphenyl phosphine oxide-1 (DPO-1). We tested the hypothesis that DPO-1-sensitive K_{DR} channels regulate the tone and reactivity of resistance-sized vessels from rat brain (middle cerebral artery) and skeletal muscle (gracilis artery).

Methods—Middle cerebral and gracilis arteries were isolated and subjected to three kinds of experimental analysis: a) Western blot/immunocytochemistry; b) patch clamp electrophysiology; and c) pressure myography.

Results—Western blot and immunocytochemistry experiments demonstrated $K_V1.5$ immunoreactivity in arteries and smooth muscle cells isolated from them. Whole-cell patch clamp experiments revealed smooth muscle cells from resistance-sized arteries to possess a K_{DR} current that was blocked by DPO-1. Resistance arteries constricted in response to increasing concentrations of DPO-1. DPO-1 enhanced constrictions to phenylephrine and serotonin in gracilis and middle cerebral arteries, respectively. When examining the myogenic response, we found that DPO-1 reduced the diameter at any given pressure. Dilations in response to acetylcholine and sodium nitroprusside were reduced by DPO-1.

Conclusion—We suggest that $K_V1.5$, a DPO-1-sensitive K_{DR} channel, plays a major role in determining microvascular tone and the response to vasoconstrictors and vasodilators.

Keywords

diphenyl phosphine oxide-1; delayed rectifier potassium current; KCNA5; $K_V1.5$; smooth muscle

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Introduction

Resistance vessels regulate tissue perfusion by integrating a variety of stimuli. Microvascular adjustments include: a) myogenic responses; b) metabolic vasodilation; c) vasoconstriction in response to neurohumoral factors; and d) vasodilation due to flow and paracrine agents. While some of the mechanisms involve endothelial cells and sympathetic nerves, it is the contractile state of smooth muscle that is the final element in any of the pathways. When it comes to smooth muscle, however, a knowledge gap exists regarding the end effectors controlling membrane potential and, thus, the intracellular Ca^{2+} concentration and vascular tone. K^+ channels are known to regulate this process of electromechanical coupling, but the type of K^+ channel(s) involved is less clear. It is our supposition that voltage-dependent K^+ (K_V) channels, especially the delayed rectifier (K_{DR}) type of K_V channels, are critically important for regulating arteriolar vascular reactivity.

Vascular smooth muscle cells express a variety of K^+ channels, including K_{DR} channels [11]. The K^+ channels of microvascular smooth muscle have been reviewed previously [17,18]. K_{DR} channels produce a prominent current in the physiological voltage range [13,40]. Evidence suggests that these K_{DR} channels are important for the membrane potential and reactivity of smooth muscle [23] in regulating tissue blood flow [10]. There are 100-plus K^+ channel gene loci in the human genome and more than one-third of them encode K_V channels (including both pore-forming α subunits and modulatory subunits). Therefore, based on the sheer number of candidates, it has been difficult to determine the molecular entities underlying the K_{DR} channels of smooth muscle. Excellent evidence, however, supports a role for the K_V1 subfamily [1,3,4,33], particularly $K_V1.5$ [7,21,38].

Recently, novel and relatively selective $K_V1.5$ channel inhibitors have become available, including diphenyl phosphine oxide-1 (DPO-1) [25,35,37]. DPO-1 allows us to test whether K_{DR} channels of arteriolar smooth muscle contain $K_V1.5$ as a major component. Further, it lets us test whether DPO-1-sensitive K_{DR} channels control the tone and reactivity of resistance-sized arteries from brain (middle cerebral artery; MCA) and skeletal muscle (gracilis artery; GA). In the present study, we describe the presence of $K_V1.5$ immunoreactivity in rat MCA and GA as well as DPO-1-sensitive K_{DR} current in smooth muscle cells isolated from MCA and GA. Further, we provide functional data indicating that inhibition of K_{DR} by DPO-1 increases contraction to phenylephrine (PE) and serotonin (5-HT) and reduces vasodilation to acetylcholine (ACh) and sodium nitroprusside (SNP). These data lead us to suggest that DPO-1-sensitive $K_V1.5$ channels play a major role in determining microvascular tone and the arteriolar response to vasoconstrictors and vasodilators.

Methods

Animal care and use

Animal studies were approved by an institutional Animal Care and Use Committee and conformed to recommendations of the National Research Council [31]. Male Sprague Dawley rats (200–250 g) were given access to standard chow and water *ad libitum*. Rats were anesthetized with sodium pentobarbital (150 mg/kg, i.p.). A carotid artery was

cannulated to record mean arterial pressure, as this value was required to calculate the appropriate distending pressure for pressure myography experiments. Animals were euthanized and the MCA and GA were removed. Arteries were stored at −80 °C for molecular analysis or used the same day for patch clamp electrophysiology and pressure myography.

In another set of experiments designed to test the specificity of DPO-1, we used smooth muscle cells isolated from the aortae of wild type and $K_V1.5$ knockout (KO; $KCNA5^{-/-}$) mice. Tissues taken from these animals were kindly provided by Dr. William Chilian (Northeast Ohio Medical University; Rootstown, OH) [32].

Western blots

MCA and GA lysates (20 μg of protein from a single resistance artery) were loaded onto 4–12% polyacrylamide gels, separated by electrophoresis, and transferred to polyvinylidene difluoride membranes. A rabbit primary antibody targeting the intracellular C-terminus of $K_V1.5$ (Alomone; 1:200) and an anti-rabbit secondary antibody conjugated to horseradish peroxide were used. Membranes were incubated with SuperSignal West Dura substrate (Thermo Scientific) and bands were imaged using a G-Box detection system (Syngene).

Immunocytochemistry

To isolate individual smooth muscle cells, rat MCA and GA were treated with a low Ca^{2+} physiological saline solution (PSS) supplemented with (mg/ml) 2 collagenase, 1 elastase, 1 soybean trypsin inhibitor and 1 bovine serum albumin at 37°C for 30 minutes. Low Ca^{2+} PSS contained (mM) 135 NaCl, 5 KCl, 0.36 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, and 5 Tris; pH 7.4. Arteries were then gently passed through the tip of a fire-polished Pasteur pipet to isolate single smooth muscle cells. Smooth muscle cells were pelleted at 400 g for 5 minutes and resuspended in PSS.

Smooth muscle cell suspension (20–30 μL) was allowed to air dry on glass slides at room temperature. Cells were fixed with cold 4% paraformaldehyde in PBS for 20 minutes at 4° C and washed with cold PBS. Fixed cells were permeabilized with 0.3% TritonX in PBS for 30 minutes at room temperature. Detergent was removed with gentle washing in cold PBS. Slides were blocked in PBS containing 1% BSA for at least 1 hr at 4° C. Slides were completely immersed in PBS + 0.5% BSA with or without rabbit primary Kv1.5 antibody (Alomone; 1:200) and kept overnight at 4° C. Slides were washed several times and then completely immersed in PBS containing fluorescently labeled anti-rabbit secondary antibody (Alexa Fluor; 1:500) for 2 hours in the dark at room temperature. After several washes in PBS, the slides were treated with the nuclear stain DAPI and washed again. Smooth muscle cells were imaged at 40x magnification with DIC optics (Zeiss). A xenon arc lamp and rhodamine filter were used.

Patch clamp electrophysiology

Mouse aortae and rat MCA and GA were enzymatically digested as described above, and a drop of cell suspension was placed on a glass chip, cells were allowed to adhere, and then the chip was placed into the recording chamber (volume approximately 300 μl) atop an

inverted microscope. PSS flowed through the chamber at approximately 3 ml/min. The bath solution contained (in mM) 135 Na-isethionate, 5 K-gluconate, 2 $MnCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, 5 Tris; pH 7.4. Whole-cell patch access was obtained using heat polished borosilicate glass pipets with resistances of 2–5 MΩ. Pipets were filled with a solution containing (in mM) 140 K-gluconate, 1 $MgCl_2$, 3 Mg-ATP, 1 Na-GTP, 10 BAPTA, 10 HEPES, and 5 Tris, pH 7.1. Ca^{2+}-activated currents were limited by the nominally Ca^{2+}-free PSS and the 10 mM BAPTA pipette. Cl^- currents were minimized by the anion replacement. Penitrem A (1 μM) and glibenclamide (10 μM) further minimized the contribution of BK and K_{ATP} currents, so that K_{DR} could be studied in relative isolation. Upon whole-cell access, series resistance and capacitance were compensated as fully as possible and a voltage template of −60 mV to +20 mV was applied in 10 mV steps. Currents were filtered at 1 kHz and digitized at 5 kHz.

Pressure myography

Resistance arteries were cannulated and pressurized to 80% of mean arterial pressure (83 ± 3 mm Hg) and visualized using a video microscope, as described previously [2]. Measurements of artery diameter were made using video calipers before and after DPO-1 (Tocris; stock solutions prepared in ethanol). Experiments included determining the response of arteries to increasing concentrations of DPO-1 and the effect of DPO-1 on: a) myogenic responses (40–140 mmHg in random order 20 mmHg steps); b) constrictions to 5-HT in MCA and PE in GA); and 3) dilations to ACh and SNP.

Statistics

Two-way repeated measures analysis of variance (2RM-ANOVA) was used to analyze data, including: a) current-voltage (I–V) relationships in microvascular smooth muscle cells before and after addition of DPO-1 and b) arterial responses to constrictors, pressure, and dilators before and after DPO-1. A value of $p < 0.05$ was considered significantly different and Bonferroni post-hoc analysis followed 2RM-ANOVA to identify specific differences when appropriate.

Results

Expression of $K_V1.5$

Fig. 1 shows $K_V1.5$ protein expression in MCA and GA. Probing for $K_V1.5$ protein in MCA and GA lysates detected a single band between 50 and 70 kDa (Fig. 1A). The predicted molecular weight of $K_V1.5$ is approximately 66 kDa. $K_V1.5$ immunoreactivity was reproducibly detected in MCA and GA (Fig. 1B), and the antibody did not produce non-specific bands (e.g., relatively clear lanes in Fig. 1A and specificity for $K_V1.5$ expressed in HEK 293 cells; Fig. 1C). It is important to note, however, that immunoblot results from whole arterioles can be confounded by the expression of $K_V1.5$ in a number of cell types in the vascular wall. While smooth muscle cells make up the majority of cells in these resistance arteries, the experiments of Fig. 1 do not demonstrate that the detection of $K_V1.5$ is actually due to its presence in vascular smooth muscle. Experiments illustrated in Fig. 2 demonstrate that $K_V1.5$ immunoreactivity is present in isolated vascular smooth muscle cells. Thus, the bands in Western blots are due, at least in part, to a $K_V1.5$ signal derived

from smooth muscle. $K_V1.5$ immunoreactivity was observed in smooth muscle cells from both the MCA (Fig. 2A) and the GA (Fig. 2B).

K_{DR} channels are blocked by DPO-1

To determine the specificity of DPO-1 as a pharmacological tool to inhibit $K_V1.5$-containing K_{DR} channels, we measured whole-cell currents of smooth muscle cells isolated from the aortae of wild type and $K_V1.5$ KO mice (Figs. 3A and B). Current was significantly smaller in cells from $K_V1.5$ KO mice (Fig. 3C), indicating that $K_V1.5$ subunits are important for forming K_{DR} channels in vascular smooth muscle. DPO-1 (1–10 μM) significantly inhibited current in smooth muscle cells both wild type and $K_V1.5$ KO mice (Figs. 3D, 3E, and 3F); however, the effect was much greater in cells from wild type mice. Thus, these data suggest that DPO-1, while not entirely selective, preferentially inhibits $K_V1.5$.

We performed whole-cell patch clamp experiments to investigate the effect of DPO-1 on K_{DR} channel currents in vascular smooth muscle cells isolated from rat MCA and GA. Depolarization elicited robust K_{DR} currents in cells from the MCA and GA (Fig. 4A and B, control). DPO-1 (10 μM) inhibited K_{DR} current in smooth muscle cells from both the MCA and GA (Fig. 4A and B, DPO-1). Fig. 4C and D contain the current-voltage relationships for cells isolated from the MCA and GA, respectively. In cells from the MCA, 10 μM DPO-1 inhibited current significantly at potentials greater than or equal to −20 mV (e.g., current block at 0 mV was 74 ± 4%, n = 6). Similarly, in cells from the GA, 10 μM DPO-1 inhibited current significantly at potentials greater than or equal to −10 mV (e.g., current block at 0 mV was 61 ± 7%, n = 4).

Inhibition of K_{DR} channels constricts resistance arteries

We investigated the effect of K_{DR} channel inhibition on the diameter of resistance arteries with active tone. Additionally, we aimed to determine whether any effects of DPO-1 were mediated by the endothelium, smooth muscle, or both. MCA and GA with and without functional endothelium (arteries were denuded by passing air through the lumen) were cannulated and pressurized to 80% of mean arterial pressure. The resting diameters of intact MCA and GA were 105 ± 3 and 105 ± 1 μm, respectively (n = 4 each), while denuded MCA and GA had resting diameters of 104 ± 1 and 105 ± 1 μm, respectively (n = 3 each). Denuded arteries did not relax significantly upon stimulation with 100 μM acetylcholine (diameter change of −0.3 ± 2.0 and −2.0 ± 1.3 μm in MCA and GA, respectively; compare to Fig. 8 below), indicating functional denudation. When increasing concentrations of DPO-1 (10 nM, 100 nM, 1 μM, and 10 μM) were added to intact or denuded MCA, diameter decreased significantly at every concentration (Fig. 5A; $p < 0.0001$ for effect of DPO-1 on diameter). The presence or absence of endothelium had no effect on DPO-1-induced vasoconstriction (Fig. 5A; $p = 0.79$ for interaction of DPO-1 and endothelium). Similarly, when the same increasing concentrations of DPO-1 were added to GA, diameter decreased at every concentration tested but the endothelium did not influence this constriction (Fig. 5B; $p < 0.0001$ for DPO-1 effect and $p = 0.88$ for interaction with endothelium).

DPO-1 and the responses to vasoconstrictors

5-HT and PE were used to constrict MCA and GA, respectively. We performed concentration-response experiments with these constrictors before and after arteries were treated with 10 μM DPO-1. When pressurized to 80% of mean arterial pressure, MCA diameter was 101 ± 3 μm (n = 8). Compared to control, MCA diameter in the presence of DPO-1 was significantly different at baseline and every 5-HT concentration. GA diameter under control conditions was 105 ± 2 μm (n = 8). DPO-1 constricted MCA to 79 ± 2 μm. GA diameter in the presence of DPO-1 was significantly different at baseline and every PE concentration when compared to the control condition. We observed no tachyphylaxis to repeated challenges with 5-HT and PE, nor did vehicle have any effect on vasoconstriction (Fig. 6C and D; n = 3 each).

DPO-1 and myogenic tone

We investigated the effect of K_{DR} channel inhibition on the myogenic responses of MCA and GA. Arteries (n = 3) were cannulated and subjected to intraluminal pressures between 40 and 140 mmHg (randomized in 20 mmHg steps). Diameter was measured in the steady-state: a) under control conditions (with vehicle); b) after the addition of 1 μM DPO-1; and c) in a Ca^{2+}-free buffer to determine the passive properties. When pressure was lowered, both MCA and GA dilated (Fig. 7A and B). Conversely, when pressure was increased, both MCA and GA constricted (Fig. 7A and B). DPO-1 constricted MCA and GA from their respective control diameters, but myogenic behavior was still observed as described above (Fig. 7A and B). Diameter in the presence of DPO-1 was significantly reduced at any given pressure for both MCA and GA. DPO-1 significantly increased the myogenic tone of both MCA and GA at any given pressure (Fig. 7C and D).

We performed additional myogenic response experiments with another K^+ channel blocker, iberiotoxin, to investigate the possibility that smooth muscle cell depolarization reproduces the effects of DPO-1. GA (n = 5) were pressurized between 40 and 140 mmHg before and after treatment with 1 μM iberiotoxin. With intraluminal pressures of 40, 60, 80, 100, 120, and 140 mmHg, the control diameters were 126 ± 4, 125 ± 4, 123 ± 3, 121 ±3, 118 ± 3, and 116 ±3 μm, respectively. In the presence of iberiotoxin, diameters at the same pressures were all significantly reduced (115 ± 3, 114 ± 3, 112 ± 3, 110 ± 4, 107 ± 4, and 105 ± 4 μm, respectively). This is similar to what we observed with DPO-1. Thus, depolarization is likely the mechanism common to both DPO-1 and iberiotoxin to produce these changes in diameter.

DPO-1 inhibits endothelium-dependent vasodilation and responses to exogenous NO

ACh was used to induce endothelium-dependent vasodilation in MCA and GA. We performed concentration-response experiments before and after DPO-1 (10 μM) treatment in pressurized arteries. In this set of experiments, the baseline diameter of MCA was 101 ± 2 μm (n = 8). DPO-1 significantly reduced MCA baseline diameter to 78 ± 2 μm and impaired vasodilation to ACh (Fig 8A). The baseline diameter for GA in this set of experiments was 103 ± 3 μm (n = 8). DPO-1 significantly reduced baseline diameter to 81 ± 2μm and impaired vasodilation to ACh (Fig. 8B). There are a number of endothelium-derived relaxing factors including nitric oxide (NO), which is responsible for a major portion of

ACh-induced vasodilation of MCA and GA. The data in Figs. 8A and B do not allow one to determine whether DPO-1 impairs the smooth muscle response to NO, or whether DPO-1 reduces the endothelial production of NO. Thus, we used SNP, a drug that releases NO, to cause vasodilation independent of the endothelium. We performed concentration-response experiments before and after DPO-1 (10 μM) treatment in pressurized MCA and GA. The baseline diameter of MCA in this set of experiments was 99 ± 1 μm (n = 3). DPO-1 significantly reduced MCA baseline diameter to 85 ± 2 μm and impaired vasodilation to SNP (Fig 8C). Regarding GA, the baseline diameter for this set of experiments was 102 ± 2 μm (n = 3). DPO-1 significantly reduced baseline diameter to 86 ± 3 μm and impaired vasodilation to SNP (Fig. 8D).

Discussion

Our data support the hypothesis that DPO-1-sensitive K_{DR} channels regulate the tone and reactivity of resistance-sized vessels from rat brain and skeletal muscle; therefore, with the assumption that DPO-1 has preferential selectivity towards $K_V1.5$ channels, the data lead us to suggest that $K_V1.5$ subunits are included in microvascular K_{DR} channels. This conclusion is based on four lines of evidence. First, we demonstrated that rat MCA and GA expressed $K_V1.5$ protein. Second, we confirmed that DPO-1 shows some selectivity towards $K_V1.5$ channels. Third, we documented DPO-1-sensitive K_{DR} currents in smooth muscle cells isolated from rat MCA and GA. Fourth, pressure myography experiments revealed powerful effects of DPO-1-sensitive K_{DR} channels to control diameter, impact myogenic reactivity, and shape responses to vasoconstrictors and vasodilators. We suggest that $K_V1.5$, a DPO-1-sensitive K_{DR} channel, plays a major role in determining microvascular tone and the arterial response to pressure, vasoconstrictors, and vasodilators. This novel finding supports and extends previous studies indicating roles for K_{DR} channels in resistance artery/arteriolar smooth muscle and microvascular tone by examining effects of 4-aminopyridine and/or correolide (Table 1).

The information in Table 1 helped firmly establish that K_{DR} channels regulate microvascular reactivity, but the molecular identity of these channels has remained unclear. Previous studies aimed at determining the molecular correlates of K_{DR} channels are available in smooth muscle from a variety of vascular beds and ranging from conduit arteries to the microcirculation. A general logical framework has evolved for differentiating two major types of K_{DR} channels and it centers on sensitivity to tetraethylammonium (TEA) [9]. TEA-sensitive K_{DR} channels most likely contain K_V2 or K_V3 family members, whereas TEA-insensitive K_{DR} channels are thought to contain K_V1 subunits (including $K_V1.5$ and perhaps also KVβ subunits). Multiple varieties of K_{DR} channels may be present in a single smooth muscle type, but our study addressed only K_V1-containing K_{DR} channels.

In the present study, we observed the expression of $K_V1.5$ protein in resistance arteries of rat brain and skeletal muscle using Western blot techniques (Fig. 1). Further, we demonstrated that $K_V1.5$ immunoreactivity is derived, at least partly, from its expression in isolated vascular smooth muscle cells (Fig. 2). This agrees with findings of Kang et al., who demonstrated $K_V1.5$ expression by Western blot in resistance arteries from rat gastrocnemius and soleus muscles [20]. Our data also correspond to immunhistochemical

data from three other groups examining $K_V1.5$ expression in microvessels or their cells. Cheong et al. observed $K_V1.5$ immunostaining in smooth muscle cells of rabbit cerebral arteries [4]. They observed immunoreactivity for $K_V1.6$ in smooth muscle as well, but $K_V1.2$ had an endothelial-only staining pattern [4]. Albarwani et al. demonstrated $K_V1.2$ and $K_V1.5$ immunoreactivity in single smooth muscle cells isolated from rat cerebral arteries [1]. McGahon et al. reported $K_V1.5$, but not $K_V1.4$, immunoreactivity in smooth muscle cells from rat retinal arterioles [30]. They also observed the expression of $K_V\beta1$ subunits, which transform $K_V1.5$ currents from the K_{DR} type to an A-type waveform [30]. Our data and the studies described above differ dramatically from another effort of Cheong et al. [5], and suggest there may be species- or vascular bed-specific differences in K_{DR} composition. Specifically, in mouse cerebral arteries, Cheong et al. observed no immunostaining for $K_V1.5$; rather they detected $K_V1.2$ in endothelium and $K_V1.3$, $K_V1.4$, and $K_V1.6$ in smooth muscle and endothelium [5]. The lack of $K_V1.5$ in mouse cerebral arteries is at odds with our data showing differences in K_{DR} current in aortic smooth muscle between wild type and $K_V1.5$. KO mice (Fig. 3). However, our data complement those of Fountain et al. who demonstrated smooth muscle-specific expression of $K_V1.5$ mRNA in mouse aorta, as well as correolide-sensitive K_{DR} current in murine aortic myocytes [12].

A major consideration for our study is whether DPO-1 is a $K_V1.5$-specific pharmacological tool. The literature would suggest that it is indeed a selective inhibitor [25,35,37], but we designed a set of experiments to address this point ourselves. Specifically, we isolated smooth muscle cells from the aortae of wild type and $K_V1.5$ KO mice and used patch clamp techniques to assess drug specificity. DPO-1 inhibited K_{DR} current in myocytes from wild type mice (Fig. 3), which suggests that $K_V1.5$ subunits are indeed expressed in murine aortic smooth muscle. However, this conclusion is tempered by the fact that we observed a much smaller, but still significant, inhibition of K_{DR} current in smooth muscle cells from $K_V1.5$ KO mice (Fig. 3). Thus, we suggest that DPO-1 is relatively $K_V1.5$-selective and our conclusion agrees with findings of Lagrutta et al., who demonstrated DPO-1 selectivity for $K_V1.5$ (and native I_{Kur}) over I_{to}, I_{K1}, I_{Kr}, I_{Ks}, and $K_V3.1$ [25]. We observed DPO-1-sensitive K_{DR} currents in smooth muscle cells isolated from rat MCA and GA (Fig. 4). We interpret that to mean that these microvascular K_{DR} channels may contain $K_V1.5$ subunits, which corroborates the expression of $K_V1.5$ protein in MCA and GA (Figs. 1 and 2). DPO-1 right-shifted the reversal of the whole-cell current-voltage relationship (indicating a theoretical depolarization; Fig. 4), which suggests that $K_V1.5$-containing K_{DR} channels may help set resting membrane potential and thus control resting vascular tone.

Accordingly, we observed that inhibition of $K_V1.5$-containing K_{DR} channels with DPO-1 significantly constricted MCA and GA, i.e., altered the vascular tone at a typical, physiological distending pressure (Fig. 5). Moreover, this effect was independent of the vascular endothelium, suggesting that it was mediated directly on vascular smooth muscle. Many of the studies in Table 1 support this idea that K_{DR} channels control resting microvascular tone, but used less selective inhibitors such as 4-AP and correolide (which were the best available tools at the time). None of the studies in Table 1 show that K_{DR} channels control microvascular reactivity to vasoconstrictors, but this is an idea worth testing, as K^+ channels are known to “dampen” or “buffer” excitation in the microvasculature [14]. Thus, we tested the reactivity to vasoconstrictors (5-HT and PE for

MCA and GA, respectively) before and after inhibition of $K_V1.5$-containing K_{DR} channels with DPO-1 (Fig. 6). With the application of DPO-1, MCA and GA had significantly smaller diameters at rest and in the presence of 5-HT or PE (Fig. 6). We interpret this to mean that $K_V1.5$-containing K_{DR} channels might provide negative feedback against vasoconstriction. While not well documented, this is not entirely surprising, as part of the mechanism of 5-HT vasoconstriction may be inhibition of $K_V1.5$ channels [8]. There are no comparable data showing PE-induced inhibition of $K_V1.5$ in the vasculature, but PE inhibits atrial I_{Kur} (thought to be $K_V1.5$) [39]. Luykenaar and colleagues have previously proposed the idea that vasoconstrictors act, in part, by inhibiting K_{DR} currents in cerebral microvascular smooth muscle [26,27]. We suggest that $K_V1.5$-containing K_{DR} channels might be important in determining microvascular responses to a variety of neural and humoral vasoconstrictors.

Another important adjustment that resistance vessels make is diameter changes in response to distending pressure (i.e., myogenic responses). Several of the studies in Table 1 demonstrate that pharmacological inhibition of microvascular K_{DR} channels augments myogenic reactivity. However, using molecular tools (dominant-negative $K_V1.5$ and $K_V1.5$ overexpression techniques), it has been more convincingly shown that $K_V1.5$ channels can affect the myogenic reactivity of rat cerebral arteries [3]. Specifically, Chen et al. have shown that: a) suppression of K_V1-containing K_{DR} channels with correolide enhances cerebral myogenic responses (similar to other studies in Table 1); b) dominant-negative $K_V1.5$ enhances cerebral myogenic responses; and c) overexpression of wild type $K_V1.5$ suppresses cerebral myogenic responses [3]. Those novel findings led us to investigate the effects of DPO-1 on the myogenic response of MCA and GA (Fig. 7). Both MCA and GA displayed typical myogenic behavior. When intraluminal pressure was reduced or increased, both types of arteries dilated or constricted appropriately. Inhibition of K_{DR} channels with DPO-1 constricted MCA and GA from their respective control diameters; however, predictable myogenic responses remained intact. In the presence of DPO-1, diameter was significantly reduced at any given pressure; therefore, the calculated myogenic tone was greater in both MCA and GA across all pressures tested. This is very similar to what has been observed previously with the inhibition of other types of K^+ channels [24].

To test the role of $K_V1.5$-containing K_{DR} channels as end effectors of vasodilation in MCA and GA, we conducted concentration-response experiments with ACh in the presence of DPO-1 (Fig. 8). With endothelial stimulation, mediators (including NO) are released that open vascular smooth muscle K^+ channels, hyperpolarize membrane potential, and dilate the vessel [41]. DPO-1 impaired the response to ACh in MCA and GA, suggesting that $K_V1.5$-containing K_{DR} channels in microvascular smooth muscle are signaling targets in endothelium-dependent vasodilation. An alternative interpretation might be that $K_V1.5$ is also expressed in endothelial cells; therefore, DPO-1 might inhibit endothelium-dependent vasodilation by depolarizing the endothelium and decreasing the driving force for Ca^{2+} entry that is required for production of some relaxing/hyperpolarizing factors. At present, it is unclear what is the individual contribution of nitric oxide, prostaglandins, or other endothelial-derived hyperpolarizing factors, but there is evidence that protein kinase A and G pathways signal to microvascular K_{DR} channels [28]. We add to this line of research by demonstrating that inhibition of DPO-1-sensitive K_{DR} channels attenuates the vasodilator

response to exogenous NO (Fig. 8). This suggests that $K_V1.5$ channels in vascular smooth muscle may be the targets of NO/cyclic guanosine monophosphate/protein kinase G signaling.

We conclude that DPO-1 inhibits K_{DR} channels in microvascular smooth muscle, indicating that these channels contain $K_V1.5$ subunits. Our data indicate that these $K_V1.5$-containing K_{DR} channels help set resting arterial tone and play an important role in microvascular reactivity to pressure and vasoactive agents. Further investigation of $K_V1.5$-containing K_{DR} channels and how they function to regulate arterial tone and reactivity could help us understand how systemic blood pressure is maintained as well shed light on how tissue blood flow is controlled. Additionally, further study of $K_V1.5$-containing K_{DR} channels may reveal therapeutic targets for treating conditions such as hypertension, angina, ischemia, and peripheral artery disease.

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Perspectives

The present study contributes to an existing body of literature concerning the regulation of resistance artery function by K^+ channels, which suggests that Kv1 family members are major components of K_{DR} channels in microvascular smooth muscle. We add to this knowledge by demonstrating that a specific member of the Kv1 family, Kv1.5, is a prominent subunit in microvascular smooth muscle, and moreover, that these K_V1.5-containing channels regulate resistance vessel tone and reactivity. Information about specific K_V channel members in resistance vessels could identify new therapeutic targets in treating cardiovascular diseases. Possibilities include the development of subunit-specific drugs that could be used to modulate inappropriate microvascular tone (e.g., K_V-specific openers to ameliorate hypertension or K_V-specific antagonists to treat hypotension associated with sepsis).

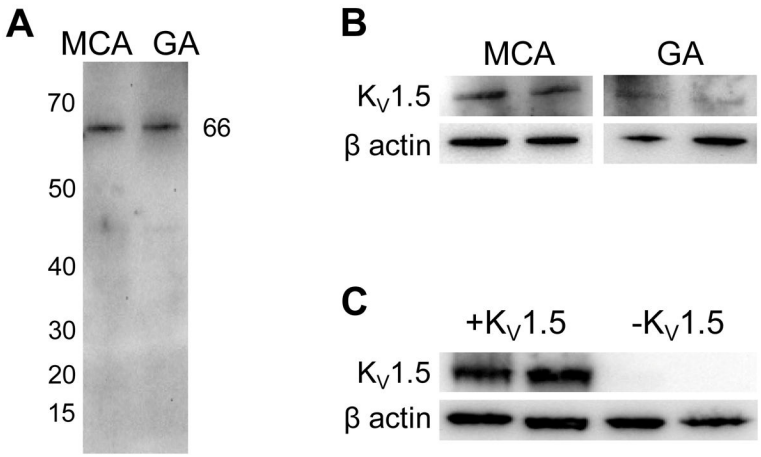

Fig. 1.

K_V1.5 protein in resistance arteries. Panel A contains a representative Western blot. Lysates from single resistance arteries were used. The numbers on the left indicate molecular weights of markers in kDa. A single band of K_V1.5 immunoreactivity was detected near 66 kDa in MCA and GA. Panel B shows results from additional resistance arteries (2 more MCA and 2 more GA) as well as a protein loading control (β actin). Panel C illustrates positive and negative controls for the antibody, as lysates from HEK 293 cells were analyzed. +K_V1.5 indicates cells transfected with human K_V1.5, while −K_V1.5 cells were not.

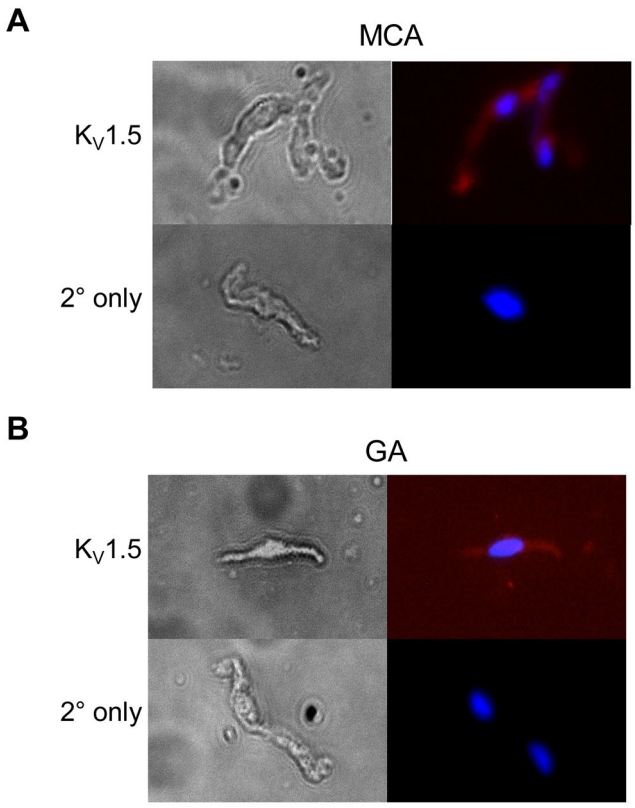

Fig. 2.

K_V1.5 immunoreactivity is present in isolated vascular smooth muscle cells. Panels A and B contain images of cells from the MCA and GA, respectively. DIC images on the left in each panel. Images on the right in each panel are merged fluorescence of K_V1.5 (red) and the nuclear stain, DAPI (blue). The bottom row in each panel shows a control experiment where the primary antibody was omitted.

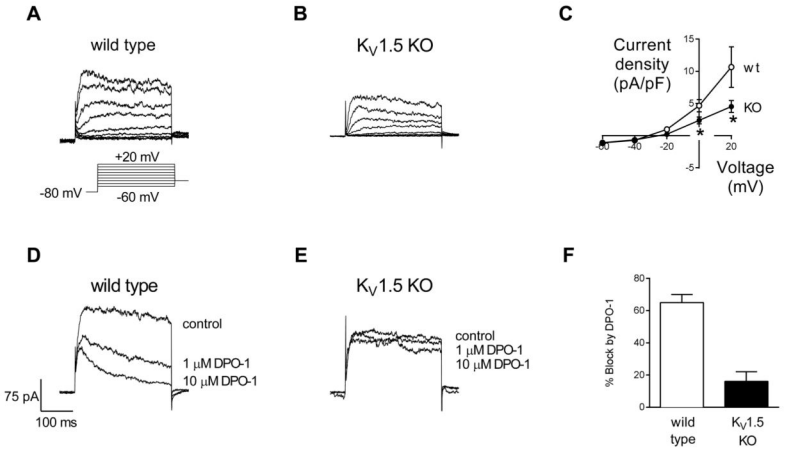

Fig. 3.

DPO-1-sensitive currents are less in smooth muscle cells from K_V1.5 knockout mice. Panels A and B contain current traces from aortic myocytes isolated from wild type and K_V1.5 knockout mice. The voltage template is shown below A. Panel C contains group data (n = 4 mice in each group) illustrating reduced current density in cells from K_V1.5 knockout mice (asterisks indicate $p < 0.05$ by two way ANOVA). Panels D and E contain currents elicited at +20 mV and the effects of 1 and 10 μM DPO-1. Panel F shows group data for inhibition by 1 μM DPO-1. There is significant inhibition in both groups, but the effect is much larger in cells from wild type mice. Traces in panels A, B, D, and E are on same scale, and share the same scale bars in panel D.

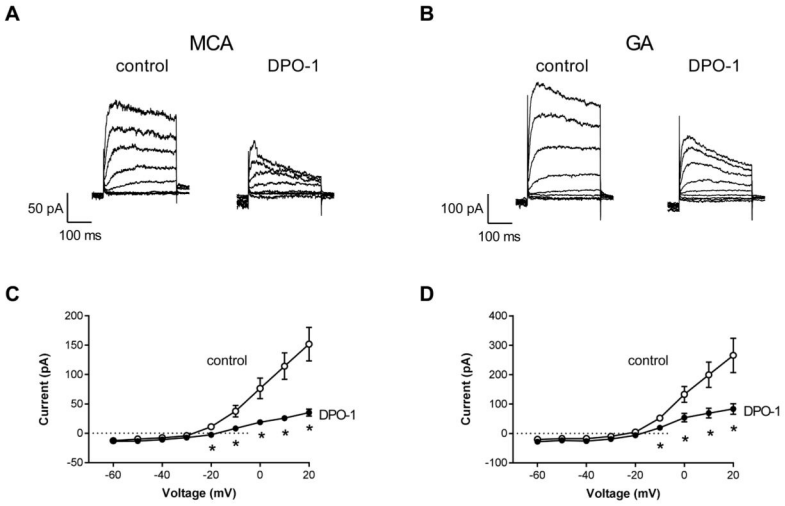

Fig. 4.

DPO-1-sensitive K_{DR} current in smooth muscle from resistance arteries. Current traces from representative smooth muscle cells are shown in panels A (MCA) and B (GA) before and after the addition of 10 μM DPO-1. The membrane potential of cells was stepped in 10 mV increments from −60 mV to +20 mV (holding potential was −80 mV). DPO-1 (10 μM) inhibited K_{DR} currents in smooth muscle cells from both types of arteries. Panels C and D contain group I–V data for cells from the MCA (n = 6) and GA (n = 4). Asterisks indicate voltages at which 10 μM DPO-1 significantly reduced K_{DR} current.

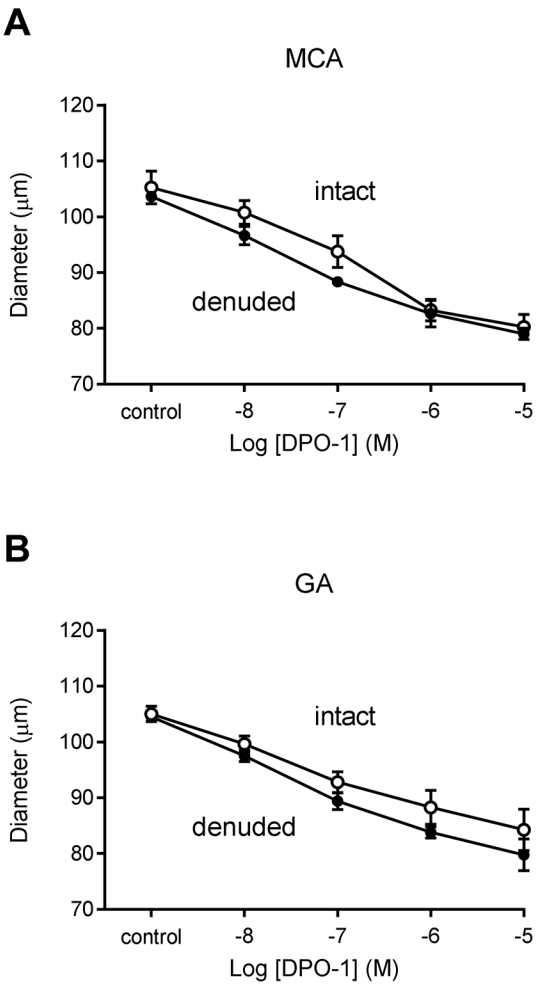

Fig. 5.

Concentration-dependent effect of DPO-1 on artery diameter. MCA (panel A; intact n = 4 and denuded n = 3) and GA (panel B; intact n = 4 and denuded n = 3) were pressurized to 80% of mean arterial pressure for video measurements of diameter. Arteries were treated with increasing concentrations of DPO-1, which elicited constriction. DPO-1 elicited statistically significantly constrictions at every concentration and the endothelium did not influence this response.

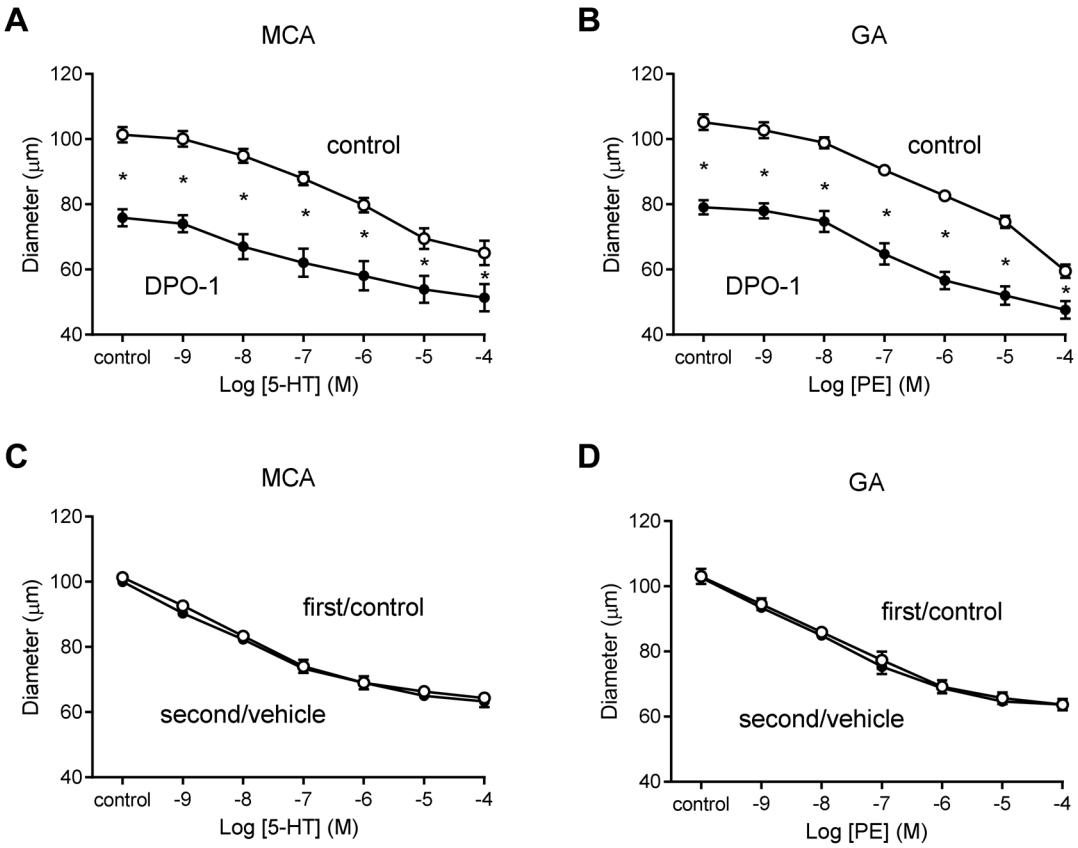

Fig. 6.

DPO-1 and contractions elicited by serotonin and phenylephrine. Inhibition of K_{DR} by DPO-1 (10 μM) augmented constriction to 5-HT in MCA (Panel A; n = 8) and to PE in GA (Panel B; n = 8). Panels C and D contain time and vehicle control experiments (n = 3 arteries in each panel). It was possible to obtain reproducible responses to repeated challenges with 5-HT or PE and the presence or absence of vehicle was without effect.

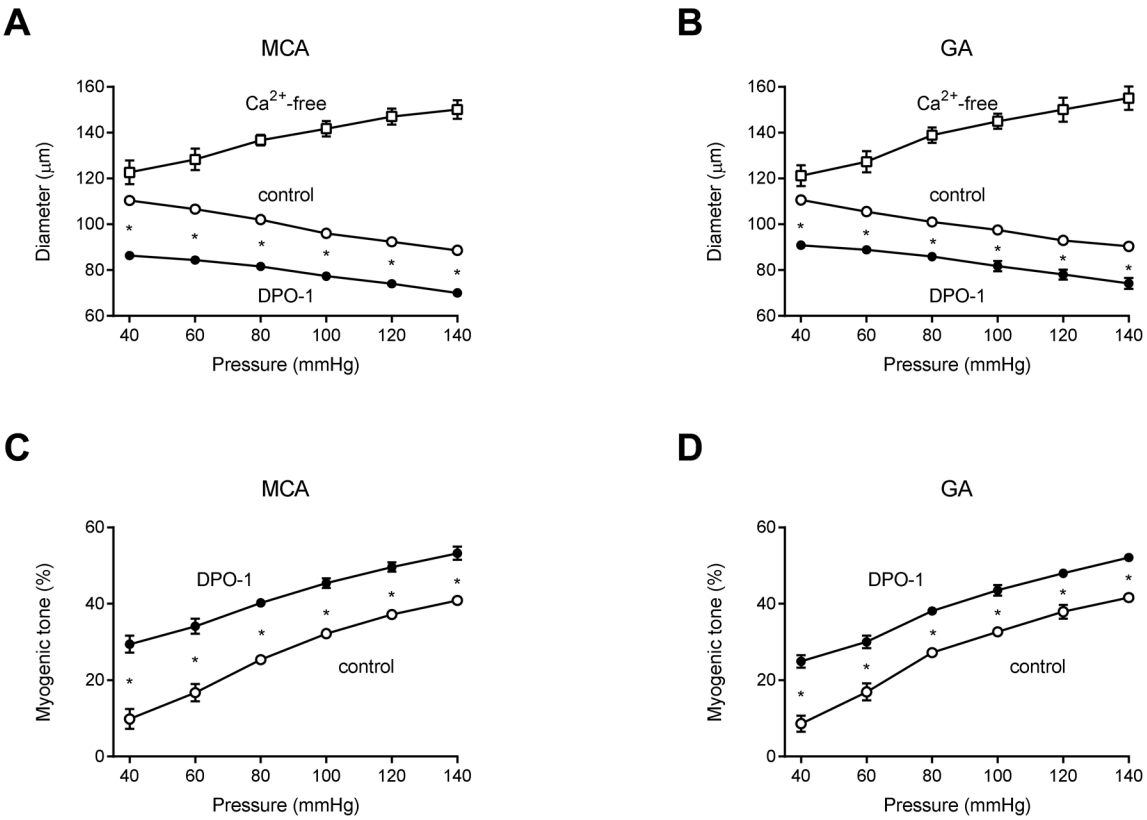

Fig. 7.

DPO-1 increases myogenic tone. Diameters of MCA (Panel A; n = 3) and GA (Panel B; n = 3) were measured when intraluminal pressure was changed between 40 and 140 mmHg. This was done: a) under control conditions (with vehicle); b) in the presence of 1 μM DPO-1; and c) in a Ca^{2+}-free buffer. When DPO-1 was added, diameter was reduced from control at any given pressure (asterisks indicate $p < 0.0001$ by 2-way RM ANOVA). DPO-1 increased myogenic tone at any given pressure (asterisks indicate $p < 0.0001$ by 2-way RM ANOVA) in both MCA (Panel C) and GA (Panel D).

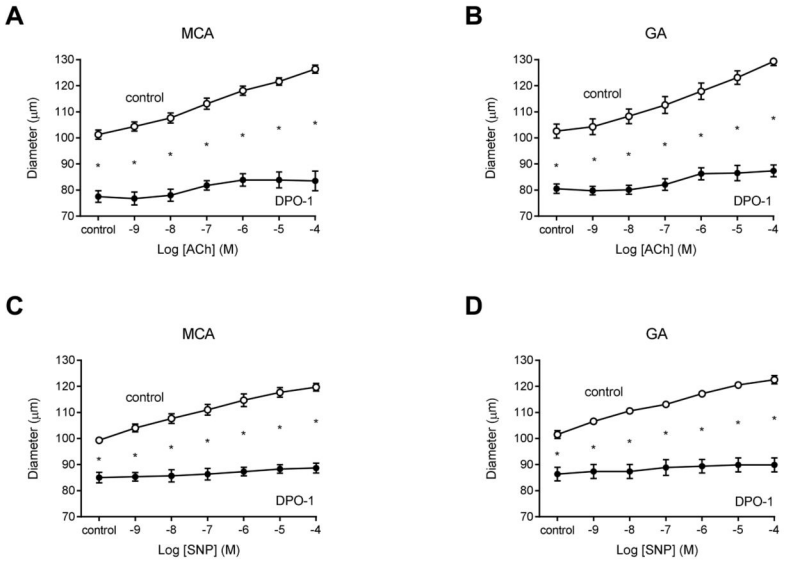

Fig. 8.

Relaxations to ACh and SNP are impaired by DPO-1. Panels A and B contain data for ACh-induced relaxation in MCA and GA, respectively (n = 8 arteries for both panels). 10 μM DPO-1 significantly reduced vasodilation to each concentration of ACh in MCA and GA (asterisks indicate $p < 0.0001$ by 2-way RM ANOVA). SNP-induced vasodilation results for MCA and GA are shown in panels C and D, respectively (n = 3 arteries for both panels). Vasodilation in response to SNP was significantly reduced by 10 μM DPO-1 in MCA and GA (asterisks indicate $p < 0.0001$ by 2-way RM ANOVA).

Table 1

Effects of K_V channel inhibitors on microvascular smooth muscle

Reference	Species	Vessel or bed	Inhibitor	Effect
Knot and Nelson 1995 [23]	Rabbit	Cerebral arterioles	3,4-DAP 4-AP	Depolarize, constrict, alter myogenic reactivity
Jackson et al. 1996 [19]	Rat and hamster	Cremasteric arterioles	4-AP	Block current, depolarize
Kirton and Loutzenhiser 1998 [22]	Rat	Renal afferent arterioles	4-AP	Constrict
Platts et al. 1998 [34]	Rat	Cremasteric arterioles	4-AP	Inhibit dilation
Horiuchi et al. 2001 [16]	Rat	Cerebral arterioles	4-AP	Constrict
Cheong et al. 2001 [4]	Rabbit	Cerebral arterioles	4-AP correolide	Block current, depolarize, constrict
Heaps and Bowles 2002 [15]	Pig	Coronary arterioles	4-AP	Inhibit dilation
Cheong et al. 2002 [6]	Rabbit	Cerebral arterioles	3,4-DAP	Block current, constrict
Albarwani et al. 2003 [1]	Rat	Cerebral arterioles	Correolide 4-AP	Block current, depolarize
Mayhan et al. 2004 [29]	Rat	Cerebral arterioles	4-AP	Constrict
Plane et al. 2005 [33]	Rat	Mesenteric arterioles	4-AP correolide	Block current, alter myogenic reactivity
Rogers et al. 2006 [36]	Rat Dog	Coronary arterioles Coronary blood flow	4-AP	Inhibit dilation
Dick et al. 2008 [10]	Dog	Coronary arterioles Coronary blood flow	4-AP correolide	Inhibit dilation, constrict
Kang et al. 2009 [20]	Rat	Skeletal muscle arterioles	4-AP	Constrict, alter myogenic reactivity