Hydrogen sulfide dilates cerebral arterioles by activating smooth muscle cell plasma membrane K<sub>ATP</sub> channels

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Liang GH, Adebiyi A, Leo MD, McNally EM, Leffler CW, Jaggar JH. Hydrogen sulfide dilates cerebral arterioles by activating smooth muscle cell plasma membrane K<sub>ATP</sub> channels. Am J Physiol Heart Circ Physiol 300: H2088–H2095, 2011. First published March 18, 2011; doi:10.1152/ajpheart.01290.2010.—Hydrogen sulfide (H<sub>2</sub>S) is a gaseous signaling molecule that appears to contribute to the regulation of vascular tone and blood pressure. Multiple potential mechanisms of vascular regulation by H<sub>2</sub>S exist. Here, we tested the hypothesis that piglet cerebral arteriole smooth muscle cells generate ATP-sensitive K<sup>+</sup> (K<sub>ATP</sub>) currents and that H<sub>2</sub>S induces vasodilatation by activating K<sub>ATP</sub> currents. Gas chromatography/mass spectrometry data demonstrated that after placing Na<sub>2</sub>S, an H<sub>2</sub>S donor, in solution, it rapidly (1 min) converts to H<sub>2</sub>S. Patch-clamp electrophysiology indicated that pinacidil (a K<sub>ATP</sub> channel activator), Na<sub>2</sub>S, and Na<sub>HS</sub> (another H<sub>2</sub>S donor) activated K<sup>+</sup> currents at physiological steady-state voltage (~50 mV) in isolated cerebral arteriole smooth muscle cells. Glibenclamide, a selective K<sub>ATP</sub> channel inhibitor, fully reversed pinacidil-induced K<sup>+</sup> currents and partially reversed (~58%) H<sub>2</sub>S-induced K<sup>+</sup> currents. Western blot analysis indicated that piglet arterioles expressed inwardly rectifying K<sup>+</sup> currents (Kir6.1 or Kir6.2) channel subunits, to-gether with four sulfonylurea receptors (SURs), which are hetero-octameric complexes containing four pore-forming, inwardly rectifying (Kir6.1 or Kir6.2) channel subunits, together with four sulfonylurea receptors (SURs), which are ATP-binding cassette family proteins (43, 45, 46). Molecular diversity exists between tissues regarding their K<sub>a</sub> and SUR isof orm (SUR1, SUR2A, and SUR2B) expression and channel composition (4). A number of pharmacological studies have provided functional evidence that smooth muscle cell K<sub>ATP</sub> channels regulate the tone of adult cerebral arteries in vitro and in vivo (20, 21, 33). We recently reported that exogenous and endogenous H<sub>2</sub>S dilates piglet cerebral arterioles in vivo and that this vasodilation is inhibited by glibenclamide, suggesting the involvement of K<sub>ATP</sub> channels (26). Newborn piglet cerebral arteriole smooth muscle cell K<sub>ATP</sub> currents have not been described nor has diameter regulation by these channels been studied in vitro. This study aimed to determine whether newborn arterial smooth muscle cells generate K<sub>ATP</sub> currents. Additional objectives of this study were to determine the effects of H<sub>2</sub>S on K<sub>ATP</sub> currents and to investigate the contribution of K<sub>ATP</sub> channels to H<sub>2</sub>S-induced dilation of arterioles isolated from other influences present in the intact brain.

We show that both pinacidil, a K<sub>ATP</sub> channel activator, and H<sub>2</sub>S activate cerebral arteriole smooth muscle cell K<sub>ATP</sub> currents, leading to vasodilation. Glibenclamide, a plasma membrane and mitochondrial K<sub>ATP</sub> channel inhibitor, reversed pinacidil and H<sub>2</sub>S-induced K<sup>+</sup> current activation and vasodilation. H<sub>2</sub>S-induced vasodilation was attenuated in cerebral arteries of SUR2 null mice, indicating that plasma-membrane K<sub>ATP</sub> channels mediate this response. These data indicate that exogenous H<sub>2</sub>S activates arteriole smooth muscle cell plasma membrane K<sub>ATP</sub> channels containing SUR2, leading to vasodilation.

MATERIALS AND METHODS

Tissue preparation and arteriole smooth muscle cell isolation. All procedures involving animals were approved by the University of Tennessee Health Science Center Animal Care and Use Committee. Newborn pigs (1–3 days old, 1.5–2.5 kg; Nichols Hog Farm; Olive Branch, MS) were anesthetized with ketamine hydrochloride (33 mg/kg im) and acepromazine (3.3 mg/kg im). Mice with a targeted deletion of exons 14–18 in the Abcc9 gene encoding SUR2 (SUR2

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null mice) were generated as previously described (8, 9). Male and female mice (~20 g) were euthanized with an overdose of pentobarbital sodium (130 mg/kg ip). Brains were removed and placed into oxygenated ice-cold (4°C) physiological saline solution (PSS) of the following composition: (in mM) 112 NaCl, 4.8 KCl, 24 NaHCO3, 1.8 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, and 10 glucose, which was gassed with 21% O2-5% CO2-74% N2 to pH 7.4. Piglet pial arterioles (100–250 μm in diameter) were dissected from the cerebral cortical surface. Murine posterior cerebral and cerebellar arteries (~200 μm in diameter) were collected. Arterioles and arteries were cleaned of connective tissue. Individual smooth muscle cells were enzymatically dissociated from cerebral arterioles using a procedure previously described (17). Cells were maintained in ice-cold (4°C) isolation solution and used for experiments within 6 h.

**H2S measurements using gas chromatography/mass spectrometry.** Detection and quantification of H2S released from Na2S and NaHS was made by comparison to standards with known concentrations of H2S. These standards were made by dilution from water saturated with H2S. Saturated H2S solution (10−1 M at 25°C) was made by placing water in a vial (50% of the volume) with the headspace filled with 100% H2S gas. The vial was vortexed well and the headspace gas replaced with 100% H2S three times at 5-min intervals with vortexing to ensure equilibration between the gas and liquid. Aliquots of the H2S-saturated water were used to make standard curves. Because the total H2S in the vial and the volume of liquid are known, the mass spectral peak of the headspace gas would be the same as that of a 300-μl sample containing the same molarity as the standard. H2S in 20 μl of the headspace gas was identified and quantified by gas chromatography/mass spectrometry (GC/MS; 5975C series; Agilent Technologies, Santa Clara, CA). Gas chromatography was run on a 0.32 mm × 30 m fused silica HP-PLOT-U column (Agilent Technologies). The gas chromatography was programmed with an initial oven temperature of 45°C, a ramp to 75°C over 30 s, hold for 1 min, ramp to 85°C over 6 s, and hold for 4 min, before returning to 45°C. The carrier gas was H2 at 3.0 ml/min. H2S area counts against molarity (0.37, 0.74, 1.9, 3.7, 5.6, 9.2, and 18.5 μM in 300 μl buffer) were linear and passed through zero/zero (r2 = 0.998). H2S concentrations in Na2S samples were calculated based on the counts from known H2S samples freshly diluted from saturated stock for each experiment.

Different concentrations of Na2S (1, 10, and 100 μM) were made by dissolving Na2S in buffer containing (in mM) 134 NaCl, 6 KCl, 2 CaCl2, 1 MgCl2, 10 HEPES, and 10 glucose (pH 7.4) at room temperature (25°C). Three hundred microliters of each Na2S sample concentration was placed immediately into a separate 2-ml air-tight vial. To obtain a time course of H2S production, each Na2S sample was vortexed well and kept upside down at 25°C for 1, 10, 20, or 40 min to allow H2S release from Na2S and equilibration of H2S between liquid and headspace gas.

**Patch-clamp electrophysiology.** Isolated arteriole smooth muscle cells were placed into an experimental chamber containing Ca2+-free HEPES buffer and allowed to settle for 10 min. Potassium currents were measured using the whole cell patch-clamp configuration (Axopatch 200B and Clampex 9.2; Molecular Devices, Downingtown, PA). To minimize voltage-dependent K+ currents, KATP currents were recorded at a steady membrane potential of −50 mV using a continuous gap-free acquisition protocol. The pipette solution contained (in mM) 10 HEPES, 102 KCl, 38 KOH, 10 EGTA, 1 MgCl2, 1 CaCl2, 0.1 NaATP, 0.1 NaADP, 0.2 NaGTP, and 10 glucose (pH 7.2 with HCl). The calculated free Ca2+ concentration in this solution is 13 nM (WEBMAXC, Stanford University, Stanford, CA). The bath solution contained (in mM) 10 HEPES, 60 KCl, 80 NaCl, 1 MgCl2, 0.1 CaCl2, 1 tetraethylammonium, and 10 glucose (pH 7.4 with KOH) (Fig. 2, C and D) or (in mM) 10 HEPES, 140 KCl, 1 MgCl2, 0.1 CaCl2, 1 tetraethylammonium, and 10 glucose (pH 7.4 with KOH) (Fig. 2A, B and D). Data analysis was performed off-line using Clampfit 9.2 (Molecular Devices). The bath solution was continuously perfused to maintain constant H2S concentrations in the chamber.

**Pressurized artery diameter measurements.** An arteriole segment ~2 mm in length was cannulated at each end in a temperature-controlled perfusion chamber (Living Systems Instrumentation, Burlington, VT). The chamber was continuously perfused with PSS, equilibrated with a mixture of 21% O2-5% CO2-74% N2, and maintained at 35°C. Arterioles were observed with a charge-coupled device camera attached to an inverted microscope (Nikon TE 200). Arteriole diameter was measured by using the automatic edge-detection function of IonWizard software (Ionoptix; Milton, MA) and digitized at 1 Hz using a personal computer. Steady-state changes in intravascular pressure were achieved by elevating and lowering an attached reservoir and monitored using a pressure transducer. Intraluminal PSS was static during experiments. Tested compounds were applied via chamber perfusion. The presence of intact endothelium was confirmed by observing bradykinin (10 nM)-induced vasodilation.

**Western blot analysis.** To determine total protein, arteries were homogenized in a lysis buffer of the following composition: 50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, and 0.1% SDS. Cellular debris was removed by centrifugation at 8,000 rpm for 10 min. Samples were denatured by adding 5× Laemmli buffer containing 2-mercaptoethanol (2%) and boiled for 3 min. Total protein was loaded onto 7.5% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. Membranes were cut so that the
same lysate could be probed for both Kir6.1 and SUR2B proteins. Membranes were incubated either with goat polyclonal anti-Kir6.1 (1:100, Santa Cruz Biotechnology, CA) or rabbit polyclonal anti-SUR2B (BNJ-40, 1:500, a kind gift from Dr. Jonathan Makielski, University of Wisconsin, Ref. 37) primary antibodies overnight at 4°C in Tris-buffered solution (TBS) containing 0.1% Tween 20 (TBS-T) and 5% nonfat dry milk. After being washed with TBS-T, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 2 h, followed by washing with TBS-T. The membranes were then developed using Supersignal West Pico chemiluminescent substrate (Thermo Scientific), and digital images were obtained using a Kodak FX Pro imaging system.

Chemicals. Glibenclamide and pinacidil were purchased from Tocris (Ellisville, MO). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO).

Data analysis. Values are given as means ± SE. Student’s t-test was used to compare two groups of paired or unpaired data, as appropriate. P < 0.05 was considered significant. Concentration-response curves were fit with a Boltzmann equation to obtain the half-maximal effective concentration (EC50) value.

RESULTS

H2S production from Na2S and reaction time course. GC/MS was used to measure the amount and rate of H2S generation by Na2S, a H2S donor. With a pH of 7.4 and at a temperature of 25°C, Na2S concentrations of 1, 10, and 100 μM produced -0.47, 4.84, and 49.83 μM of gaseous H2S, respectively (Fig. 1A). The regression of H2S concentration versus Na2S concentration was linear (Fig. 1A). After Na2S was dissolved in HEPES-buffered PSS, H2S concentrations in the gas phase reached a maximum level by the first time point (1 min), and there was no significant difference when compared with longer reaction times (10, 20, and 40 min) (Fig. 1B). These data indicate that H2S production from Na2S is very fast with H2S reaching stable levels within 1 min of Na2S addition. Assuming a complete dissociation of Na2S, these data suggest that at pH 7.4 with 1.7 ml gas and 300 μl liquid, gaseous H2S constitutes almost 50% of the total sulfide.

H2S activates KATP currents in cerebral arteriole smooth muscle cells. To test the hypothesis that KATP channels are a target of H2S, K+ currents were measured in isolated newborn piglet arteriole smooth muscle cells using the conventional whole cell patch-clamp configuration. Currents were measured at a physiological steady-state voltage of −50 mV using solutions designed to limit large-conductance Ca2+ -activated K+ channel activity. With 140 mM [K+] in the bath solution, pinacidil (10 μM), a KATP channel activator, increased mean inward current by −75 pA (Fig. 2, A and D). Glibenclamide (10 μM), a KATP channel inhibitor, did not alter K+ currents when applied alone but abolished pinacidil-activated K+ currents (Fig. 2, A and D). Na2S (10 μM) increased mean inward current by −48 pA (Fig. 2, B and D). Subsequent application of glibenclamide reduced mean Na2S-induced K+ currents by −28 pA or by −58% (Fig. 2, B and D). Similar data were obtained when using NaHS, another H2S-releasing molecule, which activated whole cell K+ currents that were partially inhibited by glibenclamide (20 μM) (Fig. 2, C and D). These data indicate that two different H2S donors activate KATP currents in cerebral arteriole smooth muscle cells.

Piglet cerebral arterioles express Kir6.1 and SUR2B subunits and are regulated by KATP channel modulators. Expression of KATP channel subunits in piglet cerebral arterioles was unclear. Western blot analysis of piglet cerebral arteriole protein lysate revealed expression of both SUR2B (−135 kDa mol mass) and Kir6.1 (−51 kDa mol mass) subunits (Fig. 3A). We next studied the myogenic response of piglet cerebral arterioles. At 40 mmHg, arterioles constricted from a mean passive diameter of 239 ± 11 μm to a myogenic diameter of 138 ± 6 μm or by 42% (n = 21). To establish the functional significance of smooth muscle cell KATP channels containing SUR2B subunits and are regulated by KATP channel modulators.

![Fig. 2. Piglet cerebral arteriole smooth muscle cells generate ATP-sensitive K+ (KATP) currents that are activated by H2S. Pinacidil (10 μM, A), Na2S (10 μM, B), and NaHS (20 μM, C) activate glibenclamide (Glib)-sensitive K+ currents. D: mean data: Glib, n = 4; pinacidil and pinacidil + Glib (10 μM), n = 6 for each; Na2S and Na2S + Glib (10 μM), n = 6 for each; and NaHS and NaHS + Glib (20 μM), n = 6 for each. All currents were recorded at a steady holding potential of −50 mV. *P < 0.05 compared with pinacidil, Na2S, or NaHS, respectively.](http://ajpheart.physiology.org/)
Physiological functions of H_{2}S were studied in pressurized (40 mmHg) piglet cerebral arterioles. Pinacidil (10 \mu M) induced a mean vasodilation of \sim 33 \mu m (Fig. 3, B and C). Glibenclamide (10 \mu M) did not alter arteriole diameter when applied alone but fully reversed pinacidil-induced vasodilation (Fig. 3, B and C). These data indicate that smooth muscle cell K_{ATP} channel subunits are expressed and that K_{ATP} channels control diameter in piglet cerebral arterioles.

Glibenclamide attenuates H_{2}S-induced vasodilation in piglet cerebral arterioles. Physiological functions of H_{2}S were studied in pressurized (40 mmHg) piglet cerebral arterioles. Pinacidil (10 \mu M) induced a mean vasodilation of \sim 20 \mu m that was reversible upon washout (Fig. 4, A and C). Na_{2}S-induced vasodilation was also repeatable (a second Na_{2}S-induced vasodilation was 107 \pm 20 \% of the vasodilation induced by a prior Na_{2}S application, \textit{n} = 5, \textit{P} > 0.05, Fig. 4A). Performing a concentration-response curve to Na_{2}S (0.1–1,000 \mu M) indicated an \textit{EC}_{50} of 30.4 \pm 5.3 \mu M (Fig. 4B). Na_{2}S-induced vasodilation was partially (by \sim 55\%) reversed by glibenclamide (10 \mu M) (Fig. 4, C and D). In contrast, glibenclamide did not alter vasodilation induced by isoproterenol, an adrenergic receptor agonist and smooth muscle-specific vasodilator of piglet cerebral arterioles (\textit{P} > 0.05) (22, 28). These data indicate that H_{2}S induces vasodilation via K_{ATP} channel-dependent and -independent mechanisms in isolated newborn cerebral arterioles. In contrast, isoproterenol causes vasodilation via K_{ATP} channel-independent mechanisms.

Na_{2}S- and pinacidil-induced vasodilation is attenuated in cerebral arteries of SUR2 null mice. To investigate molecular targets of H_{2}S-induced vasodilation, we used cerebral arterioles of SUR2 null mice. Pressurized (60 mmHg) arteries from wild-type and SUR2 null mice developed similar levels of myogenic tone (wild-type, 17 \pm 1\%, \textit{n} = 19; and SUR2 null mice, 15 \pm 4\%, \textit{n} = 8, \textit{P} > 0.05). Wild-type and SUR2 null mouse arteries also had similar mean passive diameters of 200 \pm 5 (\textit{n} = 19) and 196 \pm 10 \mu m, respectively, as determined by removal of bath Ca^{2+} at 60 mmHg (\textit{n} = 8, \textit{P} > 0.05). Pinacidil and Na_{2}S diluted pressurized wild-type cerebral arteries by \sim 11 and 10 \mu m, respectively (Fig. 5). In contrast, pinacidil and Na_{2}S diluted pressurized SUR2 null arteries by \sim 2 and 5 \mu m, respectively (Fig. 5). Therefore, mean pinacidil- and Na_{2}S-induced vasodilation was \sim 18 and \sim 50\% of that in wild-type controls. These data indicate that pinacidil- and H_{2}S-induced cerebral artery dilation occurs through plasma membrane K_{ATP} channels containing SUR2.

DISCUSSION

Novel findings of this study are as follows: 1) upon dissolution in aqueous medium, Na_{2}S rapidly converts to gaseous H_{2}S; 2) pinacidil- and glibenclamide-sensitive K_{ATP} currents are present in newborn piglet cerebral artery smooth muscle cells and regulate arteriole contractility; 3) piglet cerebral arterioles express Kir6.1 and SUR2B subunits; 4) exogenous H_{2}S derived from Na_{2}S or NaHS activates K_{ATP} currents in isolated piglet cerebral artery smooth muscle cells; 5) H_{2}S dilates pressurized piglet cerebral arterioles by activating K_{ATP} channels; and 6) pinacidil- and H_{2}S-induced vasodilations are attenuated in cerebral arteries from SUR2 null mice. These data indicate that H_{2}S dilates cerebral arteries and arterioles in part by activating smooth muscle cell K_{ATP} channels containing SUR2 subunits.

In the majority of studies published to date, NaHS has been used as an exogenous H_{2}S donor, with Na_{2}S used to a lesser extent (5). Recently, phase 1 clinical trials in humans have used pharmaceutical grade Na_{2}S for therapeutic treatment of myocardial infarction (31). Here, we used both Na_{2}S and NaHS as H_{2}S donors. These molecules are effective donors that contribute only micromolar Na_{2}S as an additional atom and dilate similarly to H_{2}S gas in vivo (27). For consideration of H_{2}S generated, we used GC/MS of the headspace gas in a sealed vial. With inclusion of a gas phase, the amount dissolved in the liquid will be reduced. In the liquid phase there is dissolved H_{2}S gas, HS^{-} and S^{2-} (H_{2}S+HS^{-}+S^{2-}). The quantity of total sulfide in the vial will remain the same, but the reaction will be shifted to the left as H_{2}S diffuses to the gas phase at equilibrium (equal partial pressures of H_{2}S in gas and liquid). The amount in gas and liquid will be determined by the
quantity of the gas space relative to the liquid quantity, the pH (lower pH moves reaction left), and temperature (assuming barometric pressure is constant). We used GC/MS to measure H$_2$S in headspace gas to determine both rate of production and levels of H$_2$S generated by Na$_2$S. Our quantitative analysis results indicate that Na$_2$S produces H$_2$S quickly, reaching a maximum level in headspace gas within 1 min of being dissolved in pH 7.4 buffer. Based on these data, in the present study cells or pressurized arteries were continuously perfused with fresh donors during experiments to maintain constant H$_2$S concentrations in experimental chambers. With the assumption that all Na$_2$S was ionized, ~50% was gaseous H$_2$S at all Na$_2$S concentrations tested between 1 and 100 $\mu$M. These measurements are somewhat higher than previous reports for dissolved H$_2$S concentrations at neutral pH (33%) and pH 7.4 (40%) in physiological solutions at 25°C (14, 47). The difference could be explained by the experimental conditions. In these experiments the gas volume was 1,700 µl, the liquid volume was 300 µl, the temperature was constant, and the pH was 7.4. Under these conditions the total sulfide was distributed ~50% between the liquid and gas.

Earlier studies and reviews have measured a variety of H$_2$S concentrations in blood from different species: ~10 $\mu$M H$_2$S in Wistar rat blood, ~50 $\mu$M H$_2$S in Sprague-Dawley rat blood, and 10–100 $\mu$M H$_2$S in human blood (11, 30, 51). Millimolar concentrations of H$_2$S or H$_2$S donors have typically been used to study cardiac and pulmonary protective effects of H$_2$S (6, 23). Our data demonstrate that low micromolar H$_2$S concentrations activate K$_{ATP}$ currents in cerebral arteriole smooth muscle cells and induce vasodilation in cerebral arterioles and arteries. We found that 300 $\mu$M and 1 mM Na$_2$S induce an alkaline shift in pH from 7.4 to 7.54 and 7.75, respectively, whereas lower concentrations of H$_2$S donors did not alter pH. Therefore, physiological functions of H$_2$S described here cannot be attributed to pH changes. However, such alterations in pH may explain some results in studies where high H$_2$S concentrations have been used.

K$_{ATP}$ channels regulate the contractility of a wide variety of smooth muscle cells, including those from coronary, pulmonary, mesenteric, and cerebral arteries, as well as bladder and airway (3, 23, 38). To date, no electrophysiological studies have identified K$_{ATP}$ currents in newborn cerebral arteriole smooth muscle cells. Here, we demonstrate that glibenclamide alone did not alter baseline K$^+$ currents or arteriole diameter, indicating that smooth muscle cell K$_{ATP}$ channels do not contribute to baseline K$^+$ currents or contractility in pressurized newborn cerebral arteries. However, pinacidil activated K$^+$ currents and dilated pressurized piglet cerebral arteries and these effects were abolished by glibenclamide. Therefore, we provide the first direct evidence that K$_{ATP}$ channels can generate K$^+$ currents in newborn cerebral arteriole smooth muscle cells and that activation of these currents induces vasodilation.

H$_2$S stimulates K$_{ATP}$ currents in rat mesenteric artery and aortic smooth muscle cells and pancreatic β-cells (45). Our data are the first to demonstrate that H$_2$S activates K$_{ATP}$ currents in cerebral arteriole smooth muscle cells and dilates cerebral arterioles and arteries. Na$_2$S diluted pressurized cerebral arterioles with an EC$_{50}$ of ~30 $\mu$M. In contrast, glibenclamide did not alter isoproterenol-induced vasodilation, indicating that K$_{ATP}$ channels do not contribute to effects of this smooth muscle-specific vasodilator. Recently, using GC/MS, we measured basal H$_2$S concentrations of ~600 nM in piglet cortical surface cerebrospinal fluid (26). Dilator concentrations measured here in vitro are near the range we have also measured in cortical surface cerebrospinal fluid during hyper-
arterioles to topical H₂S had a threshold of consistent with those in vivo.

4-aminopyridine, a voltage-gated K⁺ channel blocker, also attenuated NaHS-induced relaxation in aortic rings (6). XE991, a KCNQ channel blocker, also attenuated NaHS-induced relaxation in aortic rings (39). However, in vivo glibenclamide completely blocked pial arteriole dilation to topical H₂S (26). Therefore, concentrations of H₂S that induced dilation here are greater effectiveness of glibenclamide in blocking dilation to H₂S in vivo than in vitro are not known. One explanation for the slightly different glibenclamide sensitivity may be the size of the arterioles studied in vivo (50 μm) and in vitro (200 μm).

In addition, the relative contribution of K_ATP currents to vasodilation may depend on the H₂S concentration used. Additional experiments will be required to test these hypotheses.

K_ATP channels are comprised of a Kᵦ₆.1/6.2 channel and a SUR1, 2A, or 2B subunit (15). H₂S directly activates recombinant K_ATP channels composed of Kᵦ₆.1 and SUR1 subunits by interacting with NH₂-terminal cysteine residues (18). However, arterial smooth muscle cell plasma membrane K_ATP channels are comprised of Kᵦ₆.1 and SUR2B subunits, as supported by data here and in previous studies (1, 40). In addition, smooth muscle cells likely employ signaling pathways that are distinct from those present in immortalized cells used to express recombinant proteins. Thus H₂S-induced K_ATP channel activation in arterial smooth muscle cells may occur because of direct or indirect mechanisms (18, 45). K_ATP channels are located both on the plasma membrane and on mitochondria (12, 16). Glibenclamide inhibits both plasma membrane and mitochondrial K_ATP channels (49). Therefore, H₂S may have activated arteriole smooth muscle cell K⁺ currents and induced vasodilation by activating plasma membrane and/or mitochondrial K_ATP channels, particularly since the effects of H₂S on both K_ATP channels and mitochondrial function have been described (10, 35, 41). SUR2B does not appear to be a functional component of arterial smooth muscle cell mitochondrial K_ATP channels (1). Therefore, to study K_ATP channels activated by H₂S, we measured the functional responses in cerebral arteries of SUR2 null mice. Vascular smooth muscle cell K_ATP currents are abolished in SUR2 null mice (19). We have previously studied vasoregulation by intravascular pressure and by different K_ATP channel openers in mesenteric arteries of SUR2 null mice (1), but to our knowledge this study is the first to examine functional responses in SUR2 null cerebral arteries. Data indicate that SUR2 null mice cerebral and mesenteric arteries develop similar levels of myogenic tone to wild-type controls, indicating that K_ATP channels do not contribute to the myogenic response (1). Here, we show that wild-type and SUR2 null mouse cerebral arteries dilate similarly to removal of bath Ca²⁺ and have similar passive diameters, consistent with data in mesenteric arteries. These data indicate that SUR2 null arteries do not exhibit generalized alterations in vasodilation. Pinacidil-induced vasodilation was significantly smaller, but not abolished, in SUR2 null cerebral arteries, consistent with data in mesenteric arteries where vasodilation to this K_ATP channel opener was 10% of that in wild-type controls (1). The mechanism for the residual dilation is unclear but suggests a SUR2-independent target for pinacidil in arterial smooth muscle cells. K_ATP channel-independent mechanisms proposed to mediate pinacidil-induced vasodilation include stimulation of plasma membrane Ca²⁺ extrusion, a reduction in contractile apparatus Ca²⁺ sensitivity, and inhibition of GTP-binding protein-coupled phosphatidylinositol turnover (2, 34). Data with SUR2 null arteries indicate that approximately half of the H₂S-induced vasodilation occurs because of activation of arterial smooth muscle cell SUR2-containing K_ATP channels, results that support our evidence using pharmacological modulators. The H₂S-induced vasodilation present in SUR2 null arteries to topical H₂S had a threshold of consistent with those in vivo.

Fig. 5. Pinacidil- and Na₂S-induced vasodilation is attenuated in pressurized (60 mmHg) cerebral arteries of SUR2 null mice. A and B: representative traces illustrating pinacidil (5 μM)- and Na₂S (5 μM)-induced vasodilation in cerebral arteries of wild-type (WT) and SUR2 null mice. C: mean data for pinacidil (5 μM; n = 15 for WT, n = 6 for SUR2 null) and Na₂S (5 μM; n = 14 for WT, n = 4 for SUR2 null). *P < 0.05 compared with WT mice.
arteries is consistent with our data indicating that although glibenclamide fully blocked pinacidil-induced K⁺ current activation and vasodilation, H₂S-induced K⁺ current activation and vasodilation were only partially reversed by glibenclamide. These findings indicate that H₂S also activates K⁺ channels other than K<sub>ATP</sub>, leading to vasodilation. Future studies are needed to identify additional K⁺ channel(s) activated by Na₂S and whether Na₂S also causes vasodilation through K⁺ channel-independent mechanisms.

Murine cardiac myocytes express one full-length (130 kDa) and three short (28, 55, and 68 kDa) SUR2 forms (37, 50). Full-length SUR2 contains two nucleotide-binding domains and together with K<sub>ᵦ</sub>₆.1 or -6.2 generate glibenclamide-sensitive K<sub>ATP</sub> channels. All three SUR2 short forms lack nucleotide-binding domain 1. The 55 kDa form together with K<sub>ᵦ</sub>₆.2 generates glibenclamide-insensitive K<sub>ATP</sub> currents in cardiac myocytes. SUR2 null mouse cardiac myocytes lack full-length SUR2 but express the 55 kDa form in addition to the other two short forms (37). It is unclear whether wild-type and SUR2 null mouse cerebral artery smooth muscle cells express SUR2 short forms. However, glibenclamide partially reversed H₂S-induced vasodilation in wild-type arteries and H₂S-induced vasodilation was attenuated in SUR2 null arteries. Therefore, our data indicate that H₂S-induced vasodilation occurs because of the activation of K<sub>ATP</sub> channels containing full-length SUR2B and K<sub>ᵦ</sub>₆.1. Whether SUR2 short forms contribute to the glibenclamide-insensitive component of H₂S-induced vasodilation and the H₂S-induced vasodilation present in SUR2 null cerebral arteries remains to be determined.

At pH 7.4, H₂S is permeable to plasma membranes because its solubility in lipophilic solvents is about fivefold greater than in water (47). H₂S dissociates in aqueous solution to make the anionic species HS⁻. Both H₂S and HS⁻ may react with many intracellular targets, including peroxynitrite, peroxide, superoxide, metalloproteins, and cytochrome c (36). Therefore, both H₂S and HS⁻ may participate in cerebral arteriole dilation.

In conclusion, our data indicate that functional plasma membrane K<sub>ATP</sub> channels are expressed in newborn piglet cerebral arteriole smooth muscle cells and that H₂S activates cerebral arteriole smooth muscle cell K<sub>ᵦ</sub>₆.1- and SUR2B-containing K<sub>ATP</sub> channels, leading to vasodilation. Our data also suggest that K⁺ channels other than K<sub>ATP</sub> also contribute to H₂S-induced vasodilation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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