Hydrogen sulfide and cerebral microvascular tone in newborn pigs


Laboratory for Research in Neonatal Physiology, Department of Physiology, University of Tennessee Health Science Center, Memphis, Tennessee

Submitted 21 July 2010; accepted in final form 22 November 2010

Hydrogen sulfide (H2S) is a gaseous, endogenously produced, signaling molecule joining NO and CO as gasotransmitters. Data are accumulating that endogenous H2S is involved in control of blood pressure and tone of arteries from certain vascular beds (3, 26, 40, 51, 53). While there are reports linking H2S to neuronal function (18, 20, 22), nothing is known about H2S in regulation of blood flow in the brain.

Knowledge of the role of H2S in regulation of newborn cerebral microvascular circulation and effects of exogenous H2S on cerebral arteriolar tone is particularly important because of potential therapeutic applications of H2S and H2S-releasing molecules (34, 35). H2S is being postulated to be of value for attenuating neuronal injury in cerebral ischemia and vascular dementia because H2S can be anti-apoptotic, reduce cellular metabolism, and produce a hibernation-like state (22, 32, 37, 52).

H2S is produced by catabolism of l-cysteine to H2S, NH3, and pyruvate, primarily by cystathionine γ-lyase (CSE, EC 4.4.1.1) and cystathionine β-synthase (CBS, EC 2.5.1.22), reminiscent of l-arginine metabolism to NO. In general, CSE expression has been detected in blood vessels, and CBS and 3-mercaptopyruvate sulfur transferase (EC2.8.1.2), which can also catalyze H2S production, have been detected in the brain parenchyma (1, 20, 22).

Although there are reports on H2S in adult peripheral arteries, no data exist in the cerebral circulation, arterioles, or in the newborn period. The decrease in blood pressure caused by intravascular H2S (54) and the hypertension of CSE knockout mice (51) suggest H2S has a role in the regulation of resistance vessels.

The mechanism causing H2S-induced dilation of various peripheral arteries has been reported to involve ATP-dependent K+ (KATP) channels (36, 54), a Cl−/HCO3− exchanger (24), and/or endothelium-dependent dilators (10, 53). In adult rat aortic rings, Ca2+-dependent K+ (KCa) and voltage-gated K+ channel inhibitors had no effect on relaxation to H2S, but the relaxation was blocked by glibenclamide (54), an inhibitor of KATP channels (4, 43). Electrophysiological studies of single aortic myocytes detected H2S-induced stimulation of KATP channel currents and hyperpolarization, both of which were blocked by glibenclamide (54). A mechanism reported to be involved in cerebral vascular dilation to acidosis, which is caused by hypercapnia, is KATP channel activation (12, 35, 42, 44, 47).

The present study is designed to address the hypothesis that H2S is a functionally significant, endogenous dilator in the newborn cerebrovascular circulation. Here we report data that newborn pig cerebral arterioles in vivo dilate to H2S and l-cysteine, dilation is blocked by the KATP channel inhibitor...
glibenclamide, CSE is expressed in cerebral vessels and CBS in the brain parenchyma, inhibition of CSE reduces dilation to l-cysteine and hypercapnia, and the brain produces H2S from endogenous and exogenous substrate in sufficient amounts to produce dilation.

METHODS

All experiments involving animals were reviewed and approved by the University of Tennessee Health Science Center Animal Care and Use Committee.

Cranial windows in vivo. These experiments were conducted in vivo using α-chloralose-anesthetized, ventilated, newborn pigs equipped with surgically implanted, closed cranial windows to allow direct observation of the cerebral arterioles leading to the capillaries of the brain parenchyma (26). Briefly, a cranial window with a glass pane was implanted in a craniotomy with retracted dura mater. The space under the window was filled with artificial cerebrospinal fluid (aCSF) (pH 7.35 and PCO2 and PO2 ≈43 mmHg) via ports on the sides of the window frame. Pial arteriolar diameter measurements of one ~50-μm arteriole per piglet were used for analyses (piglet cerebral arterioles penetrate at ~30 μm). Blood pressure, body temperature, blood gases, and pH were monitored and kept within normal limits except when altered blood gases were the treatments.

Investigation of effects of H2S and l-cysteine on pial arteriolar diameter. A saturated H2S-in-water solution (10^-6 M) was diluted in aCSF (10^-6, 10^-5, 10^-4, and 2 x 10^-4 M) and injected under the cranial window. Ascending concentration-response curves were produced by replacing each concentration with the higher concentration at 5-min intervals.

For l-cysteine concentration-response curves (1, 3, 5, and 7 mM), each concentration placed under the cranial window was replaced by the higher concentration at 10-min intervals.

Effects of glibenclamide on dilation to H2S and l-cysteine. The effect of the KATP channel inhibitor glibenclamide on dilation of newborn pig pial arterioles to H2S was determined by measuring arteriolar diameter changes to topical H2S before and in the presence of glibenclamide (10^-6 M). Efficacy of glibenclamide was evaluated by blockade of dilation to the KATP channel agonist pinacidil (10^-5 M) and selectivity by absence of effects on dilations to the Ca channel agonist nNOS-1619 (2 x 10^-6 M), and the β-adrenergic agonist isoproterenol (10^-6 M).

Effects of CSE and CBS inhibition on responses to l-cysteine, hypercapnia, hypoxia, sodium nitroprusside, and isoproterenol. Responses to the CSE/CBS substrate l-cysteine, as above, were measured before and in the presence of the CSE inhibitor d,L-propargylglycine (PPG, 10 mM) and, in separate piglets, the CBS inhibitor amino-oxyacetate (AOA, 1 mM) (33, 40).

Hypercapnia was produced by ventilating for 5 min with 10% CO2, 21% O2, and 69% N2 to produce Paco2 ~75 mmHg. Hypoxia was produced by 5 min ventilation with 10% O2, 90% N2 to reduce Paco2 to ~35 mmHg, while maintaining constant Paco2. Isoproterenol (10^-6 M) and sodium nitroprusside (SNP, 2 x 10^-7 M) were applied topically under the cranial window (5 min).

Determination of the cellular localizations of CSE and CBS in cerebral tissue. For Western immunoblotting, cerebral microvessels (arteriolar branches of the middle cerebral artery, 60–300 μm in diameter) were dissected from the brain and gently cleaned of adjacent tissue. Vessel-free brain parenchyma was prepared by consecutive filtration of the piglet cortex homogenate through 300- and 60-μm nylon mesh filters; the filtrate contains neurons and astrocytes (brain parenchyma), whereas cerebral vessels (60 μm and larger) are retained on the filters. Dissected cerebral microvessels and brain parenchyma were lysed in Laemmli buffer, resolved by 12% SDS-PAGE, and electrotransferred to polyvinylidene difluoride membranes. The membranes were probed with CSE antibody and CBS antibody (1:2,000; Novus Biological, Littleton, CO) followed by peroxidase-anti-mouse IgG (1:20,000; Sigma) and visualized with Western Lightning-ECL (Perkin-Elmer, Shelton, CT).

Immunohistochemistry was performed on the slices of formalin-fixed/paraffin-embedded brain cortex using the avidin-biotin-peroxidase complex technique, as we described previously (26). CSE and CBS immunohistochemical detection was performed using monoclonal anti-human antibodies highly selective to CSE or CBS (1:100 dilution; Novus Biological). The brain sections were then counterstained with hematoxylin and viewed with an Olympus BX50 light microscope.

Measurement of H2S in cerebrospinal fluid. Collections of cerebrospinal fluid (CSF) from under cranial windows were accomplished as we have described for another gasotransmitter, CO (21, 26). CSF was collected after being under the cranial window for 5 min and the vial sealed. H2S in headspace gas was measured by gas chromatography/mass spectrometry (GC-MS).

Standards and samples (400 μl) were collected in N2-filled 2-ml vials with Teflon/rubber septum caps containing three 20 x 4-mm plastic rods to reduce the liquid volume. An 1N-saturated solution of 2% ascorbic acid was added to favor gaseous H2S over the water-soluble ion (HS⁻) and thus accumulation in the headspace gas.

GC-MS analysis of the headspace gas (80 μl) was performed using a Varian 3400cx GC connected to a Varian Saturn 3 mass spectrometer. GC was accomplished on a 30-m fused silica capillary column (0.32 mm ID) coated with SilicaPLOT (Varian CP8567) with H2 as the carrier gas and a temperature ramp of 32 to 50°C over 3.0 min, followed by 2 min of 50°C isocratic and a transfer tube temperature of 50°C. The H2S concentration in unknown samples was calculated based on standard curves diluted from saturated stock (10^-1 M at 24°C).

Also, 10^-5 M H2S was injected under the cranial window and immediately collected. Recoveries from under the cranial window were 70–80%.

Statistical analyses. Comparisons among three or more populations were made using ANOVA for repeated measures and Bonferroni post hoc tests. Comparisons between two groups used paired t-tests. Data are presented as means ± SE.

RESULTS

Exogenous H2S and the CSE/CBS substrate l-cysteine dilated newborn pig pial arterioles in vivo. Topical application of H2S in aCSF concentration-dependently diluted pial arterioles (Fig. 1). The dilation to H2S was blocked by glibenclamide (10^-6 M), which alone did not change pial arteriolar diameter.
Glibenclamide also blocked the dilation to the K_{ATP} channel activator pinacidil (10^{-5} M) but not to the K_{Ca} channel activator NS-1619 (2 × 10^{-5} M) or isoproterenol, a β-adrenergic agonist (10^{-6} M) (Fig. 2). The CSE/CBS substrate l-cysteine also dilated piglet pial arterioles (Fig. 3). The dilation to exogenous l-cysteine was blocked by the CSE inhibitor PPG (10 mM) but was unaffected by the CBS inhibitor AOA (1 mM). In addition to H_{2}S, l-cysteine metabolism produces ammonium and pyruvate. Therefore, we applied ammonium and pyruvate under the window at 2 × 10^{-4} M, the maximum concentration of H_{2}S used. Ammonium and pyruvate had apparent, minimal, dilator effects on pial arteriolar diameters (data not shown). CSE appears to be the enzyme causing H_{2}S production from exogenous substrate leading to pial arteriolar dilation.

To determine the cellular localization of H_{2}S synthesis in the brain, we used highly specific antibodies against human recombinant CSE and CBS proteins (Novus Biologicals). To test the specificity of the antibodies on Western immunoblotting, we used the liver tissue known to express CSE as the major H_{2}S-producing enzyme (20, 34). CSE, but not CBS, is highly detectable in newborn pig liver (Fig. 4). In isolated cerebral microvessels (300–60 μm), Western blots detected CSE but not CBS (Fig. 4). Conversely, CBS is the predominant enzyme in freshly isolated parenchyma (Fig. 4). CSE and CBS distribution was also detected immunohistochemically in newborn pig cerebral cortex. CBS was expressed predominantly in blood vessels, including pial and penetrating arterioles (Fig. 5). Conversely, CBS was expressed in neurons and astrocytes but was not detectable in penetrating arterioles (Fig. 5). CBS was also minimally detectable in larger pial arterioles. Overall, in the newborn brain, CSE is preferentially expressed in the vasculature, whereas CBS is the major isoform in neurons and astrocytes.

H_{2}S production was measured using GC-MS in CSF from the brain surface in vivo. Basal H_{2}S concentration was ~600 nM. Elevation of l-cysteine (5 mM) in the CSF under the cranial window increased H_{2}S concentration nearly fivefold (Fig. 6), coincident with pial arteriolar dilation (Fig. 3).

We measured dilation of newborn pig arterioles in vivo to ventilation with 10% CO_{2}, 10% O_{2}, and to topical SNP (2 × 10^{-7} M), an NO donor, before and in the presence of PPG or AOA. Pial arteriolar dilation in response to 10% CO_{2} was inhibited by PPG but not AOA (Fig. 7). Hypercapnia also increased H_{2}S concentration in CSF, an effect that was blocked by PPG (Fig. 8). In contrast, dilations to hypoxia and SNP were unaffected by PPG (Fig. 9).

**DISCUSSION**

The new findings of this study of newborn pigs include: 1) H_{2}S concentration-dependently dilates cerebral cortical pial arterioles, 2) dilation to H_{2}S is blocked by glibenclamide, 3) topical appli-
cation of l-cysteine dilates pial arterioles, 4) dilation to l-cysteine is blocked by the CSE inhibitor PPG but not by the CBS inhibitor AOA, 5) CSE is predominately expressed in cerebral vessels, 6) CBS is located mainly in neurons and astrocytes, 7) l-cysteine increases H2S in cortical CSF coincident with vasodilation, 8) pial arteriolar dilation to hypercapnia is inhibited by PPG but not by AOA, and 9) hypercapnia increases cortical CSF H2S concentration, and this increase is also inhibited by PPG. These data show for the first time a potential regulatory role of the CSE/H2S system in the cerebrovascular circulation. These data suggest that H2S is a dilator of newborn cerebral arterioles and that endogenous CSE is capable of producing sufficient H2S to decrease vascular tone.

We detected CSE in cerebral vessels and CBS in astrocytes and neurons (Figs. 4 and 5). For detection of enzyme expression in tissues, Western blot and immunocytochemistry each have limitations and strengths. With Western blot, the detected protein can be isolated to a specific molecular weight known to be the weight of the protein of interest, but the localization of the protein in the intact tissue cannot be visualized. By immunocytochemistry, the cellular localizations in the intact tissue can be visualized, but the molecular weight cannot be confirmed. Use of the same antibodies and the two methods provides greater confidence that accurate interpretation is obtained. In the present study, by Western blot, the CSE antibodies stain at the appropriate CSE molecular weight in microvessels but not brain parenchyma, which is principally neurons and glia. With the use of the same CSE antibody, prominent staining was seen in pial and penetrating microvessels in situ but not in the brain parenchyma. Conversely, in brain parenchyma, the CBS antibody bound to a protein at the appropriate molecular weight for CBS. In cerebral microvessels, no binding was detected at the appropriate molecular weight for CBS. With the use of the same antibody, CBS immunostaining was in neurons and glia in situ but not detected in cerebral microvessels. Thus, because we found consistent localizations of CSE and CBS using the same antibodies with two different methods, each with its own strengths and limitations, we feel confident in concluding that CSE is localized to the microvessels and CBS in neurons and glia. The vascular CSE expression in newborn pig brain is consistent with mouse mesenteric arteries (51).

Fig. 5. Immunohistochemistry of CSE (A–D) and CBS (E–H) in newborn pig cerebral cortex detected by the avidin-biotin-peroxidase complex technique. A and B: CSE-positive (brown) pial arterioles on the cerebral surface. B and C: CSE-positive (brown) penetrating cerebral vessels. E: CBS-negative pial arteriole on the cerebral cortical surface and an adjacent CBS-negative venule. F: CBS-negative parenchymal vessel. F and G: abundant CBS-positive (brown) neurons and astrocytes, including pyramidal cells (G). D and H: control slices treated with secondary antibody only. Counterstaining: hematoxylin. A/B, antibody.

Fig. 6. H2S concentration in cerebrospinal fluid (CSF) collected from under the cranial window in control and during application of l-cysteine (5 mM, topically). Data are means ± SE; n = 10 before PPG and n = 2 during PPG (5 mM). *P < 0.05 compared with control.
The effectiveness of CSE blockade was evaluated by determining the effects of PPG on the pial arteriolar responses to topical L-cysteine. L-Cysteine produced dose-dependent vasodilation but had no effect on pial arteriolar diameter in the presence of PPG (Fig. 3), consistent with effective inhibition of H$_2$S production. Conversely, AOA, a CBS inhibitor (33), had no effect on pial arteriolar diameter or dilation to L-cysteine (Fig. 3), suggesting the entire vasodilatory response to L-cysteine may result from metabolism catalyzed by CSE. PPG and AOA were applied at concentrations purportedly selective for CSE and CBS, respectively (33, 40). High concentrations of L-cysteine are required in intact cells and in vivo presumably because L-cysteine does not readily permeate intact cell membranes, and both CSE and CBS are intracellular. Similarly, high concentrations of L-arginine and L-arginine methyl ester are required to cause NO-induced dilation inhibited by N$^G$-nitro-L-arginine methyl ester (L-NAME; see Refs. 7 and 23). The evidence that L-cysteine must be metabolized to cause dilation is provided by blockade of the dilator response to L-cysteine by PPG (Fig. 3) and that L-cysteine is metabolized to H$_2$S is supported by a large increase in CSF H$_2$S concentration measured for the first time by GC-MS (Fig. 6). The lack of effect of AOA does not confirm CBS cannot be involved in regulation of cerebrovascular tone. Even though dilation to exogenous L-cysteine was blocked by PPG, CBS could still be active in cells that are not involved in producing the dilation to topical L-cysteine.

CSE activity is substrate-dependent (Fig. 3). Exogenous L-cysteine increased H$_2$S production (Fig. 6) and diluted newborn pig pial arterioles in vivo (Fig. 3). The CSE inhibitor PPG blocked this dilation. CSE catalytic activity also can be increased by a Ca$^{2+}$/calmodulin-dependent mechanism (51). Two other gasotransmitters, NO and CO, can also affect H$_2$S production. NO increases CSE activity in rat aorta and mesenteric arteries (52), but both NO and CO inhibit CBS activity (36). H$_2$S is metabolized via thiosulfate reductase and the sulfite oxidized to sulfate by sulfite oxidase (20). Further experimentation will be necessary to determine if such interactions are functionally significant in regulation of neonatal cerebrovascular circulation.

H$_2$S dissociates to H$^+$ and HS$^-$ in solution with more as H$_2$S in acidic solutions and more as HS$^-$ at higher pH. The actual
form of \(\text{H}_2\text{S}\) causing the physiological effect is uncertain (18) with the possibility of gaseous \(\text{H}_2\text{S}\) diffusing between cells while an ionic form may interact with channels or second messenger enzymes within cells. Of importance to the present discussion, respiratory acidosis would increase the proportion that is \(\text{H}_2\text{S}\). Increasing the proportion as \(\text{H}_2\text{S}\) would allow the gas to diffuse among cells and have gasotransmitter function.

Concentrations of \(\text{H}_2\text{S}\) detected in tissues and fluids depend upon methods employed and whether those methods measure only gaseous \(\text{H}_2\text{S}\), the total amount of sulfur ions in a liquid, or total tissue sulfur ions. The \(\text{H}_2\text{S}\) electrode used by Yang et al. (51) employs a buffer that converts \(\text{H}_2\text{S}\) and \(\text{HS}^-\) to \(\text{S}^2-\) that is detected, thereby measuring all biological forms of unbound \(\text{H}_2\text{S}\). Yang et al. (51) detected basal \(\text{H}_2\text{S}\) concentration of 4 \(\mu\text{M}\) in serum and 60 \(\mu\text{M}\) in aorta (51). Furne et al. (13) used GC with a chemiluminescence sulfur detector of headspace gas above homogenized mouse brain and liver. They concluded that whole tissue concentrations were very low [\(\sim 15\,\text{nM}\) in the tissue (13)]. Conversely, our measurements of \(\text{H}_2\text{S}\) in headspace gas by GC-MS gave considerably higher \(\text{H}_2\text{S}\) levels in piglet cortical periarachnoid CSF [\(\sim 0.5–1\,\mu\text{M};\) Figs. 6 and 8] and severalfold increases with \(\text{L}-\text{cysteine}\) and hypercapnia (Figs. 6 and 8). We use acidification after sample collection and in quantification standards to promote the gaseous form of \(\text{H}_2\text{S}\). \(\text{H}_2\text{S}\) concentration has not been measured before in CSF or intact brain so direct comparison of the present report with studies of others is not possible.

The potential functional significance of each gasotransmitter needs not be correlated with the absolute concentrations needed to cause dilation when applied topically in vivo. Instead, functional significance would be related to the concentration needed to dilate relative to the in vivo endogenous vascular smooth muscle concentration. The difference between aCSF concentration and concentration near production sites of \(\text{CO}\) will be less than the differences for \(\text{H}_2\text{S}\) and \(\text{NO}\). CO is a stable molecule that can be produced, diffuse some distance through tissue, and accumulate in extracellular fluid. Exogenous CO causes dilation with a threshold of \(\sim 50\,\text{nM}\) and the basal aCSF concentration is \(\sim 20–50\,\text{nM}\). In contrast, concentrations of \(\text{H}_2\text{S}\) and \(\text{NO}\) around the cerebrovascular smooth muscle certainly are much higher than the concentration in CSF. Neither \(\text{H}_2\text{S}\) nor \(\text{NO}\) can diffuse far before reacting in biological systems. In vivo, tissue NO concentrations are not known. Similarly, in the case of \(\text{H}_2\text{S}\), that the spillover \(\text{H}_2\text{S}\) concentration in CSF (basal \(\sim 600\,\text{nM}\)) is near the threshold (1–10 \(\mu\text{M}\)) indicates to us that \(\text{H}_2\text{S}\) concentrations near points of production are in the vasodilatory range and these sites of production may be on the brain surface, for example, endothelium of pial arterioles (Fig. 5).

\(\text{H}_2\text{S}\) and pinacidil, a \(\text{K}_{\text{ATP}}\) channel activator, caused dilation of piglet pial arterioles that was blocked by glibenclamide (Figs. 1 and 2). \(\text{H}_2\text{S}\) may affect many ion channels and receptors, including \(\text{K}_{\text{ATP}}\) channels (45, 53). Recently, \(\text{H}_2\text{S}\) was reported to activate recombinant \(\text{K}_{\text{ATP}}\) channels expressed in HEK-293 cells by interaction with the sulfonylurea receptor (SUR) 1 extracellular NH2 terminus (19). These data may provide a mechanism by which \(\text{H}_2\text{S}\) activates \(\text{K}_{\text{ATP}}\) channels to cause vasodilation, although vascular smooth muscle cell \(\text{K}_{\text{ATP}}\) channels contain SUR2B, indicating that the mechanisms may be different (2). The \(\text{K}_{\text{ATP}}\) channel mechanism for \(\text{H}_2\text{S}\)-induced cerebrovascular dilation might have applicability to our finding that \(\text{H}_2\text{S}\) appears to be involved in pial arteriolar dilation to hypercapnia (Figs. 7 and 8). The probable \(\text{H}_2\text{S}\) effectors, \(\text{K}_{\text{ATP}}\) channels, are less sensitive to pharmacological activation by lemakalim in newborn than in adult cerebral arteries in vitro (42). Therefore, maturational increases in \(\text{K}_{\text{ATP}}\) channel activity could contribute to age-related changes in cerebrovascular contractility (41), which could involve \(\text{H}_2\text{S}\).

In an earlier study, we reported that glibenclamide did not inhibit dilation of piglet cerebral arterioles in vivo to hypercapnia (28). These data do not appear to be consistent with our interpretation of the current data that glibenclamide inhibits dilation to \(\text{H}_2\text{S}\) (Fig. 1), and the CSE inhibitor PPG, which blocks dilation to the CSE substrate \(\text{L}-\text{cysteine}\), inhibits dilation to hypercapnia. We have reexamined the earlier data and concluded that experiments on the effects of glibenclamide on piglet cerebrovascular responses to hypercapnia should be repeated and expanded. While incomplete, these new data suggest glibenclamide attenuates, but does not block, dilation to hypercapnia (38), similarly to PPG in the present study (Fig. 7).

The present results suggest cerebrovascular dilation to hypoxia does not involve CSE/\(\text{H}_2\text{S}\) (Fig. 9). However, only a single level and duration of hypoxia was employed so that additional experiments to determine whether involvement of \(\text{H}_2\text{S}\) depends on the degree and length of hypoxia must be performed. Even though PPG completely blocks dilation to \(\text{L}-\text{cysteine}\), we cannot exclude the possibility that conversion of endogenous \(\text{L}-\text{cysteine}\) to \(\text{H}_2\text{S}\) catalyzed by CBS contributes to dilation to hypoxia. \(\text{H}_2\text{S}\) is involved in fish hypoxic vascular responses (39, 40). In addition, the question of whether other mechanisms are compensating for loss of \(\text{H}_2\text{S}\) responses in the dilation will require further experimentation to exclude a role for \(\text{H}_2\text{S}\).

Control of cerebrovascular circulation is complicated with multiple interacting, sometimes apparently redundant, pathways. Figure 10 is a simplified schematic diagram of two systems that appear to be involved in pial arteriolar dilation to hypoxia.
Hydrogen sulfide (H2S) has been implicated as a physiologically significant dilator in the cerebral circulation. Data are consistent with H2S being involved, but tentatively found no role for NO or CO in dilation to hypercapnia. Prostacyclin and PGE2 plays a direct role in causing hypercapnia-induced elevation of cAMP (29, 30). Whether or not the elevation of cortical CSF sufficiently that higher levels at sites of production are allowed to increase rather than being held constant. Of importance to the present discussion about H2S, the final mediator mechanism in the cerebrovascular smooth muscle cell that is enabled when prostacyclin is held constant is not known. H2S would potentially fill a critical mechanistic gap if it were to be a downstream mediator by activating KATP channels. In contrast to NO in adult rats (50), in the newborn piglet, we have consistently found no role for NO or CO in dilation to hypercapnia (25, 55). Data are consistent with H2S being involved, but interactions with prostacyclin and other pathways will require further investigation (interactions between the two systems are not shown in Fig. 10 because they are speculative and possibilities numerous).

Our data strongly suggest that CSE-derived H2S is a component of regulation of neonatal cerebral vascular circulation. These in vivo data demonstrate that H2S dilates newborn pial arterioles. H2S is detectable in cortical CSF under control conditions and increases during elevation of a dominant regulator of cerebral blood flow, CO2. A blocker of CSE inhibits the dilation to hypercapnia. These data indicate that H2S is a physiologically significant dilator in the cerebral circulation.

GRANTS

These studies were supported National Institutes of Health Grants HL-042851, HL-34059, HL-67061, HL-94378, and NS-046385.

DISCLOSURES

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

REFERENCES


