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Distinct Effects of Intravascular and Extravascular Angiotensin II on Cerebrovascular Circulation of Newborn Pigs

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Abstract

Angiotensin II (AngII) is important in regulation of vascular resistance and control of blood flow among organs and tissues. The effect of AngII on the cerebral microvasculature may be mediated or altered by endothelial-derived signals. The aim of this study is to test the hypothesis that blood AngII dilates neonatal pial arterioles via an endothelial-dependent mechanism but brain AngII can constrict pial arterioles by activating smooth muscle AT₁ receptors. Studies used anesthetized newborn pigs with surgically implanted closed cranial windows. AngII was given either by infusion into the carotid artery ipsilateral to the cranial window or topically. Intracarotid infusion of AngII dilated pial arterioles. The dilation was blocked by systemic administration of the AT₁-receptor antagonist, losartan, but unaffected by topical losartan. Topical AngII also caused dilation, but this dilation was converted to constriction by topical losartan. In piglets pretreated with the angiotensin converting enzyme (ACE) inhibitor, enalapril, topical AngII constricted, rather than dilated, pial arterioles. In enalapril treated piglets, light/dye endothelial injury blocked dilation to intracarotid AngII but did not affect constriction to topical AngII. Either indomethacin or L-NAME blocked the dilation to intraluminal AngII, but neither affected constriction to topical AngII. Chromium mesoporphyrin, that inhibits heme oxygenase, did not affect responses to either topical or intravascular AngII. These data are consistent with the hypotheses that: a) Circulating AngII dilates pial arterioles via endothelial AT₁ receptor-derived relaxing factors, notably prostanooids and NO; b) Direct AT₁ receptor activation on the brain side of the blood brain barrier (BBB) by AngII causes AT₁ receptor mediated constriction that can mask underlying AT₁ receptor-independent dilation when ACE is inhibited. Clinical manipulation of the renin-angiotensin system will have disparate actions on cerebral circulation depending upon the functional integrity of the intima and ACE.

Keywords

endothelium; AT₁ receptor; prostaglandin cyclooxygenase; nitric oxide synthase; angiotensin converting enzyme

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Statement of Author contributions Both authors participated in the design, analysis of the data, interpretation of the studies, and preparation of the manuscript. KRK conducted the experiments and KRK and CWL wrote the manuscript.

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INTRODUCTION

Angiotensin II (AngII) is important in regulation of vascular resistance and control of blood flow among organs and tissues. The strong constrictor action of AngII on systemic vascular resistance beds makes manipulation of the renin-angiotensin system a target in the treatment of congenital and acquired heart disease. In patients with biventricular and univentricular heart disease this system is commonly medically antagonized. Inhibition of the vasoconstrictive effects of AngII decreases the amount of work the heart must perform (5). Conversely, AngII infusion could be employed to increase vascular resistance and mean circulatory filling pressure to elevate both cardiac output and blood pressure, but seldom has been reported (46). Medical antagonism of the renin-angiotensin system may affect cerebral blood flow differently depending upon the extent of blood-brain barrier (BBB) integrity. Cerebral blood flow also factors into treatment of some congenital heart disease. In children born with only a single ventricle part of the surgical palliation includes routing systemic venous return directly to the lungs, bypassing the heart (39), to reduce volume loading of the ventricle and increase effective pulmonary blood flow (15). An intermediate stage in this procedure is the superior cavopulmonary anastomosis in which the superior vena cava is directly anastomosed to the pulmonary arteries providing the only source of pulmonary blood flow (6). With such circuitry pulmonary blood flow is largely dependent on cerebral blood flow as the major contribution to superior caval venous return. Thus, understanding actions of renin-angiotensin system manipulations on cerebrovascular circulation is critical for clinical management of these babies.

Between any circulating agent and the cerebral vascular smooth muscle lie the endothelial cells and internal elastic lamina that comprise the BBB. The effect of any intravascular agent on the cerebral microvasculature may be mediated or altered by endothelial-derived signals. For example, norepinephrine constricts newborn pial arterioles via alpha-adrenergic receptors when applied from the brain side of the BBB (9), but from the vascular side it dilates cerebral arterioles increasing cerebral blood flow via beta-adrenergic receptors (36).

There are four identified AngII receptors, although little is known about AT₃ and AT₄. The AT₁ receptor mediates most of the vasoactive effects of AngII (43). AT₂ is involved in organ development and can cause vasodilation (7). The AT₁ receptor antagonist losartan causes systemic vasodilation altering vascular resistance and blood pressure (17). Conversely, intravascular AngII infusion causes vasoconstriction in most systemic vascular beds via direct smooth muscle contraction of resistance arterioles (29) elevating vascular resistance and blood pressure.

As introduced above, there is reason to believe the endothelium may change the cerebrovascular response to peripherally circulating AngII. AngII has receptors in the brain (37), is known to release the prostaglandin precursor arachidonic acid from membrane phospholipids, and *in vivo* can result in endothelial-dependent vasodilation of rat cerebral arteries (18). Nitric oxide (NO) has been reported as a mediator of cerebrovascular dilation to topical AngII via the AT₂ receptor in newborn pigs (3). NO-mediated reduction of systemic vascular resistance is increased with inhibition of angiotensin converting enzyme (ACE) (28) or AT₁ receptor blockade (13). Vasodilation following blockade of AT₁ receptors may be due to the action of unmasked AT₂ receptors and/or elevation of bradykinin (13). In addition, endothelial-derived hyperpolarizing factor(s) (EDHF) has been shown to be involved in AngII dilation of isolated, pressurized, perfused rat cerebral arteries that remains following inhibition of NOS and COX (41).

The aim of this study is to test the hypothesis that blood AngII dilates neonatal pial arterioles via an endothelial-dependent mechanism but brain AngII can constrict pial

arterioles by activating smooth muscle AT₁ receptors. Thus, we anticipated that intravascular AngII would dilate pial arterioles when the endothelial barrier is intact, but cause constriction following endothelial injury. Furthermore, topical AngII was expected to produce constriction via activation of AT₁ receptors on vascular smooth muscle, but it could cause dilation via AT₂ receptors or endothelial AT₁ receptors.

METHODS

The University of Tennessee Health Science Center's Animal Care and Use Committee approved all animal procedures.

Newborn pigs (1-5 day old) (1-3.5 kg) were anesthetized with ketamine hydrochloride (33 mg/kg IM) and acepromazine (3.3 mg/kg IM), sedation was maintained with α -chloralose (50 mg/kg i.v.). The animals were intubated via tracheostomy and ventilated with air. The femoral vein was cannulated for anesthesia, fluid, and drug injections. The femoral artery was cannulated for continuous blood pressure monitoring and drawing samples for blood gas and pH analysis. The carotid artery ipsilateral to the cranial window was cannulated for antegrade saline and AngII infusion. Blood gases, pH, and body temperature were maintained within normal ranges. Blood gases were obtained prior to initiation of the protocol and following completion of each major segment of the protocol and at protocol completion.

Cranial windows

The scalp was retracted and an opening 2 cm in diameter was created in the skull over the parietal cortex. The dura was cut without touching the brain, and cut edges retracted over the bone so that the periarachnoid space was not exposed to bone or damaged membranes. A stainless steel and glass cranial window was fitted in the hole and cemented in place with dental acrylic. The windows had side needle ports so fluid under the window could be exchanged and test compounds administered topically to the cerebral vessels. The space under the window was filled with artificial cerebrospinal fluid (aCSF) equilibrated with 6% CO₂ and 6% O₂ producing gases and pH within the normal range for CSF (pH ~ 7.34, P_{CO2} and P_{O2} ~ 43 mmHg). Pial vessels were observed through the window with a dissecting microscope. Arteriole diameters were measured with a video micrometer coupled to a television camera mounted on the microscope and a video monitor. Following cannulation piglets in most groups were given a single dose of i.v. enalapril (50 μ g/kg), an ACE inhibitor, to minimize native AngII and to emulate clinical practice. In another group, responses to topical AngI and AngII were measured in piglets not treated with enalapril. All pharmaceutical agents except losartan (Merck Pharmaceuticals, Whitehouse Station, NJ) were obtained from Sigma Chemicals, St. Louis, MO.

Intravascular AngII

Angiotensin II was dissolved in normal saline to 50 μ g/ml concentration. This solution was infused antegrade into the carotid artery ipsilateral to the cranial window. Initial rate was 5 μ g/kg/min and incrementally increased at 5 min intervals to 10, 20 and 50 μ g/kg/min. These doses were selected because the 50 μ g/kg had been shown to improve cerebral blood flow during CPR in immature pigs (29). Blood pressure was continually monitored and kept stable by controlled exsanguination to avoid arteriole diameter changes due to autoregulation. Exsanguinated blood was mixed with heparin and kept warm then returned to the animal following completion of AngII infusion. To control for effects of flow and/or pressure due to the infusion, normal saline was infused into the carotid artery prior to administration of AngII. The rate of saline infusion was slightly higher than the maximum rate to be used for infusion of AngII for each animal.

Topical AngII

AngII was dissolved in aCSF to concentrations of 10^{-8} M, 10^{-7} M and 10^{-6} M. Beginning with the 10^{-8} M each concentration was sequentially instilled under the cranial window in a volume sufficient to fill the space. Washout with aCSF free of AngII was done after each concentration. Vessel diameter was measured and recorded following each instillation to generate a dose-response curve.

AT₁-Blockade

To differentiate between effects of AngII receptors, the AT₁-specific antagonist, losartan, was administered to two groups of piglets. The first group was administered a single 3mg/kg dose i.v., this dose is hemodynamically significant in pigs (22). Ten minutes were allowed to elapse to stabilize blood pressure and pulse. A separate group had 10^{-5} M losartan dissolved in aCSF instilled under the cranial window in a volume sufficient to fill the space. During the topical protocol losartan was re-instilled with the AngII to ensure presence of both. Intravenous and topical AngII were then administered to both groups as described.

Endothelial injury

To delineate the role of vascular endothelium in effects of AngII in piglets, the endothelium was damaged *in vivo*. Sodium fluorescein (160mg/kg in 8ml/kg volume) was injected i.v. and activated with exposure to appropriately filtered light from a mercury arc lamp producing endothelial injury (25). Following injection 30 minutes elapsed to allow uptake by the endothelial cells and the light was directed through the cranial window for 8 minutes. In addition to topical and systemic AngII, piglets in this group were administered topical 10^{-6} M isoproterenol and 10^{-6} M bradykinin, before and after light-dye treatment. Washout with aCSF was done prior to each being instilled. Vasodilation due to bradykinin requires a functional endothelium while the effect of isoproterenol is by direct stimulation of smooth muscle beta-receptors, independent of endothelial cells (44). Loss of dilation to bradykinin showed loss of endothelial function while persistent arteriole reactivity was confirmed with isoproterenol. To ensure fluorescein alone did not affect endothelial function; a group of animals was injected with fluorescein but not exposed to light.

Prostanoids

To block prostanoid synthesis, indomethacin trihydrate was dissolved in normal saline and 10mg/kg was given i.v., a dose sufficient to block dilation to topical arachidonic acid (23) and constriction to topical acetylcholine (2), suggesting sufficient passage across the BBB to be effective. Intravenous and topical AngII were given as above, before and after indomethacin administration. Isoproterenol at 10^{-6} M was applied as part of the topical protocol to assure generalized alteration of vascular responsiveness had not occurred.

The same protocol was repeated in another group of piglets with indomethacin, at 10^{-4} M in aCSF, applied topically.

Nitric Oxide

L-nitroarginine methyl ester (L-NAME), at 10^{-3} M, 10^{-5} M, and 10^{-6} M in aCSF, was administered topically to inhibit NOS. The protocol administering AngII and isoproterenol was repeated before and after administration of L-NAME.

Carbon Monoxide

Chromium mesoporphyrin (CrMP), dissolved to 2×10^{-5} M in aCSF, was applied topically to inhibit production of CO via heme oxygenase (HO) in another group of piglets. This concentration has been shown to inhibit HO activity in neonatal piglets (26). This solution

was protected from light by wrapping aluminum foil around the syringe during administration to prevent inactivation. The protocol administering AngII and isoproterenol was repeated prior to and following CrMP. CrMP (2×10^{-5} M) inactivated via exposure to light for >24 hours was used to act as an additional control.

Statistical Analysis

Data were analyzed using ANOVA for repeated measures and Bonferroni post-hoc analysis to compare mean arteriolar diameter. Statistical analysis was performed using InStat software (GraphPad Software, Inc).

RESULTS

Control

Infusion of normal saline into the carotid artery, at a rate slightly greater than the maximal infusion rate of AngII, had no effect on the diameter of pial resistance arterioles (Fig 1A “Baseline” versus “Control”). Similarly, replacement of aCSF without additional agents under the cranial window did not affect pial arteriolar diameter.

Angiotensin II

Intracarotid infusion of AngII dilated pial arterioles (Fig 1A). The dilation was blocked by systemic administration of the AT1-receptor antagonist, losartan (Fig 1B), but unaffected by topical losartan (Fig 1C).

Conversely, in piglets pretreated with the ACE inhibitor, enalapril, application of AngII directly on surface pial arterioles resulted in vasoconstriction (Fig 2A). In contrast, when piglets were not given enalapril, topical AngII caused vasodilation (Fig 2B). On the other hand, topical losartan not only blocked the constrictor response of pial arterioles to AngII in piglets treated with enalapril but converted the response to concentration dependent dilation (Fig 2C). All other piglets were studied in the presence of enalapril because we were pursuing mechanisms behind the divergent responses of pial arterioles to intravascular versus topical AngII that were revealed by ACE inhibition.

Topical angiotensin I (10^{-6} M) had no effect on pial arteriolar diameter even in piglets not treated with enalapril (51 ± 3 and $51 \pm 3 \mu\text{m}$, before and during angiotensin I, respectively, $n=4$ piglets).

Endothelial Injury

Both isoproterenol and bradykinin caused pial arteriolar dilation before light-dye treatment (Fig 3). Following light-dye, dilation in response to bradykinin was blocked indicating that the endothelium was non-functional. Dilation to isoproterenol was unchanged indicating the arterioles retained the ability to dilate when given a stimulus that acts directly on the smooth muscle. In the animals given fluorescein dye only (i.e. without light activation) there was no change in the response to either bradykinin or isoproterenol (Fig 3).

In piglets with confirmed light-dye injury as in Fig 3, the endothelial injury did not affect the constrictor response to topical AngII (Fig 4A). In contrast, the vasodilatory response to intracarotid infusion of AngII was converted to constriction following light-dye endothelial injury (Fig 4B).

To ensure that uptake of fluorescein did not affect the function of the endothelial cells, a separate group of animals was injected with fluorescein intravenously but protected from light to avoid activating the fluorescein (Fig 3). The same period of time was allowed to

elapse between injection of fluorescein and exposure to AngII as in the animals exposed to light. The response to intracarotid infusion of AngII was no different before and after dye only treatment (50.8 ± 3.6 to $61.6 \pm 3.5 \mu\text{m}$ before and 51.4 ± 3.6 and $62.0 \pm 4.1 \mu\text{m}$ after dye only in response to $20 \mu\text{g}$ AngII/kg/min infusion and 50.3 ± 3.1 to $40.6 \pm 2.7 \mu\text{m}$ before and 52.9 ± 4.8 to $42.5 \pm 3.7 \mu\text{m}$ after dye only in response to topical Ang II (10^{-6}M), respectively).

Characterization of EDRF

Because the data presented above indicate that endothelial derived relaxing factor(s) (EDRF) cause dilatory responses to AngII in piglet pial arterioles, we investigated potential EDRF(s) involved. Previous data indicate that prostanoids, NO, and CO are EDRFs in the newborn cerebral circulation (3, 4, 23, 24, 26, 27, 44), so the COX/prostanoid, NOS/NO, and HO/CO systems were addressed.

Prostanoids

Indomethacin, both systemic and topical, blocked the response to intracarotid infusion of AngII (Fig 5 A & C). Conversely, the constrictor response to topical AngII was not altered by either systemic or topical indomethacin (Fig 5 B & D). There was no change in the vasodilatory response to topical isoproterenol before and after treatment with either systemic or topical indomethacin (data not shown).

Nitric Oxide

Similarly to indomethacin, topical L-NAME (10^{-3}M) blocked the vasodilatory response to intracarotid AngII infusion (Fig 6A). In contrast, 10^{-6}M L-NAME had no effect on pial arteriolar dilation to intracarotid AngII infusion (at 0, 10, and $20 \mu\text{g/kg/min}$ AngII: 59 ± 8 , 66 ± 8 , and $71 \pm 8 \mu\text{m}$ before and 60 ± 6 , 68 ± 8 , and $69 \pm 8 \mu\text{m}$ after 10^{-6}M L-NAME, respectively). However, a ten fold higher L-NAME concentration (10^{-5}M) did inhibit dilation in response to intracarotid infusion of AngII (at 0, 10, and $20 \mu\text{g/kg/min}$ AngII: 58 ± 10 , 62 ± 12 , and $66 \pm 12 \mu\text{m}$ before and 57 ± 11 , 57 ± 11 , and $57 \pm 11 \mu\text{m}$ after 10^{-5}M L-NAME, respectively).

Pial arteriolar constriction to topical AngII administration was unchanged when NOS was inhibited (Fig 6B).

L-NAME (10^{-3}M) had no effect on the vasodilation response to topical isoproterenol (data not shown).

Carbon Monoxide

Inhibition of CO production with CrMP ($2 \times 10^{-5}\text{M}$, topically (26)) had no effect on pial arteriolar responses to either topical or intracarotid AngII (Fig 7). As a control, CrMP inactivated by light was administered topically. Piglets were administered AngII topically and then systemically, first in the presence of inactivated CrMP and then, following washout with aCSF free of additional substances, with active compound, CrMP.

DISCUSSION

Novel findings of the present investigation in newborn pigs are: 1) Intravascular AngII dilates pial arterioles via an endothelial and AT_1 receptor-dependent mechanism; 2) Topical AngII causes pial arteriole dilation in untreated piglets, but AT_1 receptor-dependent constriction following ACE inhibition; 3) Either COX or NOS inhibition blocks pial arteriolar dilation to intraluminal AngII, but neither affects vasoconstriction to topical AngII; 4) Heme oxygenase inhibition has no effect on pial arteriolar responses to either intraluminal or topical AngII. These data are consistent with the hypotheses that: a)

Circulating AngII dilates pial arterioles via endothelial AT₁ receptor-derived relaxing factors, notably prostanoids and NO; b) Direct AT₁ receptor activation on the brain side of the BBB by AngII causes AT₁ receptor mediated constriction that can mask underlying AT₁ receptor-independent dilation.

These are the first data regarding the effects of intra-arterial infusion of AngII on cerebrovascular circulation *in vivo*. Our results suggest that AT₁-receptor inhibition to decrease after-load on a failing heart (Introduction) may block AngII-induced cerebral arteriole dilation potentially decreasing cerebral blood flow. This is especially pertinent in children whose pulmonary blood flow is heavily dependent on cerebral venous return. In addition, cerebrovascular endothelial injury, as can be caused by numerous pathological conditions involved in hemodynamic instability in pediatric critical care settings, will likely block the AT₁ receptor-induced cerebrovascular dilation, causing AngII-induced constriction and compromising cerebral blood flow.

Previous studies have reported on the effect of topical AngII on pial resistance arterioles. Similarly to the present report in piglets without ACE inhibition, Meng and Busija (30) and Baranov and Armstead (3, 4) found topical AngII caused pial arteriolar dilation. This dilation was blocked by inhibition of AT₂ receptors (3). These studies also reported contributions of prostanoids and NO to AngII-induced pial arteriolar dilation (3, 4, 30). We found dilation mediated by relaxing factors also, but vasoconstriction in response to topical AngII application following ACE inhibition with enalapril. Present data indicate this constriction is not mediated by the endothelium. Neither indomethacin nor L-NAME affected the constriction to topical AngII in enalapril treated piglets. In contrast, both indomethacin and L-NAME inhibited the endothelium-mediated dilator response to systemic AngII.

The appropriate dose of L-NAME for topical application to the brain *in vivo* is difficult to determine. We have typically used 10⁻³M because it blocks dilation to L-NAME without altering dilation to hypercapnia or isoproterenol, suggesting selectivity (10). Similarly, Pelligrino et al used 10⁻³ M in adult rats that blocks dilation to acetylcholine but doesn't change dilation to either SNP or moderate hypoxia (32). Others have used 10⁻⁶M (N^ω-nitro-L-arginine (L-NNA) rather than L-NAME) (3) or 10⁻⁴ M (12). Of applicability to the present discussion, Faraci and Heistad (14) found concentration-dependent, 10⁻⁵ M versus 10⁻⁴ M L-NAME, inhibition of basilar artery dilation to acetylcholine, suggesting topical 10⁻⁵ M L-NAME was insufficient to maximally inhibit NOS of a large cerebral artery *in vivo*. In the present study, 10⁻³ M and 10⁻⁵M, but not 10⁻⁶ M L-NAME, inhibited dilation to intravascular AngII but did not alter basal pial arteriolar diameter or dilation to isoproterenol. The difference between the inhibition of dilation to AngII by L-NAME in the present study and the lack of effect of L-NNA observed by Banarov and Armstead (3) could result from the different concentrations of the NOS inhibitors used rather than the route of AngII administration.

The responses of pial arterioles to agonists, inhibitors, and receptor blockers are dependent upon the side of the BBB to which they are applied and the integrity of the barrier. Thus, intravascular AngII dilated pial arterioles and this dilation could be blocked by systemic losartan but not by topical losartan, suggesting the AT₁ receptors providing the dilatory signal may be on the luminal endothelial face. While losartan can cross the BBB with chronic administration, losartan crosses the BBB more slowly than other more lipophilic AT₁ receptor blockers, requiring hours not minutes for efficacy (8, 35, 42). Losartan has no partial agonist effect and approximately 1000-fold greater affinity for AT₁ than AT₂ (33). The constriction to topical AngII seen in enalapril pretreated piglets was reversed to dilation with topical losartan, a response identical to that seen in piglets not pretreated with the ACE

inhibitor. The action of enalapril to convert constriction to dilation must be on the endothelium because enalapril does not cross the BBB (11,34). That the endothelium generates the dilator signals was confirmed by converting the vasodilator response to intravascular Ang II to constriction by selective endothelial injury. Efficient endothelial injury in the pial arterioles under the cranial window was confirmed by total loss of dilation to the endothelial-dependent dilator, bradykinin (44), but no effect on the dilation to endothelial-independent dilator, isoproterenol (25), that dilates via activating of vascular smooth muscle β -adrenergic receptors. Consistent with the present functional studies, Wackenfors et al (41) detected AT₁ and AT₂ receptors on endothelium. AngII receptors appear to be on the vascular smooth muscle also because AngII causes constriction following endothelial injury (Fig 4).

The mechanism(s) by which enalapril alters the pial arteriolar responses to AngII on the brain side of the BBB are not known. ACE-inhibitors are known to effect cerebral autoregulation in the short term, decreasing the lower limit and decreasing or leaving unchanged the upper limit (31). Enalapril increases cerebral blood flow in adult patients in heart failure without an increase in cardiac output (20). While we controlled for the effect of autoregulation by keeping systemic mean arterial pressure constant, enalapril may effect local renin-angiotensin action that is somehow involved in the dilation. However, as the effect of ACE would be in the amount of AngII available to receptors it seems unlikely that enzyme inhibition would change the response of a receptor when stimulated. Activation of AT₂ receptors can cause vasodilation independent of AT₁ and prostanoids in piglets (3). Therefore, stimulation of the AT₂ receptor may cause vasodilation in contrast to AT₁-mediated constriction, as found in the present study with topical application of AngII in losartan treated piglets. Similarly, in adult mice topical AngII caused AT₁ receptor mediated pial arterial constriction, but vasodilation could be evoked by AT₂ receptor stimulation (16). AngII can attenuate endothelial-derived vasodilator responses (16), but the effect of the endothelium on action of AngII had not been previously clarified. ACE inhibition has been shown to amplify the endothelial derived dilator responses in hypertensive rats reducing reactive oxygen species (28) and could also involve elevations of bradykinin (13) that dilate piglet pial arterioles via endothelial produced prostacyclin that increases cAMP (44).

In the search for EDRFs involved in the dilatory action of intraluminal AngII, we found pathways either of whose inhibition totally blocked the pial arteriolar dilation to AngII, COX/prostanoid (presumably prostacyclin) and NOS/NO. The role of NO in dilation of piglet pial arterioles to AngII has been described before, and that was via AT₂ receptors (3). There are several reasonable possibilities for either COX or NOS inhibition alone to completely block dilation to AngII. COX products could stimulate NOS to elevate NO that produces dilation or conversely NO may stimulate COX. While possible, we have no evidence for this hypothesis. Also, the newborn cerebral circulation is very much prostanoid dominant with NO becoming of more significance with age (44). It is conceivable that the effect of removal of one pathway is masked by increased activity of the other. We do have evidence that removal of COX elevates the role of NO in the newborn cerebral circulation, but on a time scale of days not minutes (47). A third possibility has more supporting evidence. In the newborn pig cerebrovascular circulation CO is a functionally highly significant dilator (27). However, either indomethacin or LNA will completely block dilation to CO (24). Constant levels of either NO or prostacyclin will completely restore dose-dependent dilation to CO following indomethacin and/or LNA. These permissive roles of PGI₂ and NO are both accomplished via protein kinase G (21, 27). However, in the present dilation to AngII, HO/CO is not involved because CrMP had no effect on the dilation to AngII. In addition to prostanoids, NO, and CO, other endothelial dependent dilator mechanisms potentially contribute to regulation of cerebrovascular circulation. Of

particular note is (are) EDHF that cause(s) dilation of cerebral arteries and arterioles by hyperpolarization and involves K^+ channel activation and gap junctions (1). EDHF has been shown to be involved in AngII dilation of isolated, pressurized, perfused rat cerebral arteries that remains following inhibition of NOS and COX (41). In the present studies, the complete blockade of dilation to intravascular AngII by either LNA or Indo may suggest a permissive role for either or both of these mediators leaving open the possibility that additional endothelial-derived relaxant mechanisms, such as those underlying EDH activity, could also be involved in the AT1-mediated dilation.

In summary, the endothelium alters the response of pial arterioles to AngII in neonatal piglets. Topical AngII can cause either constriction or dilation depending on whether ACE is blocked or not, respectively. Intraluminal AngII produces AT1 receptor mediated, endothelium-dependent dilation that is totally blocked by either COX or NOS, but not HO, inhibition. Clinical manipulation of the renin-angiotensin system will have disparate actions on cerebral circulation depending upon the integrity of the BBB and ACE that requires further attention.

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Fig 1.

Effect of intracarotid AngII infusion on pial arteriolar diameters of newborn pigs. Baseline is before and Control is during saline vehicle infusion. A) Without losartan. B) Following 3mg/kg losartan, i.v. C) During topical losartan (10^{-5} M). Mean $\pm$ SEM. n=6, 6, 5 piglets in A), B), and C), respectively. * $p < 0.05$ from control.

Fig 2.

Effect of topical AngII on pial arteriolar diameters of newborn pigs. A) Piglets pretreated with enalapril (50 μg/kg, i.v.). B) Untreated piglets. C) Piglets treated with enalapril (50 μg/kg, i.v.) and topical losartan (10^{-5} M). Mean ± SEM. n=6, 4, and 6 piglets in A), B), and C), respectively. * $p < 0.05$ from baseline.

Fig 3.

Effect of sodium fluorescein (FI)(160 mg/kg, i.v.) and light from a 100W mercury arc lamp filtered for maximal transmission between 420 and 430 nm for 8 min (Light) on pial arteriolar responses to topical isoproterenol (10^{-6} M) and bradykinin (10^{-6} M). Controls were before treatment and Light/dye (FI&Light) is following dye and light (n=3). In separate animals (n=4), responses were measured before and after fluorescein alone without light activation (FI only). Mean $\pm$ SEM. *p<0.05 from respective baseline

Fig 4.

Effect of light-dye endothelial injury on pial arteriolar response to AngII. AngII at increasing concentration was A) applied topically or B) infused intracarotidly prior to and following exposure to fluorescein and light. Mean $\pm$ SEM. n=3 piglets. *p<0.05 from baseline

Fig 5.

Effect of indomethacin on pial arteriolar responses to AngII. Indomethacin was administered intravenously (10mg/kg) (A and B, n=6) or topically (10⁻⁴M) topically (C and D, n=5).

AngII was infused intracarotidly (A and C) or applied topically (B and D). Mean ± SEM.

*p<0.05 from baseline.

Fig 6. Effect of topical L-NAME (10⁻³M) on pial arteriolar responses to A. intracarotid AngII and B. topical AngII. Mean ± SEM. n=3 piglets. *p<0.05 compared to appropriate baseline.

Fig 7.

Effect of inhibition of HO with topical chromium mesoporphyrin (2×10^{-5} M, CrMP) on piglet pial arteriolar responses to A. intracarotid AngII or B. topical AngII. The effect of inactivated CrMP was determined before active CrMP in the same piglets. Mean $\pm$ SEM. n = 5. *p<0.05 from baseline.