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Selective Blockade of AT1 Receptor Attenuates Impairment of Hypotensive Autoregulation and Improves Cerebral Blood Flow after Brain Injury in the Newborn Pig

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Background: **Fluid percussion injury (FPI) in piglets produces vasoconstriction of pial arteries (PAs), decreases in cerebral blood flow (CBF), and impairment of hypotensive autoregulation. Two types of angiotensin II receptors, AT1 and AT2, have been identified in the brain. This study characterized the effect of pretreatment with AT1- and AT2-selective antagonists on CBF and hypotensive autoregulation after FPI.**

Methods: **Fluid percussion injury was induced in chloralose-anesthetized newborn pigs equipped with closed cranial windows. CBF was determined by the radiolabeled microsphere technique.**

Results: **Moderate and severe hypotension (71 ± 3, 53 ± 2, and 40 ± 1 mmHg for normotension, moderate hypotension, and severe hypotension, respectively) elicited PA dilation without changes in CBF in sham control piglets. The AT1 antagonist ZD 7155 partially restored impaired hypotension-induced PA dilation after FPI (19 ± 1 and 34 ± 1 *vs.* 5 ± 1 and 7 ± 1 *vs.* 12 ± 1 and 20 ± 3% for PA dilation during moderate and severe hypotension in sham control, FPI, and FPI + ZD 7155 animals, respectively). ZD 7155 also blunted the reductions in CBF during normotension and hypotension observed in untreated animals (43 ± 4, 38 ± 5, and 55 ± 3 *vs.* 32 ± 4, 19 ± 2, and 27 ± 5% CBF reductions during normotension, moderate hypotension, and severe hypotension in untreated and pretreated animals, respectively). The AT2 selective antagonist PD 123,319 did not restore hypotension-induced PA dilation and did not prevent decreases in CBF observed during normotension and moderate and severe hypotension after FPI.**

Conclusion: **These data indicate that blockade of the AT1 and not the AT2 receptor diminished the reduction in hypotensive PA dilation after FPI. AT1 blockade also blunted the decrease in CBF during normotension as well as the further decrease in CBF observed during hypotension after FPI. These data suggest that AT1 receptor activation by angiotensin II contributes to cerebrovascular dysregulation during hypotension after FPI.**

TRAUMATIC brain injury (TBI) is one of the leading causes of mortality and long-term morbidity in adults and children. In infants, however, the consequences of head injury are particularly severe, and the clinical course is different from that of adults. Infants usually die of brain injury or remain neurologically impaired.[1] Fluid percussion brain injury (FPI) is an experimental model for blunt head trauma.[2] Although the effects of brain injury have been well documented in adult animal models,[3,4] fewer data have been reported on the effects of brain injury on the brain circulation in the newborn or the mechanisms underlying such changes. For example, it has been shown that FPI in newborn pigs produces vasoconstriction of pial arteries and reduction of cerebral blood flow (CBF).[5]

Angiotensin II (AII), one of the main hormones regulating cardiovascular and fluid homeostasis as well as angiogenesis,[6] is also present and locally produced in the brain.[7] Two types of AII receptor subtypes, AT1 and AT2, have been identified in the brain, based on their differences in pharmacologic and biochemical properties.[8,9] The AT1 subtype is thought to mediate a wide variety of well-known effects of AII in the brain, such as regulation of cerebrovascular tone, thirst, and vasopressin release.[8] The effects of AT2 activation in the brain are less well understood. The density of AT2 is high in fetal brain tissue but rapidly declines in the postneonatal period.[10] However, brain ischemia and certain brain lesions have been shown to cause a dramatic up-regulation of AT2 receptor expression in the adult brain of several species.[11,12] Interestingly, inhibition of angiotensin-converting enzyme or selective AT1 long-term blockade resulted in improved outcome in stroke patients[13] and in reducing the infarct volume after experimental focal cerebral ischemia.[14] Also, experimental stroke resulted in reduced ischemic injury in AT1-deficient, as compared to AT1-overexpressing, or wild-type mice.[15] These observations indicate that AII and AT1 receptor activation might act as significant contributors to the pathophysiology of ischemic brain injury. On other hand, AII might also play a protective role in brain ischemia. AII has been demonstrated to cause dilation of cerebral arterioles[16,17] and to contribute to cerebral vasodilatation during hypoxia.[18] Infusion of AII and selective AT2 activation has been shown to decrease the mortality rate in gerbils with unilateral carotid ligation.[19] These opposing effects suggest a dual role for AII in the pathophysiology of ischemic brain injury.

The contribution of AT1 and AT2 receptor activation to cerebral hemodynamics after TBI have not been studied. A previous study[20] has shown that FPI increases the cerebrospinal fluid (CSF) concentration of AII and impairs AII-mediated cerebral vasodilation in newborn pigs. In addition, the results of this study indicate that FPI caused these changes *via* alteration in an AT1-mediated production of prostaglandins, whereas the impairment of AT2-mediated vasodilation was independent of prostaglandin metabolism.

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Therefore, this study was designed to characterize the effect of selective AT1 and AT2 blockade on pial artery hypotensive dilation, CBF, and hypotensive autoregulation after FPI in the newborn pig.

Materials and Methods

Newborn pigs (age, 1–5 days; weight, 1.9–2.2 kg) of either sex were used in these experiments. All protocols were approved by the Institutional Animal Care and Use Committee (Philadelphia, Pennsylvania). Animals were anesthetized with isoflurane (1–2 minimum alveolar concentration [MAC]). Anesthesia was maintained with α-chloralose (30–50 mg/kg, supplemented with 5 mg/kg intravenously each hour). A catheter was inserted into a femoral artery to monitor blood pressure and to sample for blood gas tensions and pH. Another catheter was placed in the left ventricle *via* the carotid artery for microsphere injection. Ligation of one carotid artery has no detectable effect on brain flow or its distribution in the normotensive or hypotensive piglet.[21] Drugs to maintain anesthesia were administered *via* a second catheter placed in a femoral vein. The trachea was cannulated, and the animals were mechanically ventilated with room air. A heating pad and a blanket were used to maintain the animals at 37–38°C.

A cranial window was placed in the parietal skull of these anesthetized animals. This window consisted of three parts: a stainless steel ring, a circular glass coverslip, and three ports consisting of 17-gauge hypodermic needles attached to three precut holes in the stainless steel ring. For placement, the dura was cut and retracted over the cut bone edge. The cranial window was placed in the opening and cemented in place with dental acrylic. The volume under the window was filled with a solution similar to CSF, of the following composition: 3.0 mM KCl, 1.5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 132 mM NaCl, 6.6 mM urea, 3.7 mM dextrose, and 24.6 mM $NaHCO_3$. This artificial CSF was warmed to 37°C and had the following chemistry: pH 7.33, partial pressure of carbon dioxide (P_{CO_2}) 46 mmHg, and partial pressure of oxygen (P_{O_2}) 43 mmHg, which was similar to that of endogenous CSF. Pial arterial vessels were observed with a dissecting microscope, a television camera mounted on the microscope, and a video output screen. Vascular diameter was measured with a video microscaler.

Cardiac output and regional blood flow distribution within the brain were measured using radioactively labeled microspheres. This method has been described previously.[22] Briefly, a known amount of radioactivity in 15-μm microspheres (300,000–800,000 spheres) was injected into the left ventricle, and the injection line was flushed with 1 ml saline. Withdrawal of reference blood samples was begun 15 s before microsphere injection and continued for 2 min after the injection. The reference withdrawal rate was 1.03 ml/min. After each experiment, the pig was killed, and the brain was removed and weighed. The brain was subdivided into major regions, and samples were counted in a gamma counter. The energy from each nuclide was separated by differential spectroscopy. Aliquots of the actual microsphere solutions injected were used for overlap calculations. The count in each milliliter per minute of blood flow was determined by dividing the counts in the reference withdrawal by the rate of reference withdrawal. Thus blood flow to any organ at the time of microsphere injection can be calculated as $Q = C \times R \times CR^{-1}$, where Q is organ blood flow (in ml/min), C is the counts per minute in the tissue sample, R is the rate of withdrawal of the reference blood sample (in ml/min), and CR is the total counts in the reference arterial blood sample.

Methods for brain FPI have been described previously.[4] A device designed by the Medical College of Virginia was used. A small opening was made in the parietal skull contralateral to the cranial window. A metal shaft was sealed into the opening on top of the intact dura. This shaft was connected to the transducer housing, which was in turn connected to the fluid percussion device. The device itself consisted of an acrylic plastic cylindrical reservoir, 60 cm long, 4.5 cm in diameter, and 0.5 cm thick. One end of the device was connected to the transducer housing, whereas the other end had an acrylic plastic piston mounted on O-rings. The exposed end of the piston was covered with a rubber pad. The entire system was filled with 0.9% saline. Two brackets mounted on a platform supported the percussion device. FPI was induced by striking the piston with a 4.8-kg pendulum. Varying the height from which the pendulum was allowed to fall controlled the intensity of the injury (usually 1.9–2.3 atm with a constant duration of 19–23 ms). The pressure pulse of the injury was recorded on a storage oscilloscope triggered photoelectrically by the fall of the pendulum. The amplitude of the pressure pulse was used to determine the intensity of the injury.

Protocol

Two types of pial arterial vessels, small arteries (resting diameter 120–160 μm), and arterioles (resting diameter 50–70 μm) were examined. Pial artery vessel diameter was determined every minute for a 10-min exposure period after infusion onto the exposed parietal cortex of artificial CSF before hypotension and after the infusion of artificial CSF after the induction of hypotension. Typically, 5–8 ml CSF was flushed through the window over a 30-s period, and excess CSF was allowed to run off through one of the needle ports.

Seven types of experiments were performed in animals randomly assigned to groups (all n = 6): (1) vascular responses and regional CBF (cerebral hemodynamics)

during normotension and moderate and severe hypotension in the absence of FPI (sham control animals); (2) cerebral hemodynamics during normotension and moderate and severe hypotension after FPI (vehicle animals); (3) cerebral hemodynamics during normotension and moderate and severe hypotension after FPI in animals pretreated with the AT1-selective antagonist ZD 7155; (4) cerebral hemodynamics during normotension and moderate and severe hypotension after FPI in animals pretreated with the AT2-selective antagonist PD 123,319; (5) vascular responses to local application of AII and the AT2 agonist CGP 42112A before and after FPI in unpretreated animals; (6) vascular responses to AII and CGP 42112A after FPI in animals pretreated with the AT1 antagonist; and (7) vascular responses to AII and CGP 42112A after FPI in animals pretreated with AT2-selective antagonist. Small arteries and arterioles were observed in each of these experimental groups. In FPI animals, responses were obtained before and 1 h after FPI, whereas such responses were obtained initially and then again 1 h later in the time-control animals. Pretreated animals received 1 mg/kg intravenous ZD 7155 (AT1-selective antagonist) or 0.5 mg/kg intravenous PD 123,319 (AT2-selective antagonist) 30 min before FPI. AII (10^{-8}, 10^{-6} M) and CGP 42112A (10^{-8}, 10^{-6} M) were applied topically on exposed parietal cortex before and 1 h after FPI. Because baseline pial vessel diameter changed as a result of the FPI intervention, data were calculated as the percent change from the baseline to normalize such differences.

Two levels of hypotension (moderate and severe) designed to lower blood pressure by approximately 25 and 45% were investigated. Moderate and severe hypotension was produced sequentially and was induced by rapidly withdrawing 5–8 or 10–15 ml blood/kg. Such a decrease in mean blood pressure was maintained constant for 10 min by titration of additional blood withdrawal (1–5 ml) to keep arterial blood pressure from increasing and by blood reinfusion (1–5 ml) to keep pressure from decreasing. The percent changes in artery diameter values were calculated on the basis of the diameter measured in the control period before hypotension. Animals were anticoagulated before hemorrhage (1,000 U heparin).

Statistical Analysis

Pial artery diameter and CBF values were analyzed using analysis of variance for repeated measures. An α level of $P < 0.05$ was considered significant. If the value was significant, the data were then analyzed by a Dunnett test with Bonferroni correction. Values are represented as mean ± SD of the absolute values or as percent change from control.

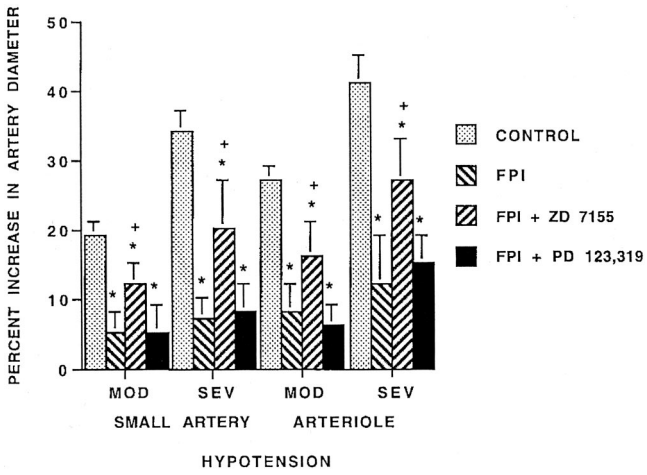

Fig. 1. Influence of hypotension (moderate [MOD], severe [SEV]) on pial small artery and arteriole diameter before fluid percussion injury (FPI) (control), after FPI, and after FPI in ZD 7155– and PD 123,319–pretreated animals (n = 6). *$P < 0.05$ compared to control; +$P < 0.05$ compared to FPI nonpretreated value.

Results

Role of the AT1 Receptor in Altered Pial Artery Dilation and Autoregulation of CBF during Hypotension after FPI

Induction of moderate and severe hypotension elicited reproducible graded pial small artery and arteriole dilation in newborn pigs in the sham control group (data not shown). Correspondingly, blood flow in the brain was reproducibly unchanged during moderate and severe hypotension in sham control animals (data not shown). Mean arterial blood pressure was 71 ± 3, 53 ± 2, and 40 ± 1 mmHg for normotension, moderate hypotension, and severe hypotension, respectively. In contrast, pial artery dilation was blunted within 1 h after FPI (fig. 1). On a percentage basis, FPI blunted pial small artery dilation in response to moderate and severe hypotension by 73 ± 6 and 79 ± 4%, respectively. There was no statistical difference in FPI-induced impairment of vascular responsiveness between pial small arteries and arterioles (data not shown). Regional CBF was unchanged during moderate and severe hypotension before FPI in vehicle-treated animals (fig. 2 and table 1). However, after FPI, CBF in the cerebrum was reduced by 43 ± 3% during normotension and further decreased by 38 ± 5 and 55 ± 3% (as compared to normotension) during moderate and severe hypotension, respectively (fig. 2A). There were similar changes in other areas of the brain (table 1).

Systemic pretreatment with ZD 7155 (1 mg/kg intravenous) 30 min before injury partially restored hypotension-induced pial artery dilation, which was impaired after FPI (fig. 1). On a percentage basis, pial small artery dilation was diminished only by 39 ± 6 and 37 ± 8% during moderate and severe hypotension, respectively. These values were significantly less than the respective

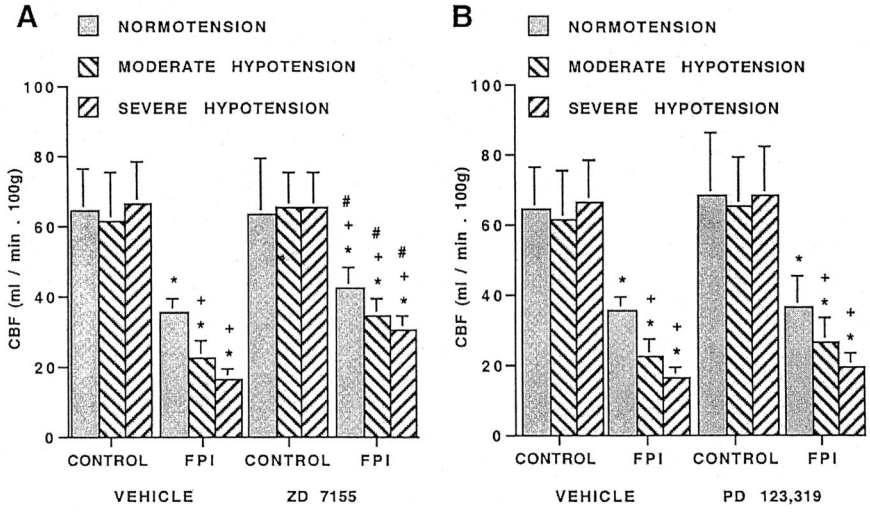

Fig. 2. (*A*) Influence of fluid percussion injury (FPI) on cerebral blood flow in the cerebrum during normotension, moderate hypotension, and severe hypotension in untreated (vehicle) and ZD 7155 (1 mg/kg intravenous)–pretreated animals. (*B*) Influence of FPI on cerebral blood flow in the cerebrum during normotension, moderate hypotension, and severe hypotension in untreated (vehicle) and PD 123,319 (0.5 mg/kg intravenous)–pretreated animals (n = 6). **P* < 0.05 compared with corresponding preinjury value (control); +*P* < 0.05 compared with corresponding normotension value; #*P* < 0.05 compared with corresponding non–ZD 7155-pretreated value.

values for untreated animals described above. Similar effects were observed in pial arterioles. Pretreatment with ZD 7155 also partially prevented the decrease in CBF observed after FPI in normotensive untreated animals (figs. 2A and table 1). Furthermore, ZD 7155 partially prevented the reduction of CBF during subsequent moderate and severe hypotension (fig. 2A and table 1). On a percentage basis, blood flow in the cerebrum after FPI was diminished only by 32 ± 4% during normotension and further decreased by 19 ± 2 and 27 ± 5% compared to normotension during moderate and severe hypotension in pretreated animals. Such values were statistically different from those obtained in unpretreated animals. Similar partial protection was observed in other brain regions.

Role of the AT2 Receptor in Altered Pial Artery Dilation and Autoregulation of CBF during Hypotension after FPI

Systemic pretreatment with PD 123,319 (0.5 mg/kg intravenous) 30 min before injury did not restore hypotension-induced pial artery dilation impaired after FPI (fig. 1). It also did not prevent the reduction in CBF observed in untreated normotensive animals after FPI (fig. 2B and table 1). Impairment of hypotensive autoregulation observed after FPI in untreated animals was also unaffected by systemic administration of PD 123,319 (fig. 2B and table 1).

Influence of Systemic Pretreatment with ZD 7155 and PD 123,319 on AII- and CGP 42112A–induced Vasodilation

Systemic pretreatment with ZD 7155 (1 mg/kg intravenous) effectively blocked pial artery and arteriole dilation to topical AII (10^{-8}, 10^{-6} M) after FPI (fig. 3). However, it did not blunt pial vasodilation elicited by topical application of the selective AT2 agonist CGP 42112A (10^{-8}, 10^{-6} M) after FPI (fig. 4). Systemic pretreatment with PD 123,319 (0.5 mg/kg) effectively blocked pial vasodilation caused by topical application of CGP 42112A (10^{-8}, 10^{-6} M) after FPI (fig. 4). Conversely, it did not blunt pial dilation elicited by topical

Table 1. Regional CBF Changes after FPI in Newborn Pigs Untreated and Pretreated with ZD 7155 and PD 123,319 during Normotension and Moderate and Severe Hypotension

	Control			Vehicle + FPI			ZD 7155 + FPI			PD 123,319 + FPI		
Region	NOR	MOD	SEV	NOR	MOD	SEV	NOR	MOD	SEV	NOR	MOD	SEV
Caudate	60 ± 6	64 ± 10	63 ± 13	35 ± 4*	23 ± 4*†	18 ± 3*†	44 ± 5*‡	33 ± 4*†‡	30 ± 5*†‡	34 ± 5*	25 ± 5*†	18 ± 4*†
Diencephalon–mesencephalon	59 ± 14	64 ± 10	65 ± 9	36 ± 6*	25 ± 4*†	19 ± 3*†	45 ± 4*‡	36 ± 3*†‡	31 ± 4*†‡	37 ± 7*	29 ± 6*†	19 ± 5*†
Gray	61 ± 10	63 ± 9	67 ± 10	35 ± 4*	23 ± 8*†	19 ± 6*†	43 ± 7*‡	35 ± 5*†‡	31 ± 4*†‡	35 ± 6*	24 ± 5*†	20 ± 7*†
White	64 ± 8	66 ± 6	65 ± 14	33 ± 3*	21 ± 6*†	18 ± 6*†	39 ± 5*‡	34 ± 6*†‡	30 ± 5*†‡	37 ± 7*	27 ± 5*†	20 ± 4*†
Medulla	60 ± 12	65 ± 8	69 ± 11	36 ± 5*	22 ± 8*†	17 ± 7*†	41 ± 5*‡	34 ± 5*†‡	33 ± 5*†‡	34 ± 6*	25 ± 5*†	20 ± 5*†
Pons	67 ± 10	63 ± 8	66 ± 13	36 ± 6*	23 ± 7*†	16 ± 6*†	42 ± 5*‡	32 ± 6*†‡	30 ± 6*†‡	36 ± 6*	28 ± 5*†	22 ± 4*†
Cerebellum	62 ± 11	64 ± 7	68 ± 11	34 ± 4*	24 ± 7*†	18 ± 6*†	39 ± 4*‡	31 ± 2*†‡	28 ± 4*†‡	34 ± 7*	25 ± 4*†	16 ± 6*†

Pretreated animals received 1 mg/kg ZD 7155 or 0.5 mg/kg PD 123,319. n = 6.

* $P < 0.05$ compared with corresponding preinjury value (control). † $P < 0.05$ compared with corresponding normotension value. ‡ $P < 0.05$ compared with corresponding non–ZD 7155 pretreated value.

CBF = cerebral blood flow; FPI = fluid percussion injury; MOD = moderate hypotension; NOR = normotension; SEV = severe hypotension; vehicle = untreated animals.

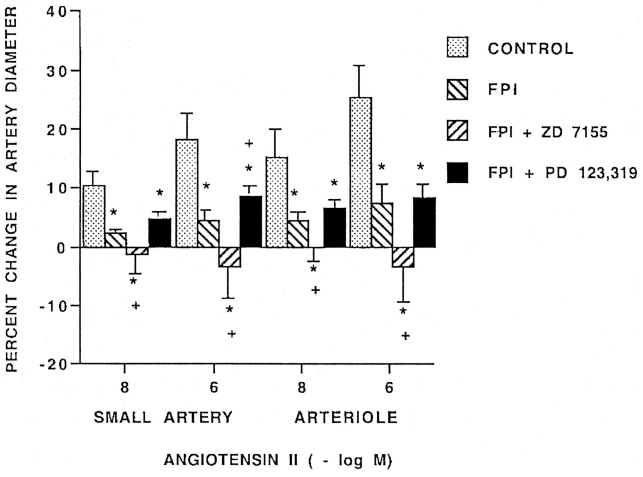

Fig. 3. Influence of angiotensin II (10^{-8}, 10^{-6} M) on pial small artery and arteriole diameters before fluid percussion injury (FPI) (control), after FPI, and after FPI in ZD 7155 (1 mg/kg intravenous)– and PD 123,319 (0.5 mg/kg intravenous)–pretreated animals (n = 6). *$P < 0.05$ compared to control; +$P < 0.05$ compared to FPI nonpretreated value.

application of AII (10^{-8}, 10^{-6} M) (fig. 3). Pial artery diameter data before FPI (control) and after FPI were previously published.[20]

Blood Chemistry

Blood chemistry values were obtained at the beginning and end of all experiments. These values were 7.48 ± 0.05, 33 ± 5, and 99 ± 8 *versus* 7.45 ± 0.05, 33 ± 2, and 97 ± 8 mmHg for pH, P_{CO_2}, and P_{O_2}, respectively. Such values were not statistically different from one another. There were also no group differences. The amplitude of the pressure pulse, used as an index of injury intensity, was equivalent in all groups (2.0 ± 0.1 atm).

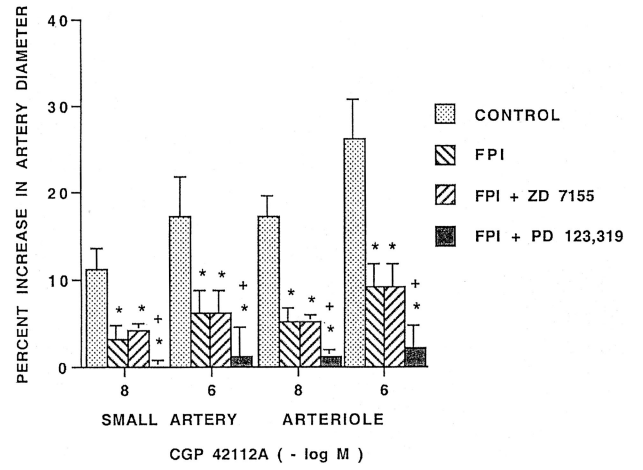

Fig. 4. Influence of CGP 42112A (10^{-8}, 10^{-6} M) on pial small artery and arteriole diameters before fluid percussion injury (FPI) (control), after FPI, and after FPI in ZD 7155 (1 mg/kg intravenous)– and PD 123,319 (0.5 mg/kg intravenous)–pretreated animals (n = 6). *$P < 0.05$ compared to control; +$P < 0.05$ compared to FPI nonpretreated value.

Discussion

The results of this study indicate that selective blockade of the AT1 and not the AT2 receptor diminishes the reduction in hypotensive pial artery dilation observed within 1 h after FPI in the newborn pig. Correspondingly, AT1-selective blockade partially restored diminished CBF during normotension and alleviated further decreases in CBF observed during induced hypotension after FPI. Thus, these data suggest that AT1 receptor activation by AII in the brain of the newborn pig contributes to cerebral hypoperfusion and impairment of hypotensive cerebral autoregulation after FPI. Because similar effects were observed in pial small arteries and arterioles, these data suggest that there are minimal regional vascular differences in the effects of AII and selective AII agonists.

Traumatic brain injury results in a very high morbidity and mortality in all age groups, with enormous human and economic impact.[23] However, the consequences of severe TBI are especially grave in newborns and infants.[1] Earlier reports indicate that cerebral hypoperfusion after TBI frequently produces inadequate CBF, resulting in secondary ischemic brain injury.[24,25] The adverse effects of even mild systemic hypotension on outcome after TBI have been documented.[26] Systemic hypotension is present in 15–30% of patients with severe head injury.[26] In recent years, the understanding that posttraumatic impairment of cerebral vasodilatory responses might be a major contributor to secondary brain injury drew greater attention to the issue of optimal management of cerebral hemodynamics after TBI.[27] Whereas some investigators emphasized the effectiveness of aggressive cerebral perfusion pressure support,[28] others explored the ways to restore the impaired cerebral vasodilation.[29,30] However, few studies have examined the mechanisms for posttraumatic impairment of cerebrovascular responses or therapeutic strategies aimed at these derangements in the newborn. The current study in combination with our previous report,[20] suggest that AII acting *via* AT1 and AT2 receptors in the brain vasculature may play a significant role in the posttraumatic impairment of cerebral hemodynamics in the newborn. These data suggest that AT1 receptor blockade may present an important avenue in developing therapeutic strategies directed at prevention and alleviation of secondary brain injury after severe TBI.

To reproduce the biomechanical effects of closed head injury in the newborn, the lateral FPI model was used. Diffuse TBI and diffuse cerebral swelling are more common in children than in adults,[31] and the lateral FPI technique is thought to mimic the shaken impact syndrome, an example of child abuse–related head injury.[2] It was observed in the previous studies that FPI was associated with pial artery vasoconstriction and reductions in CBF in newborn and juvenile pigs.[5] In addition,

FPI significantly diminishes hypotensive pial vasodilation. Correspondingly, FPI impairs normal cerebral autoregulation, resulting in decreases in CBF in response to hypotension.[3,32] Interestingly, these effects were more pronounced and lasted longer in the newborn than the juvenile pig.[32] The results of the current study are consistent with previous observations regarding the effects of FPI on hypotensive cerebral responses in the newborn pig.

In a previous study,[20] we demonstrated that topically applied AII elicited pial artery dilation in the newborn pig, which was consistent with reports by other investigators.[17] This vasodilation seemed to be dependent on release of dilator prostaglandins.[16,17,20] However, the effects of AII on cerebral vessels are not uniform. AII has been reported to display vasoconstricting and vasodilating properties, depending on the species, size, and location of the vessel investigated and method of AII administration.[16,17,33–35] The difference in the cerebrovascular responses to AII reported in the literature might be partially accounted for by variation between species and experimental methods. However, sufficient evidence exists in the literature that indicates that the heterogenicity of AII-induced vascular responses could be explained by activation AT1 and AT2 receptors. These two distinct populations of AII receptors have been shown to have different function and distribution in the brain, which greatly varies with age.[10] In our previous report, we showed that both AT1 and AT2 receptor activation produced pial artery dilation in newborn pig.[20] However, AT2 contributed significantly to AII-induced vasodilation only at high, pathophysiologic concentrations, whereas at lower, physiologic concentrations, AII produced vasodilation mainly *via* activation of AT1 receptor.[20] In that study, we had also shown that FPI impairs vascular responses caused by activation AT1 and AT2 receptors. Accentuated release of the constrictor prostaglandin TXA2 coupled with blocked release of the dilator prostaglandin PGI2 after FPI contributed to posttraumatic impairment of the AT1 activation–induced vasodilation. However, blunted vasodilation in response to AT2 activation was independent of CSF production of prostaglandins. These differences might explain the results of the current study, in which selective blockade of AT1 and not AT2 receptors led to attenuation of posttraumatic impairment of cerebral hemodynamics in newborn pigs.

Additional experiments were designed to determine the specificity and efficacy of the chosen systemic doses for AT1- and AT2-selective antagonists, ZD7155 and PD 123,319, respectively. We have shown previously that ZD 7155 and PD123,319 had selectively blocked AII (predominantly AT1 agonist at chosen concentrations)– and CGP 42112A (selective AT2 agonist)–induced vasodilation, respectively, in uninjured pigs.[20] However, it was not clear whether after brain injury these systemically administered AII antagonists would preserve their subtype selectivity on AII vascular activity. Consistent with our previous report, FPI blunted AII (10^{-8}, 10^{-6} M)–induced cerebral vasodilation. This vasodilation was completely blocked by systemic pretreatment with ZD 7155 but not with PD 123,319. On other hand, systemic pretreatment with PD 123,319 and not with ZD 7155 nearly completely blocked CGP 42112A–induced cerebral vasodilation. These results indicate that ZD 7155 and PD 123,319, administered selectively in the doses reported, were efficacious and selective in antagonizing cerebral vascular activity of AII after FPI. Topical AII in these experiments could interact with receptors on neurons or endothelial or vascular smooth muscle cells. However, the current experimental paradigm does not allow for any conclusion with respect to cellular site of action.

Previous reports in the literature indicate that AT1 receptor antagonism might play a role in reducing the extent of ischemic injury and improving outcome in a stroke animal model.[14,36] This study is the first to investigate the effects of selective AT1 and AT2 receptor antagonism on cerebral hemodynamics after brain injury.

In conclusion, the results of this study indicate that activation of the AT1 receptor subtype contributes to cerebral hypoperfusion and impairment of hypotensive cerebral autoregulation observed in the newborn pig after brain injury. These effects of brain injury could be partially ameliorated by pretreatment with AT1 but not AT2 subtype–selective blockade in the newborn pig. Therefore, these results suggest selective AT1 blockade as a potential strategy in the treatment of cerebral hemodynamic dysregulation caused by TBI.

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References

1. Duhaime AC, Gennarelli TA, Thibault LE, Bruce DA, Margulies SS, Wiser R: The shaken baby syndrome: A clinical, pathological, and biomechanical study. J Neurosurg 1987; 66:409–15

2. Gennarelli TA: Animate models of human head injury. J Neurotrauma 1994; 11:357–68

3. DeWitt DS, Prough DS, Taylor CL, Whitley JM, Deal DD, Vines SM: Regional cerebrovascular responses to progressive hypotension after traumatic brain injury in cats. Am J Physiol 1992; 263:H1276–84

4. McIntosh TK, Vink R, Noble L, Yamakami I, Fernyak S, Soares H, Faden AL: Traumatic brain injury in the rat: Characterization of a lateral fluid-percussion model. Neuroscience 1989; 28:233–44

5. Armstead WM, Kurth CD: Different cerebral hemodynamic responses following fluid percussion brain injury in the newborn and juvenile pig. J Neurotrauma 1994; 11:487–97

6. Amaral SL, Papanek PE, Greene AS: Angiotensin II and VEGF are involved in angiogenesis induced by short-term exercise training. Am J Physiol 2001; 281: H1163–9

7. Daul CB, Heath RG, Garey RE: Angiotensin-forming enzyme in human brain. Neuropharmacology 1975; 14:75–80

8. Phillips MI, Sumners C: Angiotensin II in central nervous system physiology. Regul Pept 1998; 78:1–11

9. Allen AM, MacGregor DP, McKinley MJ, Mendelsohn FA: Angiotensin II receptors in the human brain. Regul Pept 1999; 79:1–7

10. Grady EF, Sechi LA, Griffin CA, Schambelan M, Kalinyak JE: Expression of AT2 receptors in the developing rat fetus. J Clin Invest 1991; 88:921–33

11. Makino I, Shibata K, Ohgami Y, Fujiwara M, Furukawa T: Transient upregulation of the AT2 receptor mRNA level after global ischemia in the rat brain. Neuropeptides 1996; 30:596–601

12. Viswanathan M, de Oliveira AM, Correa FM, Saavedra JM: Expression of a novel non-angiotensin II [125I]CGP 42112 binding site in healing wounds of the rat brain. Brain Res 1994; 658:265–70

13. Mark KS, Davis TP: Stroke: development, prevention and treatment with peptidase inhibitors. Peptides 2000; 21:1965–73

14. Ito T, Yamakawa H, Bregonzio C, Terron JA, Falcon-Neri A, Saavedra JM: Protection against ischemia and improvement of cerebral blood flow in genetically hypertensive rats by chronic pretreatment with an angiotensin II AT1 antagonist. Stroke 2002; 33:2297–303

15. Walther T, Olah L, Harms C, Maul B, Bader M, Hortnagl H, Schultheiss HP, Mies G: Ischemic injury in experimental stroke depends on angiotensin II. FASEB J 2002; 16:169–76

16. Haberl RL, Decker PJ, Einhaupl KM: Angiotensin degradation products mediate endothelium-dependent dilation of rabbit brain arterioles. Circ Res 1991; 68:1621–7

17. Meng W, Busija DW: Comparative effects of angiotensin-(1-7) and angiotensin II on piglet pial arterioles. Stroke 1993; 24:2041–4

18. Maktabi MA, Todd MM, Stachovic G: Angiotensin II contributes to cerebral vasodilatation during hypoxia in the rabbit. Stroke 1995; 26:1871–6

19. Fernandez LA, Caride VJ, Stromberg C, Naveri L, Wicke JD: Angiotensin AT2 receptor stimulation increases survival in gerbils with abrupt unilateral carotid ligation. J Cardiovasc Pharmacol 1994; 24:937–40

20. Baranov D, Armstead WM: Prostaglandins contribute to impaired angiotensin II-induced cerebral vasodilation after brain injury. J Neurotrauma 2002; 19:1457–66

21. Laptook AR, Stonestreet BS, Oh W: The effect of carotid artery ligation on brain blood flow in newborn piglets. Brain Res 1983; 276:51–4

22. Armstead WM, Mirro R, Busija DW, Leffler CW: Opioids and the prostanoid system in the control of cerebral blood flow in hypotensive piglets. J Cereb Blood Flow Metab 1991; 11:380–7

23. Kraus JF, McArthur DL: Epidemiologic aspects of brain injury. Neurol Clin 1996; 14:435–50

24. Bouma GJ, Muizelaar JP, Choi SC, Newlon PG, Young HF: Cerebral circulation and metabolism after severe traumatic brain injury: The elusive role of ischemia. J Neurosurg 1991; 75:685–93

25. Bouma GJ, Muizelaar JP, Stringer WA, Choi SC, Fatouros P, Young HF: Ultra-early evaluation of regional cerebral blood flow in severely head-injured patients using xenon-enhanced computerized tomography. J Neurosurg 1992; 77:360–8

26. Chesnut RM, Marshall SB, Piek J, Blunt BA, Klauber MR, Marshall LF: Early and late systemic hypotension as a frequent and fundamental source of cerebral ischemia following severe brain injury in the Traumatic Coma Data Bank. Acta Neurochir Suppl (Wien) 1993; 59:121–5

27. Robertson CS: Management of cerebral perfusion pressure after traumatic brain injury. ANESTHESIOLOGY 2001; 95:1513–7

28. Rosner MJ, Rosner SD, Johnson AH: Cerebral perfusion pressure: Management protocol and clinical results. J Neurosurg 1995; 83:949–62

29. DeWitt DS, Smith TG, Deyo DJ, Miller KR, Uchida T, Prough DS: L-arginine and superoxide dismutase prevent or reverse cerebral hypoperfusion after fluid-percussion traumatic brain injury. J Neurotrauma 1997; 14:223–33

30. Cherian L, Chacko G, Goodman JC, Robertson CS: Cerebral hemodynamic effects of phenylephrine and L-arginine after cortical impact injury. Crit Care Med 1999; 27:2512–7

31. Adelson PD: Animal models of traumatic brain injury in the immature: A review. Exp Toxicol Pathol 1999; 51:130–6

32. Armstead WM: Role of endothelin-1 in age-dependent cerebrovascular hypotensive responses after brain injury. Am J Physiol 1999; 277:H1884–94

33. Joyner WL, Young R, Blank D, Eccleston-Joyner CA, Gilmore JP: In vivo microscopy of the cerebral microcirculation using neonatal allografts in hamsters. Circ Res 1988; 63:758–66

34. Mayhan WG, Amundsen SM, Faraci FM, Heistad DD: Responses of cerebral arteries after ischemia and reperfusion in cats. Am J Physiol 1988; 255:H879–84

35. Reynier-Rebuffel AM, Pinard E, Aubineau PF, Meric P, Seylaz J: Generalized cerebral vasoconstriction induced by intracarotid infusion of angiotensin II in the rabbit. Brain Res 1983; 269:91–101

36. Dai WJ, Funk A, Herdegen T, Unger T, Culman J: Blockade of central angiotensin AT(1) receptors improves neurological outcome and reduces expression of AP-1 transcription factors after focal brain ischemia in rats. Stroke 1999; 30:2391–8