Hematoma-Induced Enhanced Cerebral Vasoconstrictions to Leukotriene C₄ and Endothelin-1 in Piglets: Role of Prostanoids

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ABSTRACT

Cerebral hematoma enhances vasoconstriction induced by topical application of the vasoconstrictor agents endothelin-1 (ET-1) and leukotriene C₄ (LTC₄). We investigated the influence of dilator prostanoids on vasoconstrictions induced by ET-1 and LTC₄ in piglets. Newborn pigs anesthetized with α -chloralose were fitted with closed cranial windows 4 d after cortical subarachnoid injections of artificial cerebrospinal fluid (aCSF) (control) or blood (hematoma). The responsiveness of pial arterioles to topical application of the vasoconstrictors ET-1 and LTC₄ was examined in the control and hematoma groups before and after treatment with indomethacin (5 mg/kg, i.v.). Vasoconstriction to topical application of LTC₄ and ET-1 was enhanced by hematoma compared with the control ($28 \pm 2\%$ versus $21 \pm 2\%$ for 10^{-8} M LTC₄ and by $25 \pm 2\%$ versus $15 \pm 1\%$ for 10^{-8} M ET-1, respectively). The lower dose of ET-1 (10^{-12} M) dilated pial arterioles in the control group by $6 \pm 2\%$, hematoma blocked this dilation and it was converted to constriction ($10 \pm 1\%$). Indomethacin treatment enhanced vasoconstriction to LTC₄ in the control group to a similar constriction to that observed in

the hematoma group. Indomethacin treatment also enhanced vasoconstriction to ET-1 in the control group ($25 \pm 1\%$ for 10^{-8} M) to similar constrictions to those observed in the hematoma group ($25 \pm 2\%$ for 10^{-8} M). Dilation to the lower dose of ET-1 was blocked and converted to constriction ($17 \pm 2\%$ for 10^{-12} M) by indomethacin treatment. These results show that indomethacin and cerebral hematoma similarly enhance vasoconstriction to topical application of LTC₄ and ET-1. Loss of dilator prostanoid influence caused by hematoma may result in prolonged and uninhibited constriction of pial arterioles by constrictor vasoactive agents, leading to the sustained and prolonged arteriolar wall narrowing observed after cerebral hemorrhage. (*Pediatr Res* 38: 119–123, 1995)

Abbreviations

aCSF, artificial cerebrospinal fluid
ET, endothelin
LTC₄, leukotriene C₄

Eicosanoids have been shown to contribute to the control of cerebral hemodynamics. Vasodilator products of prostaglandin H synthase are important components in the regulation of cerebral circulation in the newborn pig (1, 2), contributing to asphyxia (3, 4), hypercapnia (5), and hypotension-induced vasodilation (1, 6). Other eicosanoids such as thromboxane and peptidoleukotrienes are potent vasoconstrictors of pial arterioles (2, 7, 8). Also, ET-1 a polypeptide isolated from vascular endothelial cells (9), is a potent vasoconstrictor agent, producing slow and sustained dose-dependent vasoconstriction of pial arterioles (10, 11).

Altered metabolic profiles of eicosanoids and other vasoactive agents have been reported after cerebral hemorrhage or

other pathophysiologic conditions such as brain injury in both human and animal models (12–14). Cerebral hemorrhage may change cerebral vascular reactivity to vasoactive agents and may lead to neurologic dysfunction, causing death or permanent disability (15, 16). Decreased levels of dilator prostanoids or no change (12, 17), and elevated levels of vasoconstrictor agents have been reported consistently (16–20) in both human and experimental animal models after subarachnoid hemorrhage. Observed changes in cerebrovascular reactivity and hemorrhage-induced sustained narrowing of large cerebral arteries may be due to altered levels of vasoactive agents in the subarachnoid space.

The mechanism by which lumen narrowing develops after subarachnoid hemorrhage is incompletely understood. However, cerebral arterioles have been reported to develop resistance to vasodilator agents after subarachnoid hemorrhage (12, 21). We reported attenuation of cerebrovascular dilation to prostanoids, hypercapnia, and hypotension after subarachnoid hematoma (11, 22). Four days after experimental subarachnoid

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hemorrhage in piglets, enhanced pial arteriolar vasoconstriction to topical application of LTC_4 and ET-1 was observed (11). Vasoconstriction to ET-1 was enhanced *in vitro* in cerebral arteries from rats with subarachnoid hemorrhage (23). These findings suggest that cerebrovascular constrictions observed after hemorrhage may be due to reduced relaxant and enhanced contractile responses of cerebral arterioles to endogenous vasoactive agents. Under physiologic conditions, removal of dilator prostanoids has been reported to enhance vasoconstrictions to constrictor agents such as LTC_4 , thromboxane, prostaglandin $\text{F}_{2\alpha}$, ET-1, oxytocin, norepinephrine, and acetylcholine (24–26). Under normal conditions, a balance exists between the actions of the dilator and constrictor agents; this balance may change under pathophysiologic conditions. In the present study, we investigated the mechanism behind hemorrhage-induced enhanced cerebral vasoconstriction to LTC_4 and ET-1. This study was based on the hypothesis that enhanced vasoconstriction to ET-1 and LTC_4 results from loss of prostanoid dilator influence.

METHODS

Animal protocols were reviewed and approved by the Animal Care and Use Committee of The University of Tennessee, Memphis, and animals were maintained in an AAALAC-accredited program. Piglets 1–3 d old (1.0–2.5 kg) were used for the experiments. The piglets received either sterile aCSF (control group) or 3 mL autologous blood (hematoma group) injected onto the surface of the cerebral cortex and left for 4 d before examining responses to topical application of ET-1 and LTC_4 .

For placement of aCSF or blood, the piglets were anesthetized with 1% halothane initially which was decreased to 0.4% halothane after induction, and 45% nitrous oxide and 21% oxygen. A small hole was made in the skull over the left frontal cortex using aseptic procedures. The dura was pierced with a 22-gauge Teflon catheter (Angiocath, Deseret Co., Sandy, UT) at an angle to the surface sufficient to prevent penetration into the brain. After removal of the needle, the tip of the catheter was advanced 2 cm posteriorly under the dura to the parietal cortex. Then 3 mL of either sterile aCSF or fresh, sterile, nonheparinized blood (removed via puncture of the precava) was injected over 1–2 min. The catheter was removed, the hole filled with sterile bone wax, and the scalp sutured. The piglets were treated with gentamicin and benzathine penicillin post-operatively.

Four days after injection, piglets were anesthetized with a mixture of ketamine hydrochloride (33 mg/kg intramuscularly) and acepromazine (3.3 mg/kg intramuscularly) and maintained with α -chloralose (50 mg/kg i.v., followed by 3–5 mg/kg each hour). The femoral artery and vein were cannulated. The arterial catheter was used to monitor blood pressure and to draw blood samples for blood gas and pH analysis, whereas the venous catheter was used for the administration of anesthesia and fluid. The trachea was cannulated, and the animals were ventilated mechanically with air. Body temperature was maintained at 37–38°C with a heating pad.

For implantation of a closed cranial window, the scalp over the left parietal cortex was cut and retracted, a hole 2 cm in diameter was made in the skull, and the dura and arachnoid were removed. A stainless steel and glass cranial window with needle ports was placed in the hole and secured with dental acrylic. The space under the window (500 μL) was filled with aCSF composed of (mM) 3.0 KCl, 1.5 MgCl_2 , 1.5 CaCl_2 , 132 NaCl, 6.6 urea, 3.7 dextrose, and 24.6 NaHCO_3 ; approximately pH 7.33, Pco_2 of 6.0 kPa, and Po_2 of 5.6 kPa. Pial arterioles were observed with a dissecting microscope, a television camera mounted on the microscope, and a video monitor. A video microscaler was used to measure vascular diameter.

After confirming that blood pressure, pH, and blood gases were within normal range, control measurements of pial arteriolar diameters were taken after the placement of aCSF containing no drug under the window. Vasoreactivity responses to topical application of LTC_4 (10^{-12} , 10^{-10} , 10^{-8} M) and ET-1 (10^{-12} , 10^{-10} , 10^{-8} M) were examined in random order (before and after indomethacin injection 5 mg/kg i.v.). LTC_4 and ET-1 were purchased from Sigma Chemical Co., St. Louis MO. Water-soluble indomethacin was a gift from Merck Research Laboratories, Somerset, NJ. A cumulative dose-response study was performed for each drug by consecutively infusing the drug under the window from lower to higher dose. After infusion of these agents at each concentration, pial arteriolar diameter change was recorded every 2 min for 10 min. At least 1 h was allowed in between the use of LTC_4 and ET-1. The cortical surface under the window was repeatedly flushed slowly with fresh aCSF that was kept bubbling with a mixture of 6.3% O_2 , 5.8% CO_2 in N_2 to maintain a pH of 7.33, Pco_2 of 6.0 kPa, and Po_2 of 5.6 kPa at 37–38°C in a water. A control measurement was always taken before the use of a new drug. Between application of different agents after repeated washing, the diameters of the pial arterioles returned to predrug application diameters (+5% of the original steady-state control diameters). The order of application of the agents did not affect the vasoconstrictions induced.

All values are presented as means $\pm$ SEM. At least two arterioles was selected to study the percentage changes in pial arteriolar diameter in each piglet. The means of the measurements from these arterioles were used as data points for the statistical analysis of the results. The results of the effects of stimuli on pial arteriolar diameter were subjected to two-way analysis of variance for repeated measures with Student-Newman-Keuls tests to isolate differences between groups. A level of p less than 0.05 was considered significant.

RESULTS

The piglets did not show any behavioral changes after the procedure, and they gained weight over the 4 d they were kept in the animal facility before the experiment.

Arterial pH, Pco_2 , Po_2 , and blood pressure were not different between the control and hematoma groups. These parameters did not change throughout the course of the experiment (Table 1).

The mean diameter of pial arterioles on the cerebral cortex, circumscribed by the cranial window in contact with the

Table 1. Arterial blood gases, pH, and mean arterial pressure (MAP) of piglets before and after indomethacin treatment (5 mg/kg) in control (aCSF) and hematoma groups

Group	n	pH	Paco ₂ (kPa)	PaO ₂ (kPa)	MAP (kPa)
Before indomethacin:					
aCSF	8	7.46 ± 0.1	4.3 ± 0.4	13.4 ± 1.0	12.0 ± 0.8
Hematoma	8	7.53 ± 0.1	4.0 ± 0.3	14.0 ± 0.7	11.7 ± 0.8
After indomethacin (5 mg/kg):					
aCSF	8	7.45 ± 0.1	4.7 ± 0.4	13.4 ± 0.8	11.8 ± 0.9
Hematoma	8	7.43 ± 0.1	4.4 ± 0.2	14.8 ± 0.6	12.8 ± 1.0

instilled blood, was significantly reduced from $135 \pm 12 \mu\text{m}$ for the control to $83 \pm 10 \mu\text{m}$ in the hematoma group. This represents a 39% reduction in the pial arteriolar diameter after subarachnoid hematoma. The arterioles measured are those greater than $60 \mu\text{m}$. The baseline diameter of the pial arterioles selected for the study of vascular reactivity was $116 \pm 33 \mu\text{m}$ ($n = 8$) for the control and $118 \pm 18 \mu\text{m}$ ($n = 8$) for the hematoma group. The pial arterioles selected for this study of vascular reactivity were between 80 and $150 \mu\text{m}$. In selecting pial arterioles for each experiment, we chose one arteriole with diameter measuring 80–115 μm and another 116–150 μm .

Infusion of indomethacin (5 mg/kg) initially decreased pial arteriolar diameter by 20–25% and increased blood pressure by 25–40% of baseline values. The pial arteriolar diameter and blood pressure returned to preinfusion levels ($\pm 5\%$ of the baseline values) within 5 min after the infusion.

Effects of indomethacin treatment (5 mg/kg) on vasoconstrictions induced by LTC₄. Topical application of LTC₄ dose-dependently constricted pial arterioles in the control group ($9 \pm 1\%$ for 10^{-12} M and $21 \pm 2\%$ for 10^{-8} M). Constriction was greater in the hematoma group ($14 \pm 2\%$ for 10^{-12} M and $28 \pm 2\%$ for 10^{-8} M, respectively) compared with the control group (Fig. 1, upper panel). Indomethacin treatment of both the hematoma and control groups enhanced vasoconstriction induced by LTC₄ in the control group ($19 \pm 3\%$ for 10^{-12} M and $31 \pm 4\%$ for 10^{-8} M, respectively) to constrictions that were similar to those observed in the hematoma group ($21 \pm 3\%$ for 10^{-12} M and $35 \pm 3\%$ for 10^{-8} M). The enhanced vasoconstrictions observed in control and hematoma groups after treatment with indomethacin were not significantly different from each other (Fig. 1, lower panel).

Effects of indomethacin treatment (5 mg/kg) on vasoconstrictions induced by ET-1. Topical application of ET-1 at the lowest dose (10^{-12} M) dilated pial arterioles to $6 \pm 2\%$, whereas higher doses constricted pial arterioles dose dependently (7 ± 1 and $15 \pm 1\%$ for 10^{-10} M and 10^{-8} M, respectively) in the control (Fig. 2, upper panel). Vasodilation induced at the lowest dose of ET-1 (10^{-12} M) was blocked by hematoma and converted to constriction ($10 \pm 1\%$). Enhanced vasoconstriction to topical application of the higher doses of ET-1 was observed in the hematoma group (for example, $25 \pm 2\%$ compared with $15 \pm 1\%$ in the control for 10^{-8} M) (Fig. 2, upper panel). After indomethacin, vasoconstriction induced by ET-1 was similar in the two groups. Dilation induced by 10^{-12} M ET-1 was blocked by indomethacin treatment and converted to constriction of pial arterioles ($17 \pm 2\%$ for the control and $18 \pm 1\%$ for the hematoma group, respectively). Higher doses of ET-1 also showed enhanced dose-dependent

Figure 1. Changes in pial arteriolar diameter after topical application of LTC₄ in the absence (top panel) and presence (lower panel) of indomethacin, 5 mg/kg. * $p < 0.05$ compared with control; $n = 8$.

constrictions of pial arterioles after treatment with indomethacin ($25 \pm 1\%$ for control plus indomethacin treatment versus $15 \pm 1\%$ in the control nonindomethacin and by $32 \pm 3\%$ for the hematoma plus indomethacin group compared with $25 \pm 2\%$ for hematoma non-indomethacin at 10^{-8} M ET-1). In the group treated with indomethacin, vasoconstriction produced by topical application of ET-1 in the hematoma group was significantly greater at 10^{-10} and 10^{-8} M, respectively, compared with the control, but the differences between the hematoma group and the control group were less than before indomethacin treatment (Fig. 2, lower panel).

DISCUSSION

The present data show that the mean diameter of pial arterioles on the cerebral cortex in contact with blood 4 d after cortical injection of autologous blood was reduced by 39% compared with the control group. In addition, there was a reduction in the number of pial arterioles of large diameter (120–200 μm), whereas pial arterioles in the range of 60–120 μm were increased. These data suggesting that pial arterioles of larger diameter may be more prone to the blood-induced

Figure 2. Changes in pial arteriolar diameter after topical application of ET-1 in the absence (*top panel*) and presence (*lower panel*) of indomethacin, 5 mg/kg. * $p < 0.05$ compared with control; $n = 8$.

reduction in vascular diameter observed after cerebral hemorrhage. This result is also consistent with findings that showed significant reductions in the diameter of pial arterioles after cerebral hemorrhage in both experimental and clinical studies (12, 15, 17, 21).

Cerebral hematoma enhances constriction of pial arterioles induced by topical applications of LTC_4 and ET-1. Inhibition of dilator prostanoid synthesis by treatment with indomethacin diminished the differences in constrictor responses to ET-1 and LTC_4 between the control and hematoma piglets.

Enhanced vasoconstriction to ET-1 and LTC_4 application in the hematoma group is consistent with our previous observation *in vivo* (11). The reported enhanced vasoconstriction to ET-1 of rat cerebral artery in an *in vitro* study after cerebral hemorrhage (23) is also consistent with our observation. Indomethacin treatment enhanced the vasoconstrictions produced by topical applications of ET-1 and LTC_4 in the control to a level similar to that of the hematoma group. This is consistent with the hypothesis that removal of dilator prostanoid influences results in enhanced vasoconstriction to ET-1 and LTC_4 similarly to that caused by cerebral hematoma. The dose of indomethacin used in this study has been shown to cross the blood-brain barrier sufficiently to block cortical prostanoid production (2, 27), resulting in loss of dilation to stimuli associated with increased cerebral prostanoid synthesis (4, 27). Consistent with this observation, Busija *et al.* (28) reported that treatment with indomethacin enhanced vasoconstriction produced by oxytocin in piglets. Similarly, prostanoid inhibition has been reported to enhance vasoconstrictor responses to acetylcholine in dog lung (26). The mechanisms behind the enhanced vasoconstriction responses to vasoconstrictor agents

after treatment with indomethacin or cerebral hematoma could involve similar components. Under normal physiologic conditions, balanced synthesis and secretion of dilator and constrictor vasoactive agents are produced to maintain the cerebral arterial vasomotor tone. After clinical or experimental cerebral hemorrhage, decreased synthesis of dilator prostanoids and increased levels of spasmogenic agents have been observed in plasma and cerebrospinal fluid from patients as well as animal models (12, 29). Decreased synthesis of prostaglandin I_2 and increased production of constricting prostanoids from vaso-spastic vessels after subarachnoid hemorrhage have been reported (30, 31). Endothelial damage, along with adherence of platelets, and increased release of spasmogenic agents plus decreased synthesis of dilator prostanoids could contribute to the enhanced vasoconstrictions observed after hemorrhage by disrupting the normal balance between the constrictor and dilator vasoactive agents within the cerebral arterial wall.

Topical application of ET-1 in the indomethacin-treated group still produced significantly enhanced vasoconstrictions in the hematoma group compared with the control, although the differences were less than before indomethacin. ET-1 has been reported to stimulate the release of other vasoconstrictor agents such as catecholamines, prostaglandin $\text{F}_{2\alpha}$, peptidoleukotrienes, thromboxane, platelet-activating factors, and endothelium-derived contracting factors that could further potentiate ET-1-induced constriction of pial arterioles (32–34). In addition, increased secretions of spasmogenic agents induced by hemorrhage have been reported (12). All of these vasoactive agents may act to potentiate the actions of one another and especially of ET-1 in this study. Interactions among these vasoconstrictor agents could lead to the enhanced vasoconstrictions observed after experimental hemorrhage. Pretreatment of aortic ring with a threshold concentration of 5-hydroxytryptamine has been reported to enhance the subsequent contractile response to ET-1 (35). Similarly, ET-1 has been reported to enhance *in vitro* contraction of porcine coronary artery ring induced by 5-hydroxytryptamine (36). Thus, a variety of mechanisms in addition to reduced dilator prostanoid influence could be involved in hematoma-induced augmentation of ET-1 constriction.

Vasodilation of pial arterioles to the lowest dose of ET-1 was consistent with our previous finding (10, 11). Treatment with indomethacin blocked the dilation, confirming the speculation that dilation of pial arterioles to a low dose of ET-1 was dilator prostanoid-dependent (10). In contrast to our previous findings (10), we observed in the present study that indomethacin treatment enhanced vasoconstriction to topical application of ET-1. The differences in our observations may be due to the fact that we probably used a mixture of all the known ETs in our first study with ET. The available ET is now more refined and specific, and in this study we specifically used ET-1. ET-2 and ET-3 have been reported to be inactive or could possibly induce vasodilation of arteries (37). This might account for the discrepancy observed in the effects of indomethacin on ET-induced vasoconstrictions. However, in an *in vitro* study with dog cerebral artery, based upon ED_{50} values, ET-2 was as potent as ET-1, whereas ET-3 was about 64 times less potent than ET-1/ET-2 (38). Hence, if the ET previously used con-

tained more ET-3 than ET-1/ET-2, the composition could contribute to the differences between our two studies.

We demonstrated that treatment with indomethacin diminished the differences in vasoconstrictions to topically applied ET-1 and LTC₄, between hematoma and control groups by augmenting constrictions more in the control group. These data suggest a mechanism behind cerebral hematoma induced vasoconstriction may involve disruption of the balance between dilator prostanoid influence and vasoconstrictor agents. Thus, loss of dilator prostanoid influence caused by hematoma may contribute to more prolonged and uninhibited constriction of pial arterioles by constrictor vasoactive agents, leading to the sustained and prolonged arterial wall narrowing observed after cerebral hemorrhage.

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