Plasminogen Activators Contribute to Impairment of Hypercapnic and Hypotensive Cerebrovasodilation After Cerebral Hypoxia/Ischemia in the Newborn Pig

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Background and Purpose—Babies are frequently exposed to hypoxia and ischemia during the perinatal period as a result of stroke or problems with delivery or respiratory management post delivery. The only U.S. Food and Drug Administration-approved treatment for acute stroke is the administration of tPA. Nonetheless, basic science studies indicate that tPA exhibits both beneficial and deleterious effects on central nervous system function. Cerebral hypoxia/ischemia (H/I) impairs dilation to hypercapnia and hypotension in the newborn pig. We investigated the role of exogenous and endogenous plasminogen activators (PA) in piglet hypercapnic and hypotensive dilator impairment after H/I.

Methods—Responses to dilator stimuli were measured in chloralose-anesthetized piglets equipped with a closed cranial window before and after hypoxia (PO2 35 mm Hg) and subsequent global cerebral ischemia. Data (n=6) were analyzed by repeated-measures analysis of variance.

Results—Hypercapnic (PCO2 75 mm Hg) and hypotensive (mean arterial blood pressure decreased by 45%) pial artery dilation (PAD) was blunted after H/I and reversed to vasoconstriction in animals pretreated with tPA or uPA (10\(^{-7}\) mol/L; 26±2, 11±1, and -4±1% for hypercapnia before, after H/I, and after H/I with tPA). In animals pretreated with EEIMD (10\(^{-7}\) mol/L), a peptide that binds uPA and tPA but does not affect proteolysis or soluble uPA receptor (suPAR, 10\(^{-7}\) mol/L), which binds but does not affect the proteolytic activity of uPA. PAD induced by hypercapnia and hypotension was attenuated to a lesser extent (25±2 and 17±1% for hypercapnic PAD before and after H/I in EEIMD-pretreated animals and 21±1 and 18±2% in suPAR-pretreated animals).

Conclusions—These data show that exogenous PA administration potentiates the impairment of hypercapnic and hypotensive PAD that occurs after H/I. Inhibition of endogenous PA may ameliorate the impairment of PAD induced by hypercapnia and hypotension PAD that develops after hypoxic central nervous system injury of diverse etiologies. (Stroke. 2005;36:2265-2269.)

Key Words: cerebral circulation ■ ischemia ■ newborn

Central nervous system (CNS) ischemia and hypoxia/ischemia occur commonly during the perinatal period as a result of problems with delivery and respiratory management postdelivery or stroke, which occurs with an incidence approaching one in 4000 births. At least 30% of neonatal strokes are the result of thrombosis. CNS damage often leads to severe and permanent neurologic damage.

Most neonatal brain injury is metabolic, whether from transient ischemia/reperfusion or from defects in inherited metabolic pathways expressed after birth. Cerebrovascular dysfunction contributes to neurologic damage after stroke and other causes of cerebral ischemia. For example, hypotension causes loss of cerebrovascular regulation, creating a pressure passive cerebral circulation that impairs cerebral blood flow (CBF) and promotes tissue ischemia. Hypercapnia related to respiratory disease has also been shown to contribute to the development of the pressure passive circulation leading to periventricular leukomalacia in the perinate. Using a piglet model, we have shown that pial artery dilation in response to hypotension and hypercapnia is blunted after cerebral hypoxia/ischemia. However, the mechanism underlying loss of compensatory vasodilation and therapeutic avenues to ameliorate its deleterious effects on CNS ischemia remain uncertain.

Tissue-type plasminogen activator (tPA) is the only US Food and Drug Administration-approved treatment of acute ischemic stroke. tPA, like urokinase (uPA) is a serine protease that converts plasminogen to the active protease.
plasmin. In addition to its salutary role in reperfusion by lysing intravascular clots, tPA contributes to excitotoxic neuronal death by enhancing signaling mediated by the N-methyl-D-Aspartate glutamate receptor. tPA increased the volume of injured brain tissue in mice after stroke but is neuroprotective in zinc-induced neuronal death.

We have recently observed that tPA and uPA mediate cerebrovasodilation and increase CBF at (patho)physiological concentrations. However, little is known regarding the role of endogenous plasminogen activators, and in particular uPA, in the CNS response to hypoxia/ischemia. On the other hand, the study of the role of exogenous plasminogen activator administration, and in particular tPA, in the CNS response to hypoxia/ischemia is of clear and substantial clinical therapeutic interest. In this study, we investigated the contribution of these plasminogen activators to impairment of hypercapnic and hypotensive pial artery dilation that occurs after cerebral hypoxia/ischemia in the newborn pig. To do so, we took advantage of the fact that the PAI-1-derived peptide, EEIIMD, inhibits the vasoactivity of tPA and uPA without inhibiting its fibrinolytic activity, whereas soluble urokinase plasminogen activator receptor (suPAR) exerts a similar effect on uPA alone. To investigate the role of endogenous plasminogen activators, we measured pial artery dilation in response to hypercapnia and hypotension before and after cerebral hypoxia/ischemia in the absence and presence of suPAR and EEIIMD. To investigate the effect of exogenous plasminogen activators, responses to hypercapnia and hypotension were obtained before and after cerebral hypoxia/ischemia in the absence and presence of the topical administration of tPA or uPA.

Materials and Methods

Piglets (1 to 5 days old) were initially anesthetized with isoflurane (1 to 2 MAC) and maintained with α-chloralose (30 to 50 mg/kg supplemented with 5 mg/kg/h intravenously). Catheters were inserted into femoral arteries to monitor blood pressure and blood chemistry. The animals were mechanically ventilated with room air supplemented with 5 mg/kg/h intravenously). Catheters were inserted into femoral arteries to monitor blood pressure and blood chemistry. The animals were mechanically ventilated with room air and the temperature maintained at 37 to 39°C monitored rectally.

Total cerebral ischemia (20 minutes) was accomplished by infusing artificial cerebrospinal fluid into a hollow bolt in the cranium to maintain an intracranial pressure 15 mm Hg greater than the numerical mean of systolic and diastolic arterial blood pressure. Intracranial pressure was monitored by a sidearm of the cranial window. Blood flow in pial arteries, as viewed with a microscope and monitor, stopped completely on elevation of intracranial pressure and did not resume until the pressure was lowered. To prevent the arterial pressure from rising inordinately (Cushing response), venous blood was withdrawn as necessary to maintain mean arterial blood pressure no greater than 100 mm Hg. As the cerebral ischemic response subsided, the shed blood was returned to the animal. Hypoxia was produced for 10 minutes before ischemia by decreasing O2 through inhalation of N2, which was immediately followed by the ischemia protocol after concomitantly restoring ventilation to room air. In a subset of animals, only hypoxia was produced.

Results

Topical Exogenous Plasminogen Activator Administration and Endogenous Plasminogen Activators Contribute to Impairment of Hypercapnic and Hypotensive Pial Artery Dilation After Hypoxia/ischemia

Two levels of hypercapnia, hypotension, and isoproterenol elicited reproducible graded pial small artery (120 to 160 μm) and arteriole (50 to 70 μm) dilation in sham control animals (data not shown). Pial small artery dilation to hypercapnia and hypotension was blunted after hypoxia/ischemia, whereas responses to isoproterenol were unchanged (Figure 1). On a percentage basis, for example, pial small artery responses to hypercapnia were blunted by 57±7 and 53±5%, whereas responses to hypotension were blunted by 66±4 and 61±6%. Similar reductions in responses were seen in pial arterioles. Pretreatment with topical exogenous tPA (10−7 mol/L) 30 minutes before hypoxia/ischemia followed by continuous addition of tPA postinsult further reduced the already impaired responses to hypercapnia and hypotension (Figure 1). In fact, responses to these 2 stimuli were reversed to modest vasoconstriction after hypoxia/ischemia (Figure 1). On a percentage basis, responses to hypercapnia postinsult in the presence of exogenous tPA were now inhibited by 105±11 and 118±11%, whereas responses to hypotension were inhibited by 116±17 and 109±14%. Vasodilation to isoproterenol remained unchanged posthypoxia/ischemia in the presence of exogenously added tPA (Figure 1). Similar observations were made in pial arterioles.
To investigate the role of endogenous plasminogen activators, another group of animals was pretreated with the plasminogen activator inhibitor-derived peptide EEIIMD (10^-7 mol/L). This peptide binds to the docking site of tPA and uPA but does not inhibit their plasminogen activator activity. In EEIIMD-treated animals, vasodilation induced by hypercapnia and hypotension after hypoxia/ischemia was significantly greater than that observed postinsult in non-EEIIMD-treated animals (Figure 1). On a percentage basis, for example, responses to hypercapnia were inhibited by 31±6 and 28±7%, whereas responses to hypotension were inhibited by only 19±7 and 23±5%. Dilation in response to isoproterenol was unchanged by EEIIMD after hypoxia/ischemia (Figure 1). Also, similar observations were made in pial arterioles compared with small arteries.

In another series of experiments, exogenous topical uPA (10^-7 mol/L) was administered before hypoxia/ischemia. Pretreatment with uPA, like tPA, reversed the normal dilation response to hypercapnia and hypotension to constriction postinsult (Figure 2). Unlike tPA, uPA inhibited dilation in response to hypercapnia after hypoxia/ischemia to a significantly greater extent than it affected the response to hypotension (Figure 2). For example, on a percentage basis, responses to hypercapnia were inhibited by 162±16 and 147±16%, whereas responses to hypotension were inhibited by 96±11 and 106±10%. Pretreatment with suPAR (10^-7 mol/L), which binds uPA but does not impair its plasminogen activator activity, also enhanced dilation in response to hypercapnia and hypotension after hypoxia/ischemia, similar to the effect of EEIIMD (Figures 1 and 2). However, in contrast to EEIIMD, responses to hypercapnia and hypotension were maintained completely by pretreatment with suPAR (Figures 1 and 2). Responses to isoproterenol were unchanged by uPA or suPAR administration postinsult (Figure 2). Similar data were obtained when pial arterioles were studied (data not shown).

Topical Exogenous Plasminogen Activator Administration and Endogenous Plasminogen Activators Contribute to Impairment of Hypercapnic and Hypotensive Pial Artery Dilation After Hypoxia

A similar paradigm of administering tPA, uPA, EEIIMD, or suPAR was used in animals that were subjected to hypoxia alone instead of hypoxia/ischemia. Hypoxia alone (n=6) impaired vasodilation in response to hypercapnia and hypotension, although not to the same extent as hypoxia/ischemia. Pretreatment with tPA or uPA potentiated this impairment, and pretreatment with EEIIMD and suPAR partially restored hypoxia-induced impairment of hypercapnic and hypotensive dilation (data not shown). On a percentage basis, for example, hypoxia alone impaired dilation to hypercapnia by 30±3 and 28±3%. After pretreatment with uPA, the impairment in hypercapnic dilation was 60±11 and 61±11%, values that are significantly less than responses to hypoxia/ischemia (see previously). Responses to isoproterenol were unchanged by hypoxia alone or by pretreatment with tPA, uPA, EEIIMD, or suPAR in hypoxic animals. There were no statistical differences between data obtained in pial small arteries and that obtained in pial arterioles.

EEIIMD and suPAR Have No Effect on Hypercapnic and Hypotensive Pial Artery Dilation in Sham Control Animals

Administration of tPA and uPA elicited modest pial small artery dilation in sham control animals (9±1 and 12±1% for tPA and uPA, respectively). Similarly, neither EEIIMD nor...
suPAR had any significant effect on pial artery diameter in sham control animals. Administration of EEIIMD blocked dilation to tPA and uPA, whereas suPAR blocked dilation to uPA (data not shown). Neither EEIIMD nor suPAR affected pial artery dilation induced by hypercapnia or hypotension in sham (nonhypoxia/ischemia) animals (16±1 and 26±2 vs 15±2 and 27±3% for hypercapnia before and after EEIIMD, n=6; 15±2 and 25±3 vs 14±2 and 24±3% for hypercapnia before and after suPAR, n=6).

Blood Chemistry
Blood chemistry tests and mean arterial blood pressure values were collected before and after all experiments and during periods of hypoxia and hypercapnia. Hypoxia decreased PO2 to 35±3 mm Hg. Low hypercapnia raised PCO2 to 56±4 mm Hg, and high hypercapnia raised PCO2 to 75±5 mm Hg. Carbon dioxide levels were kept constant during periods of hypoxia and oxygen levels were kept constant during periods of hypercapnia. Before and after all experiments, the pH, PCO2, PO2, and mean arterial blood pressure were unchanged.

Figure 2. Influence of hypercapnia (low, high; PCO2 of 50 to 60 and 70 to 80 mm Hg), hypotension (moderate, severe; 25% and 45% reductions in mean arterial blood pressure), and isoproterenol (10−5, 10−6 mol/L; A–C) on pial artery diameter before (control), after hypoxia/ischemia (PO2 of 35 mm Hg for 10 minutes followed by global cerebral ischemia for 20 minutes; H/I), after H/I pretreated with uPA (10−7 mol/L) 30 minutes before H/I, and after H/I pretreated with suPAR (10−7 mol/L; n=6). *P<0.05 versus corresponding control value +P<0.05 versus corresponding nonpretreated H/I value.

Discussion
Control of cerebrovascular tone after hypoxia/ischemic injury plays a critical role in mediating CNS ischemia and neurologic damage in affected newborns. We found that hypoxia/ischemia, an all too common occurrence poststroke or delivery, impairs dilation of pial arteries in response to hypercapnia and hypotension, whereas responses to isoproterenol are unchanged, as reported previously.6–9 Of interest, pretreatment with the plasminogen activators tPA or uPA potentiates the effect of hypoxia/ischemia, not only inhibiting dilation to hypercapnia and hypotension, but actually causing vasoconstriction. This observation is particularly intriguing in that exogenous uPA and tPA administration by themselves produced modest pial artery dilation in control animals. Moreover, application of a vasodilator would have been predicted to have an additive or synergistic effect in the presence of a second dilator depending on their mechanisms of action rather than causing vasoconstriction. These data suggest that exogenous tPA and uPA may impair physiological responses to hypoxia/ischemia and contribute to the development of the pressure passive cerebrovascular circulation after ischemic injury. The fact that responses to isoproterenol after hypoxia/ischemia remained unchanged in the presence of the plasminogen activators indicates the effect of plasminogen activators is specific for hypercapnia and hypotension and likely depends on the signal transduction cascades that they activate.20

Pretreatment with the PAI-1-derived peptide EEIIMD partially prevented and suPAR completely prevented impairment of hypercapnic and hypotensive pial artery dilation after hypoxia/ischemia, likely the result of suPAR’s extraordinarily tight binding affinity for uPA (sub nmol/L). These data suggest that although both endogenous tPA and uPA may contribute to the development of a pressure passive cerebrovascular circulation after ischemic injury, uPA predominates in its contribution to hypoxic/ischemic vascular derangement. Because EEIIMD binds to the docking site of tPA and uPA but does not inhibit their plasminogen activator activity, these data also suggest that this effect is not mediated through their plasminogen activator activities. Again, the protective effect of these plasminogen activator inhibitors, however, did not result from a general nonspecific potentiation/inhibition of vascular responsiveness, because EEIIMD and suPAR had no effect on dilation induced by isoproterenol.

Topical application of EEIIMD (10−7 mol/L) blocked pial artery dilation to tPA and uPA, whereas suPAR (10−7 mol/L) blocked uPA pial dilation, consistent with our previous
findings. Vasodilator responses to the nitric oxide releaser sodium nitroprusside and the nonselective dilator papaverine were unchanged in the presence of either EEIIMD or suPAR. These data suggest that EEIIMD and suPAR are efficacious and selective inhibitors of plasminogen activator induced cerebrovasodilation. However, neither EEIIMD nor suPAR had a significant effect on pial artery diameter by themselves, suggesting that plasminogen activators make a minimal contribution to tonic cerebrovascular tone in the piglet under physiological conditions. EEIIMD inhibited the vascular activity of uPA to the same extent as PAI-1, but EEIIMD does not modulate tPA or uPA catalytic activity. These results exclude the possibility that EEIIMD signals by itself as had been suggested recently for PAI-1 and affirms that its activity is mediated through a noncatalytic effect of tPA and uPA on vascular tone. By extension, observations concerning the effects of EEIIMD and suPAR on hypercapnic and hypotensive pial artery dilation after hypoxia/ischemia suggest that protection by these agents occurs through antagonism of noncatalytic actions of endogenous plasminogen activators to selectively repress cerebral vasodilating function.

The plasminogen activator concentration used in this study was chosen based on enzyme-linked immunosorbent assay data indicating that resting physiological CSF tPA concentration is 10⁻⁷ mol/L, which increases to approximately 10⁻⁷ mol/L under pathologic conditions such as fluid percussion brain injury in the piglet. The mechanism whereby plasminogen activators modulate hypercapnic and hypotensive pial artery dilation after hypoxia/ischemia is presently uncertain. EEIIMD and suPAR partially restore pial artery dilation to NMDA receptor activation that had been reversed to vasoconstriction after fluid percussion brain injury in the piglet. Data in the present study indicate that plasminogen activator contribution to CNS damage extends well beyond the previously suggested simple interactions with the NMDA receptor. These data suggest new means to control the loss of cerebrovascular tone after hypoxia with or without subsequent ischemia/reperfusion. Importantly, these data for the first time provide information that suggests that endogenous uPA may also play an important role in the damage that develops after hypoxic CNS injury of diverse etiology. In turn, observations regarding the relative vascular impairment after hypoxia versus hypoxia/ischemia suggest new ways to modify the toxic effects of the therapeutic administration of tPA and thereby increase its benefit-to-risk ratio. Nonetheless, because the study design used topical pretreatment, caution is urged regarding clinical interpretation as to whether intravenous delivery of tPA or uPA posttrauma is detrimental or beneficial.

In conclusion, these data show that exogenous plasminogen activator administration potentiates hypoxic/ischemic impairment of hypercapnic and hypotensive pial artery dilation. These data suggest that means to block or neutralize endogenous plasminogen activator release resulting from hypoxia/ischemia may ameliorate hypercapnic and hypotensive pial artery dilator impairment, preserve cerebrovascular regulation, and lessen tissue ischemia.

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References
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