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BRAIN RESEARCH

Research Report

# uPA impairs cerebrovasodilation after hypoxia/ischemia through LRP and ERK MAPK

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ABSTRACT

We have previously observed that soluble urokinase plasminogn activator receptor (suPAR) prevents impairment of cerebrovasodilation induced by hypercapnia and hypotension after hypoxia/ischemia (H/I) in the newborn pig. In this study, we investigated the role of low-density lipoprotein-related protein (LRP) receptor and the ERK isoform of mitogen activated protein kinase (MAPK) in uPA-mediated impairment of vasodilation after H/I in piglets equipped with a closed cranial window. CSF uPA increased from 9±2 to 52±8 and 140±21 ng/ml at 1 and 4 h after H/I, respectively. The LRP antagonist receptor associated protein (RAP) and anti-LRP antibody blunted the increase in CSF uPA at 1 h (17±2 ng/ml) but not 4 h post insult. uPA detectable in sham-treated cortex by immunhistochemistry was markedly elevated 4 h after H/I. Phosphorylation (activation) of CSF ERK MAPK was detected at 1 and 4 h post H/I and blocked by RAP. Exogenous uPA administered at 4 h post H/I further stimulated ERK MAPK phosphorylation, which was blocked by RAP. Pre-treatment of piglets with RAP, anti-LRP, and suPAR completely prevented, and the ERK MAPK antagonist U 0126 partially prevented, impaired responses to hypotension and hypercapnia post H/I, but none of these antagonists affected the response to isoproterenol. These data indicate that uPA is upregulated after H/I through an LRP-dependent process and that the released uPA impairs hypercapnic and hypotensive dilation through an LRP- and ERK MAPK dependent pathway. These data suggest that modulation of uPA upregulation and/or uPA-mediated signal transduction may preserve cerebrohemodynamic control after hypoxia/ischemia.



## 1. Introduction

Perinatal cerebral hypoxia/ischemia has many causes, unclear pathophysiology, no specific mechanism-related treatment, and poor outcome. Neonatal stroke may occur in as many as 1 in 4000 births (Nelson and Lynch, 2004). In newborns with stroke, complications such as hypoxic/ischemic events are common (Ferriero, 2004). Maternal and perinatal coagulopathy

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predispose to perinatal stroke (Gunther et al., 2000; Kraus and Acheen, 1999), with 30% of neonatal strokes being due to thrombosis (DeVeber and Andrew, 2001). A better understanding of the pathophysiologic responses that occur in children after cerebral hypoxia/ischemia is needed to develop mechanism based approaches to therapy.

One contributor to neurological damage after hypoxia/ischemia is thought to be cerebrovascular dysfunction. For example, hypotension leads to loss of cerebrovascular regulation promoting tissue ischemia, while cerebrovasoconstriction associated with hypocapnia contributes to periventricular leukomalacia in the perinate (Volpe, 1998). Using a piglet model, we have shown that pial artery dilation in response to hypotension and hypercapnia is blunted after cerebral hypoxia/ischemia (Jagolino and Armstead, 2003; Leffler et al., 1989a,b). However, the mechanism underlying loss of compensatory vasodilation and therapeutic avenues to ameliorate its deleterious effects on CNS ischemia remain uncertain.

Urokinase (uPA) and tissue plasminogen activator (tPA) are serine proteases that convert plasminogen to the active protease plasmin (Bdeir et al., 2000; Collen and Lijnen, 1991). Recombinant tPA is the only FDA approved for stroke (Kim et al., 1999). However, tPA exhibits deleterious as well as beneficial effects that profoundly constrain its clinical utility. In addition to its salutary role in reperfusion, tPA contributes to excitotoxic neuronal cell death (Nicole et al., 2001) and increases stroke infarct volume in mice (Wang et al., 1998).

We have observed that exogenous tPA or uPA applied topically to the piglet cerebral cortex potentiates the impairment of pial artery dilation caused by hypercapnia and hypotension in the setting of hypoxia/ischemia (Armstead et al., 2005a). In other studies, we have shown that the endogenous plasminogen activator inhibitor-1 derived peptide, EEIIMD, inhibits tPA and

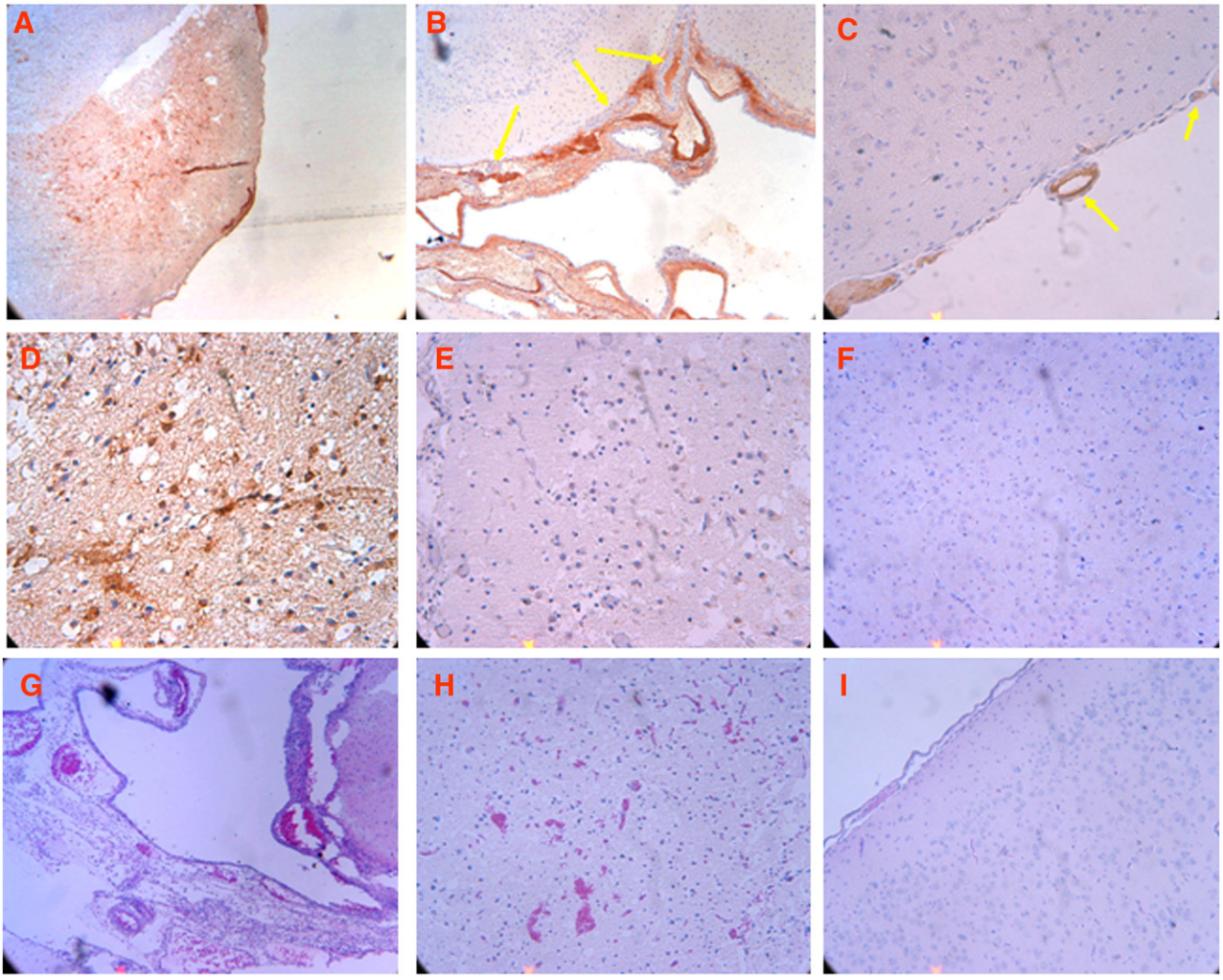


**Fig. 1 – Immunohistochemistry and histopathology 4 h after cerebral hypoxia/ischemia. Sections of the parietal cortex from piglet brains after ischemic injury (A–E) and from uninjured control animal (I and F), were subjected to antigen retrieval in citrate buffer and stained with anti-uPA monoclonal antibody (5 μg/ml) (Panels, A–D and F) or with non-immune mouse $IgG_1$ as a negative control (Panel E), secondary biotinylated anti-mouse IgG (1:200), followed by incubation with HRP-conjugated streptavidin. Magnification shown is 100× for panels A, B, G, and I, 200× for panels C, E, F, and H, 400× for panel D. Adjacent sections from the same brains exposed to ischemic injury (Panels G, H) and from uninjured controls (Panel I), were stained by H&E for histological inspection. These data reflect an *n* of 2 per experimental group.**