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## Recombinant Tissue Plasminogen Activator Causes a Significant Increase in Endogenous Urokinase Following Experimental Focal Cerebral Ischemia.

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The aim of this study was to investigate the effects of different doses of exogenous recombinant human tissue plasminogen activator (rt-PA) on the endogenous cerebral plasminogen/plasmin system in focal ischemia. Ischemia was induced using the suture model in rats subjected to 3 h ischemia (I) and 24 h reperfusion (R). Each group of rats (n=6) received either treatment (0.9, 9, or 18 mg rt-PA/kg body weight) or saline (control group) at the end of ischemia; in addition a sham-operated group of rats (n=6) was involved in the comparisons. The activity of the plasminogen activators was measured by casein dependent plasminogen zymography of the ischemic and non-ischemic basal ganglia and cortex. Compared with the contralateral non-ischemic area and the sham-operated controls, urokinase (u-PA) was significantly increased in the ischemic area. In the cortex u-PA rose from sham 91% ± 7% to ischemia 176% ± 10% ($p<0.005$). Increasing rt-PA doses led to further cortical u-PA activation: 0.9 mg: 179% ± 20%; 9 mg: 195% ± 18%; 18 mg: 249% ± 13% ($p<0.001$). An extreme increase in the u-PA activity was observed in the basal ganglia: from 97% ± 5% (sham) to 1019% ± 22% (ischemia; $p<0.001$). This increase was further aggravated by higher rt-PA doses: 0.9 mg caused 1122% ± 14%; 9 mg caused 1189% ± 33%, and 18 mg caused 1236% ± 15% ($p<0.001$). The t-PA level did not change during I3R24 from that in the sham-operated animals; however, during low and moderate doses of rt-PA, endogenous t-PA was reduced on the ischemic side. The high dose (18 mg) caused no change in t-PA expression in the ischemic cortex (104% ± 5%) but a slight increase in the ischemic basal ganglia (121% ± 6% ; $p<0.001$). In conclusion, while ischemia leads to a significant increase in u-PA, mainly in the basal ganglia, endogenous t-PA is not altered. Increasing doses of artificially administered rt-PA leads to a further elevation of u-PA activity. Thus, u-PA seems to play the major role in the endogenous plasminogen activator system following focal cerebral ischemia. This may be relevant for the development of edema and hemorrhage secondary to ischemic stroke.

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## Abnormal Vascular Responses and Cerebral Reperfusion Injury in ApoE-Deficient Mice on Western Diet.

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*Background:* Western diet fed apoE deficient mice demonstrate impaired endothelium-dependent relaxation in systemic vessels, and serve as a useful mouse model for atherogenesis. However, the effects of apoE gene deficiency and Western-diet on cerebrovascular function and response to ischemia have not been studied. We postulated that Western diet-fed apoE knockout mice would show abnormalities in cerebrovascular function and reactivity, and that these abnormalities may underlie increased susceptibility to cerebral ischemia. *Methods and Results*: Adult wild type C57 black/6 mice (WT) and apoE knockout mice were fed a Western diet (42% of calories from fat) for eight weeks prior to study. Blood flow was measured using laser Doppler flowmetry and laser speckle imaging. ApoE knockout mice showed reduced absolute cerebral blood flow (CBF), impaired cerebral autoregulation and marked blunting of the cortical barrel blood flow response to whisker stimulation. In contrast, the vasodilatory response to hypercapnia was relatively preserved. These results indicate specific defects in the function and reactivity of the cerebrovasculature in the Western diet-fed apoE knockout mice. Separate animals were subjected to transient middle cerebral artery filament occlusion for one hour, followed by reperfusion. ApoE knockout mice showed lower regional CBF than WT mice during 40 minutes of reperfusion by laser Doppler flowmetry (at 10 min: 37±10% vs. 52±17%; at 40 min: 68±21% vs 82±15%, mean±SEM, n =4 each group). ApoE knockout mice showed larger cerebral infarcts (112.5±15.7 $mm^3$) by 2,3,5-triphenyltetrazolium chloride staining than WT mice (65.6±5.5 $mm^3$ , n=3 each group, $p<0.05$) after 24 hours of reperfusion. The larger infarct size in apoE knockout mice was paralleled by functional changes in neurological deficit as compared to WT (3.6±0.8 vs 2.2±0.9, n=4 each group). *Conclusion:* Western diet-fed apoE knockout mice show specific abnormalities in cerebral autoregulation and cerebrovascular reactivity in response to whisker stimulation, reflecting vascular dysfunction. These abnormalities may underlie the increased susceptibility of these animals to focal ischemia.

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## Oxygen-Glucose Deprivation Caused Cerebral Endothelial Cells Apoptosis Involve Smac Release from Mitochondria.

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Background and Purpose: Death of cerebral endothelial cells (CECs) following hypoxia/ischemia is accompanied by cellular events suggestive of apoptosis. The second mitochondria-derived activator of caspase (Smac) cascade has been implicated in a mitochondria-dependent apoptotic signaling process. Smac is released from mitochondria into cytosol in response to death signals. Smac activates caspase 3 by binding to inhibitor of apoptosis proteins (IAPs). This study aimed to explore whether Smac release is involved in CEC death induced by hypoxia/ischemia. Methods: Cultured murine CECs were subjected to oxygen-glucose deprivation (OGD) for 0 (as control), 4 and 8 hrs. Cells were harvested and subfractionized into cytosolic and mitochondrial fractions. The extent of cell death was assessed by the MTT and LDH assays. Western blotting and immunofluorescent staining were used to detect the subcellular translocation of Smac. The binding of Smac and XIAP was detected by co-immunoprecipitation. Results: After OGD, Smac release from mitochondria into cytosol could be demonstrated by Western blotting with an increase in cytosol and decrease in mitochondria fractions in a time-dependent manner. This finding was confirmed by immunofluorescent studies. Smac was localized in mitochondria and appeared as a punctate pattern in CECs not subjected to OGD. OGD resulted in a homogenous staining of Smac, suggesting its release from mitochondria into cytosol. Co-immunoprecipitation studies showed Smac binding to XIAP after OGD. We have previously shown caspase activation under the same OGD paradigm in CECs. N-acetyl-cysteine (NAC), an antioxidant, inhibited OGD-induced Smac release from mitochondria and CEC death. Conclusions: CEC death following hypoxia/ischemia involves Smac release from mitochondria into cytosol to bind XIAP, resulting in caspase activation and CEC death. This process could be prevented by NAC, an antioxidant.

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## Erythropoietin and Erythropoietin Mimetic Peptide in Focal Cerebral Ischemia.

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Erythropoietin (EPO) is a large macromolecule with neuroprotective effects but questionable brain penetration. A smaller mimetic peptide (EMP-1) has been developed that binds to the EPO receptor. The purpose of this investigation was to determine whether EPO and EMP-1 provide neuro- and vascular protection after temporary focal cerebral ischemia. **Methods**: Sixty-one animals were subjected to either 2 hours of middle cerebral artery occlusion (MCAO) followed by 48 hours of reperfusion (n=30) or 90 minutes followed by a 7 day survival (n=31). Animals received either EPO 5000 u/kg, EMP-1 400 mcg/kg, or saline. In the acute study, infarct size and hemorrhage development were assessed and in the chronic study, infarct size and neurobehavioral outcome were endpoints. **Results**: There were no significant differences in infarct size at 48 hours but there was signficantly more hemoglobin in the ischemic hemispheres of the EMP-1 treated animals ($p<0.01$). At 7 days, the EMP-1 treated animals exhibited significantly lower infarct size than either saline ($p< 0.0001$) or EPO ($p<0.05$) animals and the EPO group was also significantly lower than saline ($p<0.05$). In addition, the EMP-1 treated animals had improved neurobehavioral scores, at least in the early days after the injury ($p=0.012$). **Conclusions**: EMP-1 may be superior to EPO in providing neuroprotection after focal cerebral ischemia. Although EPO and EMP-1 did not reduce infarct size at 48 hours, the significant decrease at 7 days may reflect a neurorestorative action. Whether the improved performance of EMP-1 over EPO is due to superior brain penetration is unknown at this time.

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## Intranasal Delivery of Nerve Growth Factor Bypasses the Blood-Brain Barrier and Protects Against Acute Ischemic Damage.

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***Objective:*** Plenty of experiments in *vivo* and in *vitro* suggest that nerve growth factor (NGF) has significant neuroprotective actions on ischemic brain damage. However NGF is a protein with large molecular weight that does not cross the blood-brain barrier (BBB) efficiently. When administered through parenteral routines such as intravenous (IV) or intramuscular injection, it is difficult to reach the central nervous system (CNS). Although intracerebroventricular and intraparenchymal injection are effective to deliver NGF to CNS, it is invasive and not practical for clinical patients. Intranasal (IN) administration targeted the CNS bypassing the BBB. This study confirmed the reliability of IN pathway by comparing the distribution of NGF in different brain regions after IN and IV injection of NGF. The potential neuroprotective effects of IN NGF on cerebral infarction were also observed. ***Methods:*** A blinded, vehicle-controlled study of IN NGF and IV NGF on acute cerebral ischemia was performed using the intraluminal suture middle cerebral artery occlusion (MCAO) model. Experiment 1 Rats were randomly divided into IN NGF, IV NGF and untreated group (n=4). The NGF concentration in the brain following IN or IV treatment was measured by ELISA. Experiment 2 Rats were assigned to 4 groups: IN vehicle, IN NGF, IV vehicle, IV NGF (n=8 each). Treatments were initiated 30 min after the onset of MCAO and then again 24h later. Three neurologic behavioral tests were used to assess the motor, sensory and balance functions at 24h and 48h after MCAO. Corrected infarct volumes were determined 48h after MCAO. ***Results:*** Olfactory bulb in IN NGF group obtained the highestconcentration, arriving at 3252pg/g. The NGF concentration of olfactory bulb and hippocampus in IN NGF group is significantly higher than that in IV NGF and control group. The infarct volume was reduced significantly by 39% after IN NGF compared with IN vehicle. The balance function of IN NGF improved significantly at 24 and 48h ($P_{24}=0.02$ and $P_{48}=0.04$, respectively). ***Conclusions:*** IN NGF could bypass BBB, reach the CNS, reduce infarct volume and improve neurological function following MCAO in rats, which provides a simpler, safer and potentially more cost effective method of delivery.