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Studies on the kallikrein-kinin system in brain tissue and vasculature in experimental focal brain ischemia

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Bradykinin (Bk), the major metabolite of the kallikrein-kinin system (KKS) affects vascular function including induction of endothelium-dependent vasodilatation in arteries and an increase of blood-brain barrier permeability leading to brain oedema and swelling. Bk acts via two types of G-protein coupled receptors: constitutively expressed B2 receptors or B1 receptors following *de novo* expression. A full KKS has been found in the brain tissue.

There is good evidence in favour of an important pathophysiological role of the KKS and Bk in cerebral disorders including focal ischemia. This role appears to include vascular (dys)regulation on the macro- as well as the microvascular level. However, the interaction of the parenchymal and the vascular system is not yet clear. Therefore, in the present study we performed the characterization of the KKS genes expression in the brain tissue, in the middle cerebral artery (MCA) and in cerebral microvessels (MVs) under normal conditions and following focal ischemia.

First, we have developed a new method of high yield high purity MV extraction from the rat brain hemisphere in a purely mechanical way at $\leq$ 4°C. Purity was controlled by hematoxilin-eosine staining and significant accumulation of specific markers of ECs (eNOS and Tie2) and pericytes (PDGF-R) on the level of gene expression. A negligible level of neuronal marker mRNA was obtained while the glial fibrillary acidic protein (GFAP used as glial cell marker) mRNA level was comparable to brain tissue (BT). Extensive immunohistochemical analyses suggest remnants of glia endfeet tightly connected with the MV as the source of GFAP mRNA. In addition, GFAP mRNA and GFAP immunoreactivity (IR) was consistently found in the wall of all pial arteries (such as the MCA) studied as well as in meningeal tissue adjacent to pial arteries. The role of GFAP in the vessel wall is not yet clear, however, one may speculate about a relationship to the expression of blood-brain barrier features in cerebral vessels.

The intraluminal suture technique was used to occlude the origin of the MCA of male Sprague-Dawley rats to result in focal brain ischemia. Occlusion of the MCA was maintained either for 2 hours followed by removal of the suture to allow reperfusion (transient MCAO) or left in place until the end of the observation period (permanent MCAO). The survival time of the animals was 8, 24 and 48h. Total RNA was extracted from brain tissue samples, individual arteries and MV extracts for subsequent measurement of gene expression levels by quantitative real time-PCR using $\Delta\Delta$Ct methodology.

Using the rat brain the present study is the first to systematically study KKS gene expression in a fully comparative manner in the parenchyma and selectively in the macro- as well as the microvasculature. Under control conditions a complete KKS (with the exception of the B1 receptor) was found in brain tissue and in cerebravasculature. The components of the KKS (except for kalikrein) appear to be predominantly expressed in vascular system, most notably in MVs. *De novo* expression of B1 receptor mRNA was demonstrated after focal ischemia in the ischemic hemisphere for all three compartment as well as in the contralateral hemisphere for BT and MCA. Similarly, a marked up-regulation of the kininogen mRNA expression was found in the tissue and in the macro- and microvasculature in the contralateral hemisphere. In the ischemic hemisphere up-regulation with the order kininogen > B2 receptor > ACE mRNA was found, again in tissue, arteries and MVs. Overall, the degree of post-ischemic up-regulation was similar in all three tissue compartments studied over the 48h observation period. Gene expression of KAL was not altered (tissue, MVs) or down-regulated (MCA) in the ischemic hemisphere. In conclusion, the present results demonstrate the suitability of our rat cerebral MV extracts for gene expression studies. Identification of all of the components of the kallikrein-kinin system in brain tissue and cerebral macro and micro cerebrovasculature may help in further elucidating the pathophysiological role of KKS stimulation in different neuropathological conditions including focal ischemia.