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Expression profile and functional importance of acid sensing ion channels in acidosis-induced vasorelaxation in an *in vitro* model of cerebral ischemia

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Extracellular acidosis is a remarkable vasomotor signal in the cerebral circulation in physiological (neurovascular coupling) and pathological conditions like cerebral ischemia. Reduction in extracellular pH in the brain leads to vasodilatation. Some cellular elements (e.g. BK and K_{ATP} channels; NO) and mechanisms involved in mediating and modulating acidosis-induced relaxation have been described in the literature. Short-term culture of arteries from various vascular beds has been employed to study vascular pathology in vessel walls and attendant alterations in gene expression profiles, second messenger pathways as well as resultant changes in vasomotor functions. The recent identification of acid sensing ion channels (ASICs) in some vascular beds suggests their possible involvement in modulating vasomotor tone.

Real time PCR was employed to study the pattern of ASIC mRNA expression in the MCA wall of male *Wistar Kyoto* rats in comparison with (i) matching brain tissue samples and (ii) arteries cultured for 24 h and 48 h. Immunofluorescence staining and confocal microscopy was done to assess ASIC protein expression and localization in the MCA wall. The functional implication regarding vasomotor response to acidosis and maintenance of resting tension was assessed using in vitro myography. Changes in $[Ca^{2+}]_i$ induced by ASICs was also studied using fluorescence-based Ca^{2+} imaging.

A robust mRNA expression of ASIC-1, -2 and -4 was found in brain tissue samples and to a lower extent in freshly isolated MCA. In the MCA wall, short term culture induced a down-regulation of ASIC-1 and -2 expression without any remarkable change in ASIC-4 expression. In freshly isolated and 24h-cultured MCA, ASIC-2 immunoreactivity was observed in the adventitial layer with only a sparse distribution in the medial layer. Considering the functional implication of the mRNA and protein expression observed, acidosis induced a pH-related relaxation of MCA ring segments, this response being more pronounced in cultured than freshly isolated MCA. Incubation with the ASIC blocker amiloride moderately enhanced acidosis-induced relaxation, in cultured MCAs somewhat stronger than in freshly isolated vessels. In addition, amiloride resulted in a decrease of resting tension, albeit only in freshly isolated MCA. In Ca^{2+} imaging studies, amiloride quenched fluorescence signals such that this part of the study had to be aborted.

Overall, this study describes ASIC subtype composition in the MCA wall in physiological condition (MCA_F) and pathological condition as modelled by short term culture which was used to simulate vascular pathology induced by cerebral ischemia. The vasomotor activities of these channels at acidic and normal pH were also described. Amiloride-induced enhancement of acidosis-induced relaxation in both groups (freshly isolated and cultured arteries) indicates that ASICs exert the opposite effect that is, moderately limiting acidosis-induced relaxation. This effect was observed to be better pronounced in the pathological model (cultured arteries) down to pH~6.5, a range of extracellular pH described in cerebral ischemia. This activity of ASIC may thus be seen as countering the vasoparalysis that has been previously described following cerebral ischemia.