

ENDOTHELIAL MODIFICATIONS INDUCED BY ARTERIAL PERFUSION WITH PRO-INFLAMMATORY CYTOKINES IN THE IN VITRO ISOLATED GUINEA PIG BRAIN PREPARATION

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The early events of brain inflammation can be ideally studied in a preparation in which both vascular and neuronal compartments are preserved. The functional preservation of the vascular system and the blood brain barrier (BBB) in the isolated guinea-pig brain preparation maintained in vitro by arterial perfusion (de Curtis, Brain Res Protoc 3: 221, 1998) allowed to study endothelial activation during cerebral inflammation determined by arterial perfusion for 1 hour with a solution containing TNF-alpha (10 ng/ml) and IL-1beta (1 ng/microl). BBB permeability, monitored by evaluating cerebral $[K^+]_o$ with K^+ -sensitive electrodes during brief arterial perfusions with a high- K^+ solution before and 4 hr after cytokine treatment revealed $[K^+]_o$ values of 7.1 ± 0.6 mM and 8.9 ± 1.8 mM in the cortex and in the underlying white matter (n=3). In untreated brains, $[K^+]_o$ was 5.1 ± 0.3 and 5.0 ± 0.1 mM after 1 and 5 hours in vitro (n=6). Cortical field potentials were not modified by the exposure to cytokines. Five hours after cytokine perfusion, brains were processed for immunocytochemistry using antibodies against endothelial adhesion molecules, neuronal cytoskeleton and extracellular matrix components. Staining intensity was compared to that of controls (unstimulated brains maintained in vitro for 5 hr or fixed in situ). A marked E-selectin staining in stimulated brain but not in the controls suggests endothelial activation by cytokines. In treated animals, higher tissue permeability to antibodies against components of vascular basal membranes (laminin 1, collagen IV and heparan sulfate proteoglycan) indicates that inflammatory agents enhance BBB permeability. Moreover, a loss of immunoreactivity for MAP2 and SMI-32 in the cytokine-stimulated brains indicates the presence of neuronal damage caused by proteases activation. The study demonstrates that the preparation is viable for studying the early mechanisms that promote neural tissue inflammation in vitro.