NEUTROPHIL-DEPENDENT CEREBRAL OEDEMA IN NORMAL AND DIABETIC RAT: ROLE OF TRANSCELLULAR SYNTHESIS OF CYSTEINIL LEUKOTRIENES

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We studied the synthesis of bioactive cysteinyl leukotrienes (cys-LT) generated by isolated neutrophils-vascular wall interactions in intact and perfused rat brain from control or diabetic animals. Induction of diabetes was obtained by intraperitoneal injection of Streptozotocin (STZ, 65mg/kg) and stated by blood glucose level >350mg/dl. 30 days after STZ injection, rat brain was isolated, weighted and perfused through the basilar artery, with an oxigenated buffer at a flow rate of 1 ml/min. Temperature was gradually elevated and finally maintained at 35°C; changes in vascular tone were recorded by mean of a pressure transducer.
Perfusion with a TXA2 receptor agonist (U46619, 0.1 microM) caused an increase in perfusion pressure from 112.1±4.4 to 221.3±16 mmHg (n=15). Histamine (1microM) induced significant relaxation of constricted vessel (-73.8 ±2.71 %; n=5) providing evidence of maintained endothelial function of the preparation; relaxation degree was much less in diabetic rats (-45.5±11.99 %, n=5).
Isolated brains were then perfused with purified human neutrophils (200.000 PMNL/ml, primed with 1nM GMCSF, 30 min) and challenged with 0.1microM fMLP while perfusing (30 min). At the end of perfusion (2h), brains were weighted (wet weigh is an index of oedema formation) and the perfusate accumulated in the organ chamber (75 ml) was collected for cys-LT detection. Challenge of PMNL-perfused preparations was accompanied by an increment of brain wet weight in both normal and diabetic rats (net increase/initial weight: 65,4±7.5 and 58.4±11.6 % respectively) as well as synthesis of cys-LT (381±64 and 140.2±20.59 pg/ml respectively) significantly higher ($P<0.01$, n=6-9) when compared to brains perfused with unchallenged PMNL (27.7±4 and 31.5±3 % weight net increase; 88.3±13.7 and 76.5±6 cys-LTpg/ml in control and diabetic respectively). When PMNL were preincubated with the 5-lipoxygenase inhibitor MK886 (1microM), both brain weight and cys-leukotrienes production were significantly inhibited (37.4±6.6% and 123.8±48 cys-LTpg/ml respectively) in normoglycemic rats (n=3). Our results suggest that while diabetic cerebral endothelium appears less responsive to vasodilating stimuli than control, in both normal and hyperglicemic animals activation of perfusing neutrophils results in oedema formation subsequent to transcellular biosynthetic events.