The role of endothelial nitric oxide synthase (eNOS) uncoupling on leukocyte-endothelial interactions in rat mesenteric postcapillary venules

Maria A. Kern, Lindon H. Young, Brian Rueter, Edward S. Iames, Qian Chen

Pathology/Microbiology/Immunology & Forensic Medicine, Philadelphia College of Osteopathic Medicine, Philadelphia, PA 19131

Abstract

BACKGROUND: Endothelial derived nitric oxide (NO) is essential in the regulation of blood pressure and attenuates leukocyte-endothelial interactions associated with vascular injury. Endothelial NO synthase (eNOS) is coupled to L-arginine in the presence of tetrahydrobiopetrin (BH_4) to produce NO. However, when BH_4 is oxidized to dihydrobiopetrin (BH_2) under conditions of oxidative stress, the ratio of BH_2 to BH_4 is increased causing the uncoupling of eNOS to use molecular oxygen as a substrate, instead of L-arginine, to produce superoxide.

METHODS: This study examined the role of eNOS uncoupling by superfusing BH_2 (100 or 200 μM) by itself and BH_2 (100 μM) combined with BH_4 (100 μM) in rat mesenteric venules on leukocyte rolling, adherence, and transmigration by using intravital microscopy. The effects of BH_2 were compared to Krebs' buffer, to NOS inhibitor N^G -nitro-L-arginine methyl ester (L-NAME, 50 μM), and to the combination of BH_2/BH_4.

RESULTS: We found that superfusion of BH_2 (100 μM n=6, 200 μM n=6, both $P<0.05$) significantly increased leukocyte rolling, adherence, and transmigration, similar to L-NAME (n=6, $P<0.05$), within a 2 hr period compared to Krebs' buffer control rats (n=6, $P<0.05$). The BH_2 induced response was significantly attenuated by BH_4 (n=6, $P<0.05$).

CONCLUSIONS: The data suggest that eNOS uncoupling may be an important mechanism mediating inflammation induced vascular injury.

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