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.

This study was supported by the Center for Chronic Disorders of Aging and the Department of Pathology/Microbiology/Immunology & Forensic Medicine at PCOM.