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

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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 tetrahydrobiopterin (BH$_4$) to produce NO. However, when BH$_4$ is oxidized to dihydrobiopterin (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 $\text{NO}^\text{G}$-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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