Endothelial-derived nitric oxide (NO) is essential in the regulation of diastolic blood pressure and promotes an antithrombotic surface which attenuates leukocyte-endothelial interactions associated with vascular injury. Endothelial nitric oxide synthase (eNOS) produces NO from L-arginine in the presence of the essential cofactor tetrahydrobiopterin (BH4) during vascular injury the ratio of BH4/BH2 is increased and this promotes eNOS to produce superoxide (SO) instead of NO and is termed eNOS uncoupling (1). Our previous studies have shown administration of BH4 promotes leukocyte-endothelial interactions (Fig. 2) in the mesenteric circulation in vivo. The pro-inflammatory effects of BH4 may be due to the increased BH4/BH2 ratio causing eNOS uncoupling and reduced endothelial-derived NO bioavailability (2). Protein kinase C epsilon (PKC ε) activates eNOS via phosphorylation on eNOS serine 1177 using PKC ε peptidyl activator (PKC ε+). Whereas, PKC ε peptide inhibitor (PKC ε-) reduces eNOS activity (3) (Fig. 3). However, the effects of PKC ε+ peptide or PKC ε- to exacerbate or attenuate BH4-induced leukocyte-endothelial interactions has not yet been determined.

**Methods**

Intravital microscopy was performed on one loop of mesentery of male Sprague-Dawley rats (275-325 g, Ace Animals, Boyertown, PA) and the mesentery was placed on a viewing pedestal to observe mesenteric vessels under light microscopy (Fig. 4). A right carotid artery cannulation was performed to monitor mean arterial blood pressure. During the experiment, test solutions (listed in the following experimental groups) were superfused over the mesentery and the number of rolling (number that passed a set reference point per minute), adhered (number that remained firmly adhered to the endothelial surface for >30 seconds within 100 µm length), and transmigrated (number that had emigrated through the endothelium within 10 µm on either side of the 100 µm length of vessel) leukocytes were recorded (4).

**Results**

We found that BH4 significantly increased leukocyte rolling, adherence, and transmigration when compared to Krebs’ control (P<0.05), and this effect was similar with BH4 + PKC ε (P<0.05). Whereas, BH4 + PKC ε- (P>0.01) significantly attenuated all three types of BH4-induced inflammation. The data suggest that eNOS uncoupling may be an important mechanism mediating inflammation-induced vascular injury, and that inhibiting uncoupled eNOS activity with PKC ε- may attenuate oxidative stress and restore vascular endothelial function. However, we found that BH4 + PKC ε- significantly reduced BH4-induced inflammation. This data suggest that PKC ε- can exert anti-inflammatory effects when the BH4/BH2 ratio is increased to promote eNOS coupling. This study outlines the importance that the BH4/BH2 ratio determines whether eNOS is in the uncoupled or coupled state. Moreover, inhibiting uncoupled eNOS activity with PKC ε- or increasing coupled eNOS activity with PKC ε+ can both exert anti-inflammatory effects. This idea provides indirect evidence as a potential strategy for attenuating endothelial dysfunction-induced inflammatory responses in various vascular diseases.

**References**