Recruitment and activation of PMNs and subsequent SO release contribute to inflammation mediated tissue injury such as that following ischemia/reperfusion (IR). Therefore, identifying mechanisms that regulate PMN SO release may lead to therapies that mitigate PMN tissue injury. Phorbol ester (PMN NAPDH oxidase) has been shown to be essential to generate SO release. There are 5 isoforms of PKC (a, θ, δ, β1 (β)), δ (δ), and ε (possibly δ) and ε (possibly δ) expressed by PMNs which regulate NADPH oxidase activity by phosphorylation of p47phox (a component of the NADPH oxidase enzyme) (Figure 4). It is known that PKC δ, a broad-spectrum PKC activator which functions as a diacylglycerol (DAG) mimetic, augments SO release in PMNs via NADPH oxidase activation principally through δ (δ), ε and to a lesser extent a (α) 1 activation [1]. Additionally, PKC δ activation is known to inhibit PMN elastase release which occurs during inflammation of activated PMNs [2]. To date, the roles of PKC δ in mediating inflammatory responses and leukocyte SO release in vivo remains unclear. Characterizing the role of various PKC isoforms would help elucidate the homeostatic balance of PKC regulating PMN NADPH oxidase and inflammation mediated injury.

We have previously shown that a cell-permeable PKC δ peptide activator (PKC δ+) facilitates the translocation of translocation of PKC δ to cell substrates via interaction with its specific receptor for activated C kinase (RACK) and PKC δ+ mediated cardioprotective effects in myocardial IR injury [4,5]. We propose that inhibiting PKC δ would augment PMN SO release. Therefore, using a PKC δ peptide inhibitor (PKC δ−) that inhibits the binding of PKC δ to its RACK, or rottlerin, a phenolic compound that is a selective inhibitor of PKC δ (IC50 3-6 μM) at its ATP binding site can be used to assess the role of PKC δ in the regulation of SO release in PMNs [5,6,7].

**Methods**

**Isolation of PMNs**

Male Sprague-Dawley rats (350-400 g, Charles River), used as PMN donors, were anesthetized with 2.5% isoflurane and given a 16 ml intraperitoneal injection of 0.5% glycopyrrolate (Sigma Chemical) dissolved in PBS. Rats were reanesthetized with isoflurane 16:8 h later, and the PMNs were harvested by peritoneal lavage in 30 ml of 0.9% NaCl, as previously described [5,8]. Peritoneal lavage fluid was centrifuged at 200 x g for 10 min at 4°C. Then, the PMNs were washed in 20 ml PBS and centrifuged at 200 x g for 10 min at 4°C. PMNs were resuspended in 2.5 ml PBS and density was calculated. The PMNs preparation were >90% pure and >95% viable according to microscopic analysis and 0.3 trypan blue exclusion, respectively.

**Measurement of SO release from Rat PMNs**

The SO release from PMNs was measured spectrophotometrically (model 260 Gilford, Nova Biotech; El Cajon, CA) by the reduction of ferricytochrome c [5,8]. The PMNs (5 x 10⁶) were resuspended in 450 μl of PBS and incubated with ferricytochrome c (100 μM, Sigma Chemical) in a total volume of 900 μl PBS in the presence or absence of PKC δ activators (Myr-MRAAEDPM, MW=1130 g/mol, 2.5-20 μM, n=10-13), PKC δ inhibitor (Myr-SFNSEYGLSM, MW=1326 g/mol, 1-20 μM, n=7-10) or rottlerin (MW=516 g/mol, 0.025-20 μM, n=6-12) for 15 min at 37°C in spectrophotometric cells. The PMNs were stimulated with 15 or 30 nM PKC δ (MW=616 g/mol, n=13-26) in a final reaction volume of 1.0 ml. Absorbance at 550 nm was measured every 30 sec for up to 420 sec for PMN and the change in absorbance measured (SO release) from PMNs was determined relative to time 0. Cell viability among all studies was determined by >0.3% trypan blue exclusion at the end of SO release assay. Viable cells remained unstained and non-viable cells stained blue.

**Statistical Analysis**

All data in the text and figures are presented as means ± S.E.M. The data were analyzed by analysis of variance using post hoc analysis with Fishers PLSD test. Probability values of <0.05 are considered to be statistically significant.