MODULATION OF NITRIC OXIDE RELEASE IN CULTURED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS BY MYRISTOYLATED-PKC EPSILON ACTIVATOR/INHIBITOR PEPTIDES

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INTRODUCTION

Protein kinase C epsilon (PKCe) is known to increase endothelial nitric oxide synthase (eNOS) activity by binding to a specific receptor for activated C kinase (RACK-1), facilitating its translocation from the cytosol to the cell membrane for phosphorylation of eNOS at serine-1177 to augment activity [1,2]. Previous in vitro and in vivo animal studies have demonstrated that PKCe activation stimulates eNOS activity, increasing nitric oxide (NO) release, whereas a peptide inhibitor that disrupts PKCe interaction with RACK-1 results in decreased NO release [3,4,5,6,7].

However, the modulation of PKC-activated NO activity (Figure 1) is not well known in human endothelial cells. Moreover, elucidating the role of PKCe in regulating eNOS activity would be essential in the clinical setting of ischemic heart disease. Re-establishing blood flow after thrombus removal results in endothelial dysfunction that is characterized by limited NO bioavailability and excess generation of reactive oxygen species (ROS) by eNOS during reperfusion (Figure 2).

Therefore, we aim to demonstrate enhancement and attenuation of NO release in cultured HUVECs using cell-permeable, myristic-acid conjugated PKCe activator (MYR-90), and inhibitor (MYR-70). No MYR-90/VLFX (MYR plus VLFX), in the absence and presence of acetylcholine (Ach). Ach is a well-established positive control to activate eNOS via calcium calmodulin (CAM) and promote NO release from vascular endothelial cells [7,8]. Determining NO release using selective cell permeable PKCe activator (MYR-90) and inhibitor peptides (MYR-70) in cultured HUVECs under normoxic conditions would provide a foundation to test these peptides under hypoxic-reoxygenation conditions when eNOS would be uncoupled and produce ROS instead of NO release.

HYPOTHESIS

We hypothesize that MYR-90/PKCe+ would increase NO release, whereas MYR-70/PKCe− would decrease NO release from cultured HUVECs in the absence and presence of Ach.

METHODS

Measurement of NO release from cultured HUVECs: Single-donor HUVECs (Lonza, Walkersville, MD) at passages 3-4 were grown to confluence in 6-well (100 cells/well) plates. NO release was measured in real time using a calibrated NO electrode following the administration of 10 μM MYR-90/PKCe+ or 10 μM MYR-70/PKCe− treatments in the absence or presence of 10 μM Ach stimulation of NO release. All treatments were prepared in endothelial growth media (EGM). Basal NO release was determined by measuring the difference between wells with and without Ach.

Statistical Analysis: All data in the figures are presented as means ± S.E.M. ANOVA analysis using Student-Neuman-Keuls was used to assess statistical difference in NO release between basal levels vs MYR-90/PKCe+ treated or MYR-70/PKCe− treated cultured HUVECs in the absence and presence of Ach stimulation (Ach or EGM). p<0.05 were considered statistically significant.

RESULTS

Basal NO release (83±12 pmol) was determined by measuring the difference between wells with and without HUVECs (n=8, p<0.05). In the absence of Ach stimulation, MYR-90/PKCe+ treatments significantly enhanced NO release to 136±13 pmol (n=6, p<0.05) and MYR-70/PKCe− attenuated total NO release to 16±27 pmol (n=7, p<0.05) compared to basal levels.

As a positive control, 10 μM Ach significantly enhanced NO release to 153±11 pmol above basal levels (p<0.05, n=20). In the presence of Ach stimulation, MYR-90/PKCe+ still significantly increased NO release above basal levels to 129±17 pmol (p<0.05, n=8) and MYR-70/PKCe− decreased NO release to 34±12 pmol (p<0.05, n=7) compared to basal levels, as shown in Figure 5.

CONCLUSION

These results suggest that in cultured HUVECs, MYR-90/PKCe+ presumably increases NO release via activation of eNOS, whereas MYR-70/PKCe− attenuates eNOS activity by inhibiting its phosphorylation; thus these peptides demonstrate comparable effects across species in regulating NO release. The understanding of the role of eNOS modulation is important for developing therapeutic interventions in the context of human organ ischemia reperfusion injury, in which uncoupled eNOS produces ROS instead of NO.

REFERENCES