PDIA1 overexpression activates acutely Nox1 NADPH oxidase in VSMC

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INTRODUCTION

Mechanisms regulating NADPH oxidase remain open and include the redox chaperone protein disulfide isomerase (PDI). We described previously that PDI transient overexpression in VSMC enhances basal Nox1 mRNA expression and activity, without any further response for AngII stimulus in ROS production.

OBJECTIVES

Here we investigated whether Nox1 activation mediated by PDI overexpression may involve AngII receptor (AT1R).

MATERIALS AND METHODS

We developed rabbit aortic VSMC with doxycycline inducible system for rat PDI (VSMC-PDI), which showed 3.5-fold increased total PDI expression after 48h of doxycycline addition.

DISCUSSION AND RESULTS

VSMC-PDI showed increased superoxide production by NADPH-triggered enriched membrane fraction (2.5±0.3 RLU/mg protein vs. without doxycyclin, lucigenin reduction), which was inhibited by Nox1/4 inhibitor (KGT136901 μM). VSMC-PDI showed higher migration trajectories vs. VSMC with no stimulus (1.8-fold, end distance from origin by single cell migration assay for 16h). Although inhibitors of AT1R (losartan and candesartan, 10-100nM for 16h) decreased intracellular superoxide (2-hydroxyethidium quantification by HPLC) or extracellular H2O2 (AmplexRed assay) productions mediated by PDI transient overexpression, there is no direct PDI interaction with AT1R, based on several confocal microscopy analyzes in both VSMC and HEK293 cells transfected with AT1R-GFP. PDI silencing did not affect AT1R mecanotransduction mediated by laminar shear in CHO cells, reinforcing the absence of direct interaction of PDI/AT1R. Since AT1R activates β-arrestin1/2 signaling, we silenced β-arrestin1/2 with further PDI induction in VSMC-PDI. Preliminary results suggest no changes in Nox1 NADPH activity. Finally, chronic VSMC-PDI (obtained after antibiotic selection of VSMC transiently transfected, 4 fold increased PDI expression) showed no more increased intracellular superoxide or Nox1 NADPH oxidase activation compared to basal VSMC.

CONCLUSIONS

The mechanisms underlying Nox1 acute activation by PDI overexpression in VSMC does not involve direct interaction with AT1R, and might not involve β-arrestin1/2. Chronic PDI overexpression may induce VSMC redox adaptations, since Nox1 NADPH oxidase activity is decayed to basal levels.

Keywords: protein disulfide isomerase, NADPH oxidase, vascular

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