学 位 論 文 の 要 旨

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学 位 論 文 名　Taurine Suppresses Platelet-Derived Growth Factor (PDGF) BB-induced PDGF-β Receptor Phosphorylation by Protein Tyrosine Phosphatase-mediated Dephosphorylation in Vascular Smooth Muscle Cells

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論 文 内 容 の 要 旨

INTRODUCTION

Platelet-derived growth factor (PDGF)-BB is one of the potent stimulators of vascular smooth muscle cells (VSMCs), and involved in pathogenic processes including the development of atherosclerosis. Taurine has prophylactic effects on atherosclerosis, although the molecular mechanism remains obscure. Previously, we found that taurine suppresses PDGF-BB-induced cell proliferation, DNA synthesis, immediate-early gene expressions and extracellular signal-regulated kinase (ERK) 1/2 phosphorylation in VSMCs. The present study was aimed to elucidate the precise molecular mechanism of taurine in PDGF-BB signaling pathway. We here show that taurine significantly suppresses PDGF-BB-induced phosphorylation of PDGF-β receptor (PDGFR-β) and activation of its downstream signaling molecules such as Ras, MAPK/ERK kinase (MEK) 1/2 and Akt. Furthermore, we show for the first time that taurine does not directly affect PDGFR-β autophosphorylation, but promotes protein tyrosine phosphatase (PTPase)-mediated PDGFR-β dephosphorylation by preventing PDGF-BB-induced decrease in PTPase activity.

MATERIALS AND METHODS

Rat VSMCs were isolated from thoracic aortae of Wistar-Kyoto rats and used for our experiments. Phosphorylations of ERK1/2, MEK1/2 and Akt were examined by Western blot analysis using specific antibodies. Phosphorylation of PDGFR-β was examined by immunoprecipitation and Western blot analysis using specific antibodies. ERK1/2 activity was determined by *in vitro* kinase assay using Elk-1 fusion protein as substrate, and phosphorylation of Elk-1 fusion protein was measured by Western blot analysis using anti-phospho-Elk-1 antibody. For Ras activation assay, activated Ras was collected by affinity precipitation with Ras-binding domain (RBD) of Raf-1-immobilized agarose beads, and the amount was measured by Western blot analysis using anti-Ras antibody. For PDGFR-β autophosphorylation assay *in vitro*, purified PDGFR-β pretreated with taurine or PDGFR-β kinase inhibitor was incubated with kinase reaction buffer containing PDGF-BB and ATP, and then examined by Western blot analysis using anti-phospho-tyrosine antibody. For cellular PTPase assay, conversion of *p*-nitrophenyl phosphate to *p*-nitrophenol with cell lysate was measured colorimetically. PDGFR-β-specific PTPase activity in cell lysate was examined by autoradiography using ^{32}P-labeled PDGFR-β as substrate.

RESULTS AND DISCUSSION

In this study, taurine dose-dependently suppressed PDGF-BB-induced PDGFR-β phosphorylation, Ras activation, MEK1/2 phosphorylation, ERK1/2 activation and Akt phosphorylation *in vivo*. These results indicate that taurine decreases PDGFR-β phosphorylation, and in turn, attenuates the subsequent activation of downstream signaling molecules. Taurine did not affect PDGFR-β independent signaling such as phorbol 12-myristate 13-acetate-induced ERK1/2 phosphorylation *in vivo*. Thus, we investigated the suppressive mechanism of taurine at PDGFR-β level. In PDGFR-β autophosphorylation assay *in vitro*, taurine did not affect PDGF-BB-induced PDGFR-β autophosphorylation,

although PDGFR-β tyrosine kinase inhibitors AG1296 and genistein, which act on substrate-binding and ATP-binding sites of PDGFR-β, respectively, inhibited the autophosphorylation. Furthermore, there was no difference in PDGFR-β autophosphorylation *in vitro* between taurine-treated cells and control cells, indicating that taurine does not directly affect PDGFR-β kinase activity. It is well established that phosphorylation states of proteins including PDGFR-β are reciprocally regulated by protein tyrosine kinase-mediated phosphorylation and PTPase-mediated dephosphorylation. Thus, we next examined the effect of taurine on PDGFR-β dephosphorylation *in vivo* using AG1296. In the presence of AG1296, taurine promoted decrease in tyrosine phosphorylation of PDGFR-β, indicating that taurine increases PDGFR-β dephosphorylation. It has been reported that PDGFR-β dephosphorylation is controlled by PDGFR-β-specific PTPase, and that PDGF-BB binding to PDGFR-β induces temporal suppression of the PTPase activity. Thus, the effects of taurine on cellular and PDGFR-β-specific PTPase activities were examined. Although PDGF-BB markedly decreased cellular PTPase activity, taurine significantly prevented PDGF-BB-induced decrease in its activity *in vivo*. Furthermore, taurine also significantly protected PDGFR-β-specific PTPase activity against PDGF-BB-induced suppression *in vivo*. Taken together, these results clearly indicate that taurine suppresses PDGF-BB-induced PDGFR-β phosphorylation by promotion of PDGFR-β-specific PTPase-mediated dephosphorylation.

CONCLUSION

We demonstrated that taurine suppresses PDGF-BB signaling pathway at PDGFR-β level by PDGFR-β-specific PTPase-mediated dephosphorylation. This mechanism provides one explanation for the prophylactic effect of taurine on atherosclerosis, and the framework for development of new therapeutic strategies.