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PLATELET DERIVED GROWTH FACTOR-MEDIATED UPREGULATION OF CONNEXIN43 IN BLADDER SMOOTH MUSCLE CELLS: A POSSIBLE ROLE IN URINARY BLADDER OVERACTIVITY

Hypothesis / aims of study

Overactive bladder (OAB) is a highly common clinical condition characterized by urinary urgency and frequency. One of the major causes of OAB is the bladder outlet obstruction (BOO) subsequent to benign prostatic hyperplasia in elderly people. Accumulating evidence indicate that elevated intercellular coupling via gap junction contributes to overactivity of myogenic bladder. However, the pathogenic factors and mechanisms involved in regulation of gap junctions are still obscure. Given that platelet derived growth factor (PDGF) is increased in stretched bladder smooth muscle cells (BSMCs) both in vivo and in vitro, we asked PDGF could be involved in the regulation of gap junction.

Study design, materials and methods

Rat BOO model was made by ligating the urethra.[1] The expression of PDGF and Cx43 were examined by histocytochemical and northern blot analysis. BSMCs were isolated from 7-wk-old female Sprague-Dawley rats and analyzed for Cx43 expression and function after stimulation with PDGF by Western blot, immunofluorescent staining and scrape-loading dye-transfer assay. The implicated signal mechanisms were characterized by using specific kinase inhibitors. Intraperitoneal injection of PDGF was employed to confirm the effect in vivo.

Results

1)Incubation of BSMCs with PDGF resulted in a time- and concentration-dependent induction of Cx43 expression, (Fig.1A) which was associated with an obviously increased membrane localization of Cx43 and elevated number of dye-coupled cells.(Fig.1B) 2) The Cx43-elevating effects of PDGF could be blocked by PDGF receptor kinase inhibitor AG1296, PI3K inhibitor LY294002 and ERK inhibitor PD98059.(Fig.2) 3) Cx43 levels in BSMCs cells were also upregulated by other growth factors, including bFGF and EGF, in a mechanism independent of PDGF signaling.(Fig.3) Combined stimulation with PDGF could synergistically augment Cx43 expression. 4) Intraperitoneal injection of PDGF also induced Cx43 protein levels in bladder.(Fig.4) 5) In a rat model of BOO, in which an increased PDGF has been previously documented, an obvious elevated level of Cx43 mRNA was detected.

Interpretation of results

Our study demonstrated that PDGF induced Cx43 expression and function in BSMCs both in vivo and in vitro. This effect of PDGF was mediated by the PDGF receptor-PI3-MER kinase signaling pathway. In addition, PDGF markedly potentiated the effects of other growth factors on Cx43 expression. These results, together with the evidence showing the coexistence of elevated PDGF and Cx43 in BOO bladder, indicate that PDGF may underlie the increased Cx43 expression and function in bladder.

Concluding message

We identified PDGF as a potent inducer of Cx43 expression and function in bladder smooth muscle cells. Targeting PDGF and PDGF-related signals could be a novel therapeutic approach for treatment of OAB.

Fig.1A

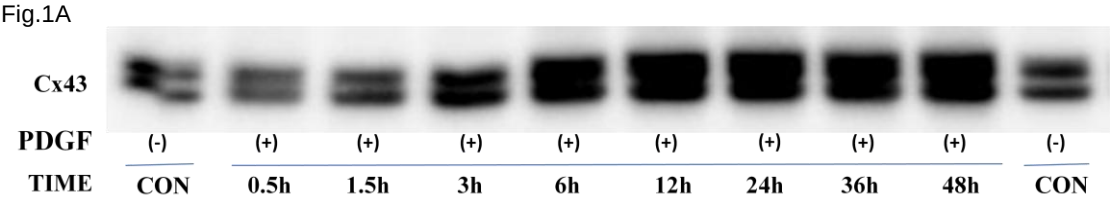


Fig.1B

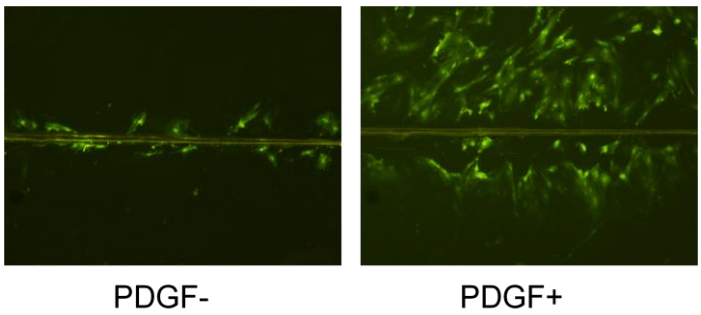


Fig.2

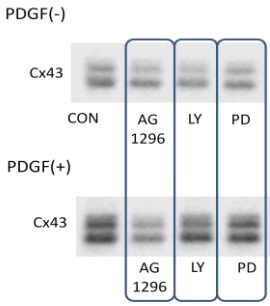


Fig.3

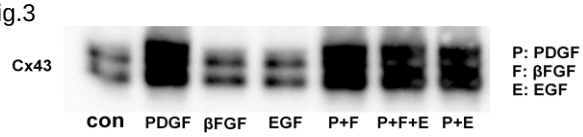


Fig.4

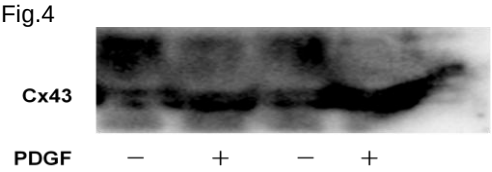
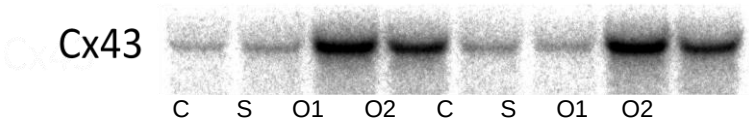


Fig.5



C:control, S;sham ope, O1,2:obstructed bladder

References

1. Fry CH et al Urology 63:3-10, 2004
2. Sawada N et al Lab Invest. 88(5):555-63, 2008

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**All animal procedures were reviewed and approved by the university of Yamanashi Animal Care and Use committee.**