491

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UPREGULATION OF CONNEXION 43 EXPRESSION AND FUNCTION IN URINARY BLADDER BY INFLAMMATORY CYTOKINES

Hypothesis / aims of study

Inflammation is a common pathological situation in bladder disorders and is thought to be one of the etiologies of overactive bladder (OAB) and interstitial cystitis. At present, little information is available regarding factors and mechanisms involved in inflammation-related bladder disorder. Given that the increased coupling of bladder smooth muscle cells (BSMCs) via gap junctions has been recognized as an important factor contributing to bladder overactivity and that the altered gap junction protein connexin43 (Cx43) has been shown to be implicated in multiple pathological situations, we asked whether gap junctions in bladder could be affected by inflammatory cytokines.

Study design, materials and methods

BSMCs were isolated from 7-wk-old female Sprague-Dawley rats and analyzed for Cx43 expression and function after stimulation with inflammatory cytokines (interleukin 1 and tumor necrosis factor alpha) by Western blot, immunofluorescent staining and scrape-loading dye-transfer assay. The implicated signal mechanisms were identified by using various kinase inhibitors and specific siRNA. In vivo mouse inflammatory model was induced by intraperitoneal injection of LPS. The expression of Cx43 in bladder was examined by histocytochemical and western blot analysis.

Results

1) Incubation of BSMCs with inflammatory cytokines IL-1 and TNF resulted in a time-dependent elevation in Cx43 protein levels. This was associated with obviously increased membrane localization of Cx43 protein and elevated number of dye-coupled cells. (Fig.1) Increment of Cx43 by the inflammatory cytokines was preceded by an activation of PKA, as revealed by VASP phosphorylation. Blockade of PKA signaling pathway with PKA inhibitor H89 or CREB siRNA could completely abolish the effect. (Fig.2) 3) The Cx43-elevating effects could also be abolished by NF b inhibitor SC514 and, partially, by iNOS inhibitor L-NAME. In support of a role of nitric oxide (NO), stimulation of cells with NO donor SNAP or SNP elevated Cx43, and lastly, intraperitoneal injection of LPS also elevated Cx43 in bladder.(Fig.3)

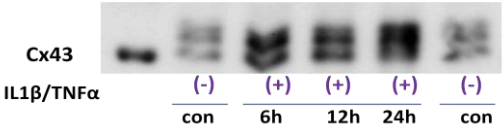
Interpretation of results

Our study demonstrated that IL1 /TNF induced Cx43 expression and function in BSMCs both in vivo and in vitro. This effect was mediated by cAMP signaling pathway. The complete abolishment of the Cx43-elevating effect by NF b indicated an involvement of NF b-regulated gene products. One of the possible candidates could be NO. This was shown by the observations that: 1) iNOS was induced by inflammatory cytokines; 2) iNOS inhibitor partially blocked the effects of the cytokines; and 3) NO donors also induced Cx43 expression.

Concluding message

In this study, we demonstrated, for the first time, that inflammatory cytokines induced Cx43 expression and function in bladder smooth muscle cells through mechanisms involving NO-mediated activation of cAMP signaling pathway. Our finding may open a new window towards our understanding of the molecular mechanisms implicated in inflammatory situations such as interstitial cystitis in bladder.

Fig.1



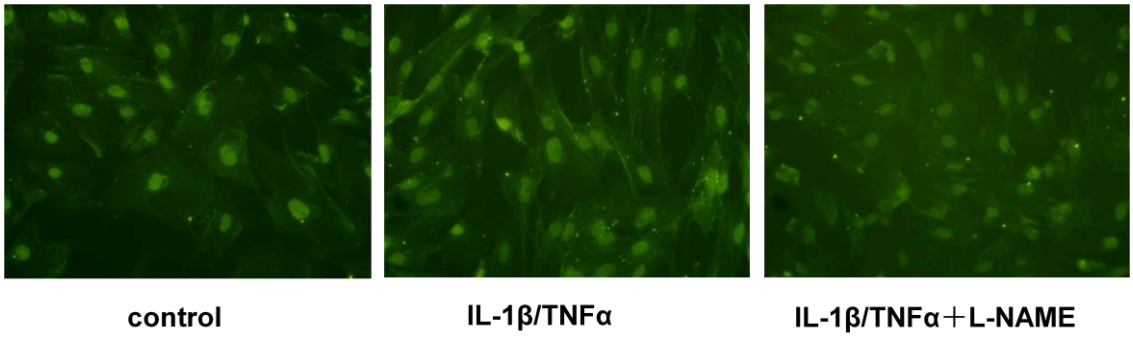


Fig.2

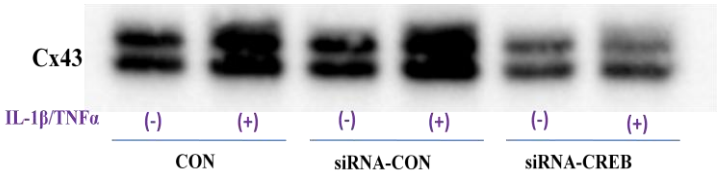
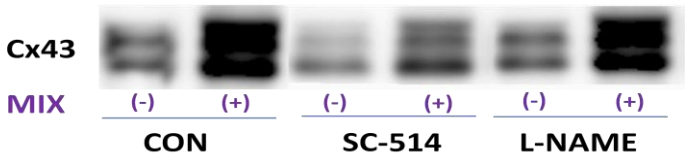
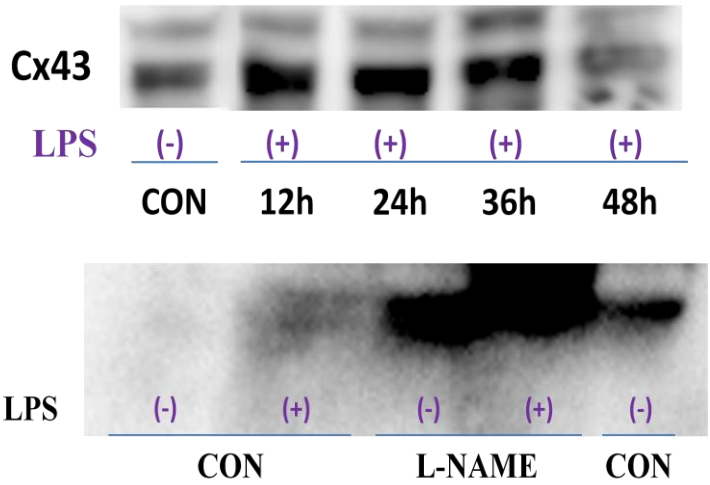


Fig.3



MIX: IL1□ /TNF□



Specify source of funding or grant	None
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. All efforts were made to minimize animal suffering and to reduce the number of animals used.