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NEURONAL DISTRIBUTION OF P2X3 RECEPTOR AND ITS INHIBITORY EFFECT ON DETRUSOR CONTRACTILITY

Hypothesis / aims of study
The significance of the purinergic receptor subtype P2X3 (P2X3R) in the urinary bladder has been historically confined to its role in afferent pathways, due to the abundant distribution of P2X3R within the urothelium and sensory nerves. In particular, P2X3R is being tested as a potential target for the therapeutic management of bladder pain. Aside from its role in modulating bladder sensation, little is known regarding its local effect on detrusor function. Few studies have investigated the association of P2X3R with parasympathetic fibers, and whether this receptor could be implicated in the modulation of detrusor efferent function. The purpose of this study was to identify the neuronal distribution pattern of P2X3R within the bladder and to investigate its potential role in integrating efferent-afferent signalling to modulate bladder contractility in stress-sensitive rats.

Study design, materials and methods
P2X3R distribution was investigated by double labeled immunofluorescence microscopy in bladder tissue from Wistar Kyoto rat (WKY). *Functional studies*: Longitudinal bladder smooth muscle tissue with denuded mucosa were placed in organ bath containing Kreb's solution at 37ºC and functionally evaluated in *ex vivo* isometric tension studies. After equilibration, nerve-mediated detrusor contractions were induced by electrical field stimulation (EFS, 20V, 2-64Hz). Frequency-response curves were generated under baseline conditions as well as after administration of two different P2X3R antagonists, NF-110 and RO51. During EFS, organ bath aliquots were collected and the amount of ATP released upon stimulation was determined by luciferin-luciferase assay. In addition, the effect of P2X3R antagonism on EFS was investigated in the presence of (i) atropine and guanethidine, (ii) capsaicin (used to desensitize C-fibers), or (iii) calcitonin gene related peptide (CGRP) receptor inhibitor.

Results
P2X3R was abundantly expressed on neuronal fibers and nerve trunks within the detrusor. P2X3R immunoreactivity partially co-localized with VAChT, a neuronal marker for parasympathetic fibers, as well as with TRPV1 and CGRP (markers for capsaicin-sensitive and peptidergic sensory fibers). In functional studies, the amplitude of EFS-induced contractions increased significantly at all frequencies tested after the administration of either P2X3R antagonist NF110 or RO51, compared to detrusor responses under baseline conditions. Contractions induced by EFS were not affected by NF110 in Sprague-Dawley rats. In WKY, the presence of NF110 significantly increased ATP release measured during EFS compared to baseline levels. The contractile response to EFS in the presence of atropine and guanethidine was not affected by P2X3R antagonist. In addition, capsaicin-desensitization and CGRP receptor inhibition prevented the NF110 mediated increase in EFS.

Interpretation of results
The distribution of P2X3R on efferent nerves and the P2X3R antagonist induced increase in neuronal ATP release together with the atropine-sensitive effect of P2X3R antagonists suggest a P2X3R mediated pre-junctional inhibition of release of excitatory neurotransmitters from parasympathetic fibers. Moreover, the prevention of the effect of P2X3R antagonist on EFS-induced contractions after CGRP receptor inhibition and C-fiber desensitization, suggest that P2X3R activation modulates peptide release from afferent fibers. Since the P2X3R contribution to EFS is more prominent in WKY relative to SD, these finding provide further rationale for thuse of the WKY strain as a model of visceral pain in which parasympathetic activity may enhance local activation of sensory fibers.

Concluding message
Our data indicate that P2X3R is localized on both afferent and efferent nerve fibers of the bladder and may participate in modulating both sensory and motor detrusor function. Thus, the local effector role of sensory fibers induced by stretch or urothelial signaling can be expanded to include a function elicited by efferent activation.

Disclosures
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