TRPV1 in GU Disorders

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Abstract: Since the first work about neuronal desensitization mediated by vanilloids, performed back in the 1970's, major advances have been made in the elucidation of TRPV1 role in the genitourinary (GU) tract. The receptor distribution in the GU tract was unveiled. Both *in vivo* and *in vitro* studies brought new insights on TRPV1 physiology. The role of TRPV1 in bladder function in both normal and pathological states was clarified. All these data allowed the development of effective TRPV1 antagonists which not only confirmed the role of TRPV1 in micturition dysfunction but also suggested new approaches for the treatment of GU pathologies.

Keywords: TRP channel, TRPV1, capsaicin, resiniferatoxin, genitourinary tract, GRC 6211, incontinence.

1. TRPV1 EXPRESSION IN THE GU TRACT

The presence of a vanilloid receptor in the GU tract was first forwarded to explain the increased bladder frequency and pain produced by capsaicin application to the urinary bladder [1-3]. Radioactive resiniferatoxin (RTX) binding to the pig urinary bladder showed for the first time the presence of the presumed receptor [3]. However, it was necessary to wait for the cloning of TRPV1 gene to get a more accurate picture of the receptor in the GU tract [4]. By doing immunohistochemistry reactions against the receptor protein, TRPV1 has been found to be expressed in varicose nerve plexus, throughout the GU mucosa and muscular layer [5-9]. In the rat urinary bladder, the great majority of the TRPV1 imunorreactive (TRPV1-IR) fibres are peptidergic [5, 10], contain protease activated receptors (PARs) [11], and have a complete co-localization with TRPA1 [10].

Besides being expressed in the nervous system, TRPV1 has also been found in urothelial cells of rodents [7, 10] and humans [8, 12]. Urothelial TRPV1 differs, however, from the neuronal TRPV1 receptor in some aspects. For instance, it is known that neuronal TRPV1 transcription is NGF-dependent, increasing in the presence of this trophic factor [13]. However, urothelial TRPV1 mRNA levels are not altered by an increase in NGF levels [12]. Human urothelial TRPV1 activation by capsaicin desensitizes [12], in contrast with the rodent receptor [7].

2. TRPV1 CONTRIBUTION TO GU FUNCTIONS IN NORMAL AND PATHOLOGICAL CONDITIONS

The role of TRPV1 in normal urinary bladder activity is still unclear. While Charrua and co-workers did not find any differences between bladder function of anaesthetised wild type and TRPV1 KO mice [14], Birder and co-workers observed that urethane-anaesthetised TRPV1 KO presented fewer voiding contractions, with a great percentage presenting overflow incontinence [15]. Furthermore, these same authors described that awake TRPV1 KO mice presented non-voiding contractions which were absent in their WT littermates [15, 16]. Similarly confounding observations were obtained after the administration of TRPV1 antagonists to intact bladder: while low doses of the TRPV1 antagonist GRC-6211 had no effect on rat bladder contractility, doses above l mg/kg of weight transiently blocked bladder reflex activity [17]. As the effect of high doses of this TRPV1 antagonist was absent in TRPV1 KO mice, it is tempting to hypothesize that TRPV1 has some mechanoreceptive properties [17]. Alternatively, TRPV1 interaction with TRPV4 may be required to express TRPV4 mechanosensitive properties [18, 19].

Neuronal TRPV1 expression is enhanced in patients that suffer from a variety of GU tract disorders, such as interstitial cystitis (IC, also known as bladder painful syndrome, BPS), neurogenic (NDO) and idiopathic detrusor overactivity (IDO) [20-23]. In bladders of IC patients there is an increase in TRPV1-expressing nerve fibres coursing in the suburothelium, when compared with the controls [20]. This increase has a positive correlation with the visual analogue pain score [20]. Changes in TRPV1 expression in the urothelium of IC patients are less clear. However, it is tempting to suggest that urothelial TRPV1 might be involved in the excess of ATP release from the urothelium of IC patients [21]. TRPV1 activation induces ATP release from human urothelial cells [12]. ATP can then activate P2X3-expressing nociceptive bladder afferents coursing underneath the urothelium [24].

In patients with involuntary bladder contractions during bladder filling of neurogenic and non-neurogenic origin,

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nerve fibres and urothelial cells in the urinary bladder were shown to overexpress TRPV1 [22, 23]. In addition to promoting involuntary bladder contractions, TRPV1 has been suggested to initiate sensory input that leads to urgency to urinate, a disabling lower urinary tract symptom that precedes urinary incontinence [25, 26]. Furthermore, TRPV1 expression in the trigonal mucosa was inversely correlated with bladder volume at which patients have their first sensation to void [27, 28].

3. TRPV1 MODULATION DURING GU TRACT DISORDERS

TRPV1 can be directly activated by lipidic pro-inflammatory molecules [29-34]. In fact, the TRPV1-mediated effect of anandamide during cystitis it is thought to contribute to bladder overactivity and pain symptoms. Nevertheless, endovanilloids are weak TRPV1 activators in the urinary bladder when compared to capsaicin and resiniferatoxin [34]. This might be overcome by TRPV1 sensitization [35]. It is known that in order for vanilloids to activate TRPV1 the receptor needs to be phosphorylated by Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) [36]. *In vitro* phosphorylation by protein kinase A (PKA) or protein kinase C (PKC) also leads to TRPV1 sensitization [36]. Receptors, such as protease-activated receptor 2 and 5-hydroxytryptamine 7 receptor, are known to sensitize TRPV1 receptor *via* PKA-mediated phosphorylation [37, 38]. Furthermore, group II metabotropic glutamate receptors [39] or mu opioid receptors [40, 41] are known to inhibit TRPV1 activation by modulation of the cAMP/PKA pathway. Receptors, such as bradykinin receptor [42-47], purinergic receptors [48-51], tyrosine kinase receptor A (trk A) [52-54], among others, are known to sensitize TRPV1 receptor through a PKC-dependent mechanism. Additionally, phospholipase C (PLC) and phospholipase A2 (PLA2) activation mediated by bradykinin receptor, may lead to the production of arachidonic acid metabolites which may activate TRPV1 receptor [55-57].

Phosphatidylinositol-4, 5-bisphosphate (PtdIns (4, 5) P2) has a dual effect on TRPV1. It sensitizes the receptor in the presence of high concentration of agonist [58, 59] and inhibits the receptor at low concentration of that agonist [60, 61]. TRPV1 receptor can also be sensitized when ATP binds to the receptor [62], increasing the response to further stimulus [62].

TRPV1 levels in neuronal cells are known to be increased during inflammation in general [63] and in cystitis in particular [64]. The increase of TRPV1 protein levels in dorsal root ganglia cells that accompanied inflammation does not seem to be accompanied by an increase of TRPV1 mRNA suggesting a post-translational regulation [63, 65]. Curiously, the receptor translation does increase in urothelial cells upon inflammation [12] suggesting different regulating mechanisms in neuronal and non-neuronal cells.

TRPV1 expression in membranes may be regulated by a SNARE-dependent trafficking of the protein docked in synaptic vesicles in the cytoplasm [66]. Indeed, an increase in TRPV1 trafficking from the cytoplasm to neuronal membrane, through a PKC-mediated mechanism was shown to occur during inflammation [66]. This might suggest that part of the TRPV1 receptor is kept inactive inside synaptic vesicles in neuronal terminals [66]. TRPV1 trafficking to the membrane is also thought to be PKA dependent, since point mutation of putative sites of PKA phosphorylation almost abolished TRPV1 cytoplasmic membrane expression [67]. The phosphoinositide 3-kinase (PI3K)-dependent pathway may be another mechanism that promotes TRPV1 trafficking to the membrane [68, 69].

TRPV1 can also be modulated by its own splice variants. The human TRPV1 splice variant, TRPV1b, produces a negative-dominant effect on TRPV1 activity [70, 71]. *In vivo* experiments conducted in rodents showed that there is a decrease in the neuronal expression of TRPV1b during cystitis [65]. Since TRPV1 mRNA levels were unchanged [65], it is tempting to hypothesize that increased TRPV1-responsiveness might also be derived from the reduction of the expression of inactive splice variants.

4. TRPV1 DESENSITIZATION FOR MANAGEMENT OF GU DISORDERS

As mentioned before, patients with NDO or IDO have an excess of TRPV1 in the urinary bladder [22, 23]. Involuntary detrusor contractions associated with spinal cord injury are triggered by sensory input conveyed by TRPV1 expressing C-fibres that project to the sacral level of the spinal cord [72]. Therefore, TRPV1 receptor desensitization has been investigated to treat patients with NDO, mainly of spinal cord origin [73, 74]. As TRPV1 excess is also present in the bladder of patients with other forms of detrusor overactivity [23, 24] and a similar reflex was observed in experimental models of IDO [75], TRPV1 desensitization by intravesical application of vanilloids has also been extensively investigated in patients that suffer from IDO [25].

Among vanilloids that can be used intravesically, resiniferatoxin (RTX) is less pungent than capsaicin although equally effective in inducing TRPV1 desensitization [76]. Therefore, RTX was the selected vanilloid to treat, by intravesical route, both neurogenic and idiopathic detrusor overactivity [25, 26, 76-85]. RTX treatment reduces the density of TRPV1 expressing fibres [23, 86] and TRPV1 expression in urothelial cells [87] and caused a marked increase in the bladder capacity of these patients as well as a marked decrease in the number of episodes of urinary incontinence associated with detrusor overactivity.

Intravesical vanilloids have also been investigated to reduced pain and urinary frequency of IC/BPS patients [88-91] who, as mentioned above, also overexpress the receptor [20]. However, contradictory information about the effect of RTX in IC patients has been presented by other authors [92]. Differences in RTX outcome in different studies might result from different ways of preparing and storage of RTX. This compound is highly unstable in plastic containers so the efficacy of the solution is lost within a few hours of its preparation.

5. TRPV1 ANTAGONISTS

Pharmacological blockade or genetic ablation of the TRPV1 receptor demonstrated that this receptor is essential for the development of bladder overactivity and noxious input in cystitis [14, 17]. The administration of the new oral specific TRPV1 antagonist GRC-6211 has reduced bladder

hyperreflexia in both acute and chronic bladder inflammation models [17]. Furthermore, GRC-6211 administration reversed the increase in spinal c-Fos expression in animals with acute inflammation [17].

TRPV1 receptor is also thought to be involved in the increase in reflex activity associated with spinal cord transection [72, 73], chronic bladder outlet obstruction [75] and idiopathic bladder overactivity [25]. The TRPV1 role in the increased spinal micturition reflex after chronic spinal cord transection is further supported by the effect of specific TRPV1 antagonists [93]. Application of the TRPV1 antagonist GRC-6211 decreased, in a dose dependent manner, the voiding frequency in rats with bladder overactivity caused by chronic spinalization [93].

To be therapeutically useful, researchers must still address the effects of TRPV1 antagonists on body temperature and acute thermal sensation. Although not observed with GRC-6211 in animal models [17], other TRPV1 antagonist was shown to cause severe hyperthermia by mobilizing blood from visceral circulation [94]. In addition, it is unclear if the decrease of peptide release in peripheral tissues might facilitate arterial vasoconstriction precipitating ischemic heart problems [95]. Nevertheless, once these setbacks are solved, the future of TRPV1 antagonist in GU dysfunction is expected to be shining.

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