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From Lab to Clinic

The Influence of Alpha$_1$-Adrenoreceptors on Neuropeptide Release from Primary Sensory Neurons of the Lower Urinary Tract

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Abstract

Objectives: Adrenergic α_1-receptors agonists and antagonists have been reported to increase and reduce, respectively, neurogenic inflammatory responses mediated by capsaicin-sensitive sensory neurons. However, the precise role and localization of the α_1-adrenoceptors involved in these effects are not known.
Methods: We have studied in the rat whether functional α_1-adrenoreceptors are expressed in primary sensory neurons, and whether they regulate neurogenic inflammation and nociceptive responses in the urinary bladder.
Results: The α_1-adrenoreceptor agonist phenylephrine (1 μmol/l) (1) mobilized intracellular Ca^{2+} in cultured lumbar and sacral dorsal root ganglia neurons, (2) caused the release of substance P (SP) from terminals of capsaicin-sensitive sensory neurons from the lumbar enlargement of the dorsal spinal cord and urinary bladder, and (3) increased plasma protein extravasation in the urinary bladder. All these effects were abolished by the α_1-adrenoceptor antagonist alfuzosin (10 μmol/l). Furthermore, alfuzosin (30 μg/kg, iv) partially, but significantly, inhibited cyclophosphamide-induced plasma protein extravasation in the rat urinary bladder. Phenylephrine-induced Ca^{2+} mobilization in cultured dorsal root ganglia neurons was exaggerated by pretreating the rats in vivo with cyclophosphamide. Finally, cyclophosphamide increased c-*fos* expression in the rat lumbar spinal cord. Also these in vitro and in vivo effects were inhibited by pretreatment with alfuzosin.
Conclusions: α_1-Adrenoceptors are functionally expressed by capsaicin-sensitive, nociceptive, primary sensory neurons of the rat urinary tract, and their activation may contribute to signal irritative and nociceptive responses arising from the urinary tract. It is possible that, at least, part of the beneficial effects of α_1-adrenoceptor antagonists in the amelioration of storage symptoms in the lower urinary tract derives from their inhibitory effect on neurogenic inflammatory responses.

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1. Introduction

Lower urinary tract symptoms (LUTS) associated with benign prostate hyperplasia (BPH) are successfully treated with adrenergic α_1-receptor antagonists [1–3]. Although reduction in smooth muscle tone of the bladder-urethral functional unit is considered responsible for the beneficial effect, various reports [4–6] suggest that amelioration of storage LUTS by α_1-adrenoceptor antagonism cannot solely originate from the reduction of bladder outlet resistance. In fact, LUTS may occur in the absence of outlet obstruction, and α_1-adrenoceptor antagonists are used for the treatment of pain and other pelvic symptoms in chronic prostatitis/chronic pelvic pain syndrome [7,8]. Finally, neurogenic lower urinary tract dysfunction (NLUTD) caused by diverse neurologic diseases are also treated with α_1-adrenoceptor antagonists [7], and an α_1-adrenoceptor antagonist has been successfully used for the treatment of the symptoms in patients with indwelling double-J urethral stents [9] or ureteral colic [10]. Moreover, it has been reported by a 6-mo open-label study that α_1-adrenoceptor blockade improves LUTS and painful ejaculation [11]. Reduction in increased intraurethral pressure, adrenoceptor blockade at the bladder neck and prostatic smooth muscle, and a modulatory effect on voiding and pain pathways in the spinal cord have been proposed [7,12] to explain the effects of α_1-adrenoceptor antagonists.

A subset of polymodal primary sensory neurons with A-δ and C fibers that respond to mechanical, thermal, and chemical stimuli are characterized for expression of the capsaicin receptor (transient receptor potential vanilloid-1 [TRPV1]) and of the neuropeptides calcitonin gene–related peptide and substance P (SP). Sensory neuropeptides are released from central and peripheral endings of these primary sensory neurons [13]. Release in the spinal cord has been associated with nociceptive transmission, whereas release from peripheral endings causes a series of local inflammatory responses referred to as neurogenic inflammation. Neurogenic inflammation includes vascular (hyperemia and plasma protein extravasation) and nonvascular responses, and has been proposed to play a major role in different diseases of the urinary tract, including cystitis and overactive bladder syndrome [14].

Sympathetic efferents are known to modulate neurogenic inflammatory responses by interacting with primary afferent terminals. Norepinephrine injection into the human skin potentiated capsaicin-induced heat hyperalgesia by activation of α_1-adrenoceptors [15,16]. Sympathectomy inhibited capsaicin-induced c-*fos* expression in dorsal horn neurons [17], and hyperalgesia induced by intradermal capsaicin was abated by α_1-adrenoceptor blockade [18]. Finally, capsaicin-induced flare in the rat skin was reduced by an α_1-adrenoceptor antagonist [19]. These studies suggest that sympathetic efferents modulate neurogenic inflammation in the skin and that peripheral α_1-adrenoceptors play a major role in this modulation. However, the precise location of α_1-adrenoceptors that activate sensory neurons is not known, nor is whether this excitatory action of α_1-adrenoceptors occurs also in the urinary system.

In the present study, we have investigated whether α_1-adrenoceptors are expressed in and excite rat TRPV1-expressing primary sensory neurons, and whether α_1-adrenoceptors contribute to neurogenic inflammatory responses and nociception that originated in the urinary tract following cyclophosphamide (CYP) treatment.

2. Materials and methods

2.1. *Animals*

A total of 120 male Sprague-Dawley rats (150–200 g; Morini, Italy) were used throughout the entire study. The study conformed to the Declaration of Helsinki, complied with the Italian guidelines, and was approved by the local ethics committee for animal studies.

When requested, CYP (150 mg/kg, ip) or its vehicle (0.9% NaCl) administered 90 min or 48 h prior to the diverse assays was used for cystitis induction.

2.2. *Intracellular Ca^{2+} measurement in cultured primary rat dorsal root ganglion neurons*

Experiments were performed, with a few modifications as previously reported [20]. Briefly, rats were pretreated with CYP or its vehicle; then 48 hours later, animals were terminally anaesthetized with diethyl ether and decapitated. Lumbar and sacral dorsal root ganglia (DRG) were removed from both animal groups and rapidly placed in cold phosphate-buffered solution (PBS) before being transferred to collagenase (10 mg/ml), trypsin (5 mg/ml), and DNAse (1 mg/ml) for 35 min at 37 °C. Ganglia, placed in cold Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, 2 mmol/l l-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin, were dissociated in single cells by several passages through a series of syringe needles. Medium and ganglia cells were filtered to remove debris, topped off with 8 ml of DMEM medium, and centrifuged. The pellet was resuspended in DMEM medium and plated on poly-l-lysine (8.3 μmol/l) and laminin (5 μmol/l) coated 25-mm glass cover slips.

For the intracellular Ca^{2+} ($[Ca^{2+}]_i$) fluorescence measurements, plated neurons (2 days old) were loaded with Fura-2-AM-ester (3 μmol/l) in a buffer solution (mmol/l: $CaCl_2$ 1.4, KCl