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Inhibition of substance P activity prevents stress-induced bladder damage

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Abstract

Substance P is a neuropeptide involved in inflammation, immune regulation and stress response. Stress may induce bladder damage by stimulating inflammatory response such as mast cell activation. We here examined the role substance P during stress-induced mast cell degranulation and urothelial injury in rat bladder. Adult Sprague-Dawley rats (200–270 g) were either exposed to cold-immobilization stress or substance P (SP) intracerebroventricularly. Different doses of substance P receptor (NK1R) antagonist CP 99994 were administered peripherally or centrally before the stress exposure. From each group, samples of the bladder were examined with light and electron microscope. Stress- and SP-injected centrally, increased the number of both granulated and degranulated mast cells. Ultrastructurally, urothelial degeneration with vacuolization in the cytoplasm and dilated intercellular spaces were seen. Both central and peripheral injection of CP 99994 prevented stress-induced urothelial degeneration as well as stress-induced mast cell degranulation. In conclusion, centrally and peripherally released substance P is involved in stress-induced bladder damage. Inhibition of NK1R prevents stress-induced pathological changes of urinary bladder and NK1R antagonist can be considered for the treatment of inflammatory bladder diseases.

Keywords: Stress-induced mast cell degranulation; SP; NK1R

1. Introduction

Physical and emotional stresses are etiological factors underlying several diseases. Chronic stress, for example, alters structure of hippocampus and disturbs GABAergic system which may underlie various stress-related psychiatric disorders [1,2]. Stress-induced psychiatric disorders are probably partly mediated by substance P (SP)-induced activation of neurokinin-1 receptors (NK1R) [3]. Pharmacological blockade of NK1R is effective in suppression of psychological and behavioral stress reactions [4] and SP or SP agonists injected intracerebroventricularly induce a stress response such as vocalization [5] and activates several stress pathways [6].

Beside psychiatric disorders, stress by altering similar mechanisms can also induce somatic diseases such as peptic ulcer, cyctitis and inflammatory skin diseases [7–9]. It is, however, not known whether centrally active SP can alter peripheral inflammatory response such as mast cell degranulation and urothelial damage. We recently demonstrated that mast cell degranulation in skin increases following central injection of SP while stress-induced mast cell degranulation in skin can be prevented by intracerebroventricular administration of NK1R antagonist [7]. One of the aims of the present study was determine the role of centrally released SP in stress-induced bladder damage.

Studies demonstrated that stress-induced release of peripheral SP may also be involved in somatic pathologies but definitive studies are missing. SP peripherally is localized in capsaicin-sensitive sensory nerve endings which are important in stress response. For example, inactivation of sensory neurons with capsaicin prevents acute stress-induced epithelial damage and mast cell proliferation in the rat bladder [8]. It is very likely that during stress, discharge of neuropeptides, particularly SP, from primary afferent fibers activates mast cells in the bladder and induces inflammation [10]. The responses to SP and other tachykinins are regulated by G protein-coupled neurokinin receptors. Of these receptors neurokinin-1 receptor (NK1R) is the predominant receptor subtype activated by SP

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which is involved in inflammation and is expressed by endothelial cells, submucosal glands, and circulating leukocytes [11] NK1R seems also to modulate plasma extravasation in the urinary bladder induced by SP [12]. The fundamental role of NK1R in cystitis was demonstrated in NK1R knockout mice; these mice were resistant to bladder inflammation induced by antigen challenge [10]. The role of NK1R activation in stress-induced urothelial damage, however, has not been directly evaluated.

Mast cells seem to be equally important in initiation and progression of inflammation in the bladder. Increased numbers of mast cells were observed in bladder biopsies obtained from patients with interstitial cystitis [13]. SP can induce mast cell degranulation [14] as well as mediates the inflammatory responses induced by mast cell tryptase [15]. These findings imply a link between SP and mast cells in the pathogenesis of bladder inflammation. The contribution of SP-induced NK1R activation to stress-induced mast cell degranulation has not been directly tested. Hence our other goal is to determine whether inactivation of NK1R prevents stress-induced urothelial damage and mast cell activation.

2. Materials and methods

Adult Sprague Dawley rats (200–270 g) were used in this study. SP antagonist, CP 99994 was generously donated by Pfizer. Both SP and CP 99994 were dissolved in physiological saline. As a vehicle, saline was tested separately in untreated animals; when appropriate saline was injected either intracerebroventricularly (i.c.v.) or intraperitoneal (i.p.). This work was approved by the Institutional Animal Committee of Marmara University, School of Medicine.

2.1. Experimental groups

1. Stress group ($n=5$): Rats were fasted for 48 h and exposed to cold-immobilization stress for 3 h after i.c.v. injection of saline.
2. SP-injected group: SP (5 μg) was injected i.c.v. to 48 h starved animals ($n=5$). Animals were sacrificed 3 h after injection.
3. Stress-exposed, SP-treated group: Animals were exposed to cold-immobilization stress after i.c.v. injection of SP (5 μg, $n=5$).
4. CP 99994 (i.c.v.) treated group: Animals were treated with CP 99994 i.c.v. (5 μg, $n=4$; 10 μg, $n=5$; and 20 μg, $n=4$) immediately before exposed to stress.
5. CP 99994 (i.p.) treated group: Peripheral injection of CP 99994 was performed (0.5 mg/kg, $n=4$; 1 mg/kg, $n=4$; 2 mg/kg, $n=4$) 10 min before stress exposure.

Cold-immobilization stress was performed as described before [8,9]. Animals were decapitated under ether anesthesia.

2.2. Microscopical preparation

Tissues were fixed with 2.5% phosphate-buffered glutaraldehyde (1 M, pH 7.2). For electron microscopic investigation, bladder samples were post fixed in 1% phosphate-buffered osmium tetroxide (0.1 M, pH 7.2), dehydrated in a graded series of alcohols and polymerized in epoxy resin. Approximately 1-

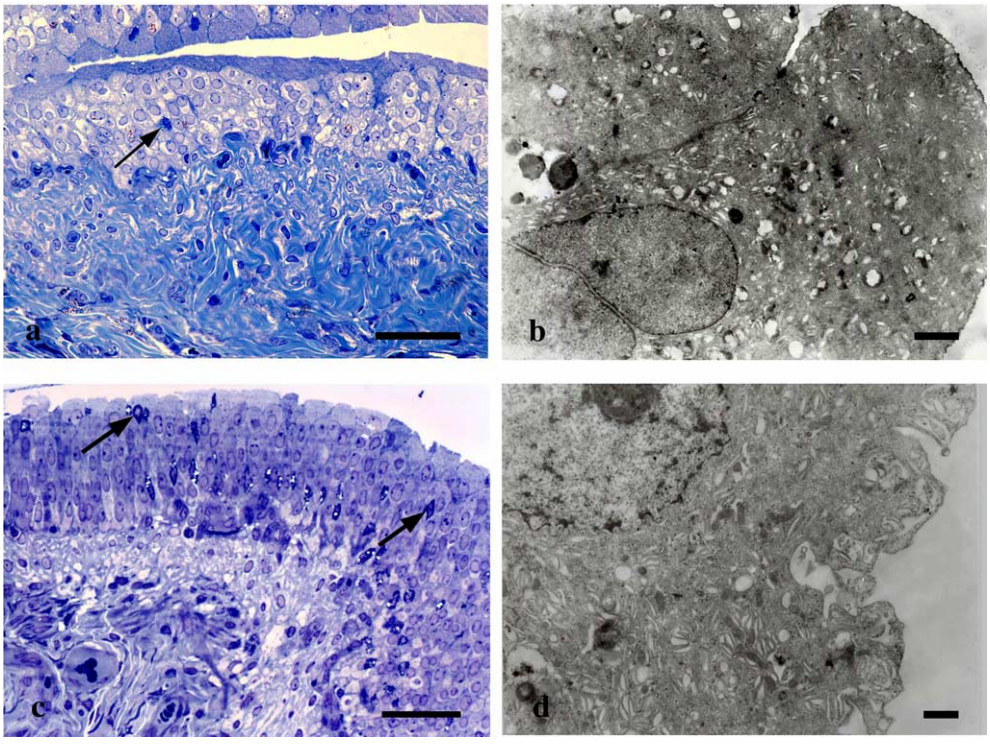

Fig. 1. *Control group:* Normal urothelium and a few number of mast cells (→) between the urothelium and normal intercellular space and urothelial cytoplasm were observed in the urinary bladder from the control rats (a and b) and starved for 48 h (c and d) and injected with saline i.c.v. Panels a and c: Toluidine blue. Scale bars: (a, c) 25 μm; (b, d) 1 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)