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The effect of acute distension on vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY) and substance P (SP) immunoreactive nerves in the female rat urinary bladder

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Summary. The effect of acute distension on vasoactive polypeptide (VIP)-, neuropeptide Y (NPY)- and substance P (SP)-immunoreactive nerves in the wall of the urinary bladder was investigated. At the age of 3 months, 25 female albino rats underwent forced diuresis combined with balloon obstruction to achieve maximal distension for 3 h. A modified, indirect immunofluorescence detection method was applied 2 days, 7 days and 21 days after distension. A marked, extensive depletion of VIP, NPY- and SP-immunoreactive nerves was observed after distension. This disturbance was reversible, and increased fluorescence of VIP-, NPY- and SP-immunoreactive nerve fibres compared with control specimens was seen in bladder specimens taken even as soon as 21 days after distension. This transient depletion of peptidergic innervation may partly explain the prolonged voiding problems that often occur after acute urinary retention. The depletion of sensory nerves containing SP shortly after distension may explain the transient benefit obtained from distension therapy in patients with painful bladder disease. It is suggested that the increased SP activity during the recovery phase may be related to neurogenic inflammation.

Key words: Neuropeptide Y – Vasoactive intestinal polypeptide – Substance P – Bladder distension – Rat

The dual autonomic innervation of the mammalian bladder has been known and accepted for some time, but a number of peptides, including vasoactive intestinal polypeptide (VIP), substance P (SP) and neuropeptide Y (NPY), have also been proposed as neurotransmitters or neuromodulators, and it has been suggested that these may represent a third peptidergic component of the autonomic nervous system [16].

VIP, which brings about vasodilatation and smooth muscle relaxation [17], is found in all layers of the human urinary bladder, but principally in the detrusor and around the blood vessels [6]. NPY has been shown to occur in nerve fibres in the walls of blood vessels and in the muscle layer [11], and perivascular nerves containing it have been shown to be identical to noradrenaline-containing adrenergic fibres [5]. SP is believed to be one of the sensory neurotransmitters related to the perception of pain [13] and has been found to produce smooth muscle contraction in vitro [18].

Changes in the concentration and distribution of these peptides have recently been observed in many clinical and experimental situations. The total amounts of VIP and SP have been demonstrated to be significantly higher in obstructed bladders [2], while reductions in the number of VIP-immunoreactive nerves and in VIP concentrations have been observed in unstable and hyper-reflexic neuropathic bladders [7, 10].

We have observed a transient cholinergic and adrenergic hypoinnervation after acute bladder distension [21, 22]. Since no investigations had been carried out on the peptidergic innervation in bladders after acute distension, the present work was aimed at studying the effect of acute bladder distension on VIP-, NPY- and SP-reactive nerves using a modified, indirect immunofluorescence method.

Materials and methods

A total of 25 adult female albino rats of the Sprague-Dawley strain were used at body weight 240–290 g and age 3 months. They had been bred at 22–24°C and were housed 3 or 4 per cage. The light cycle was 12 h L/12 h D and there was an adequate supply of tap water and commercially manufactured pellets. The rats were anaesthetized with pentobarbitone sodium, 35 mg/kg body weight i.p., and the bladder was catheterized with a Fogarty (3 F) embolectomy catheter (12-080-3F, Baxter, Santa Ana, Calif., USA), the balloon being filled with 0.05 ml water and then pulled into the bladder neck. The rats were given furosemide, 12 mg/kg body weight i.m., and 4 ml Ringer solution i.p. to induce maximal bladder distension for 3 h. Infection prophylaxis was achieved with an injection of cefuroxime, 30 mg/kg body weight. After distension for 3 h the bladders were emptied, and the rats were allowed to recover. Buprenorfine was given s.c. at 0.1–0.13 mg/kg body weight if the animals appeared to be in pain. They were then watched carefully to check bladder emptying.

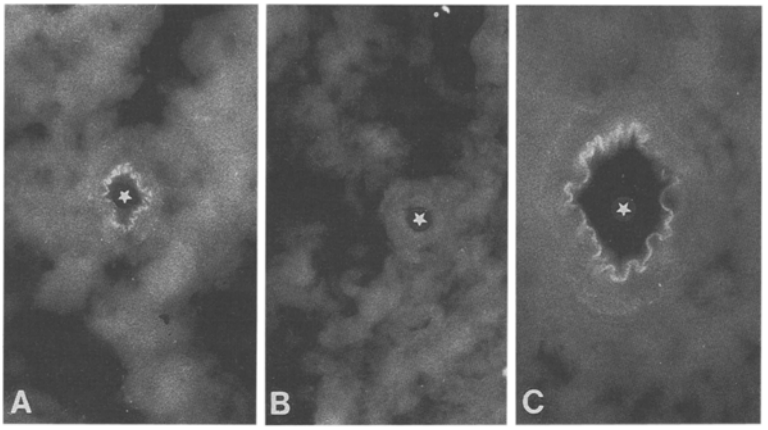

Fig. 1. A–C. Perivascular NPY-immunoreactive nerves. **A** NPY-immunoreactive fibres surrounding blood vessels (*). **B** No trace of NPY-immunoreactive perivascular fibres can be seen 2 days after distension. **C** Some NPY-immunoreactive fibres surrounding the blood vessel 21 days after distension. Original magnification ×125

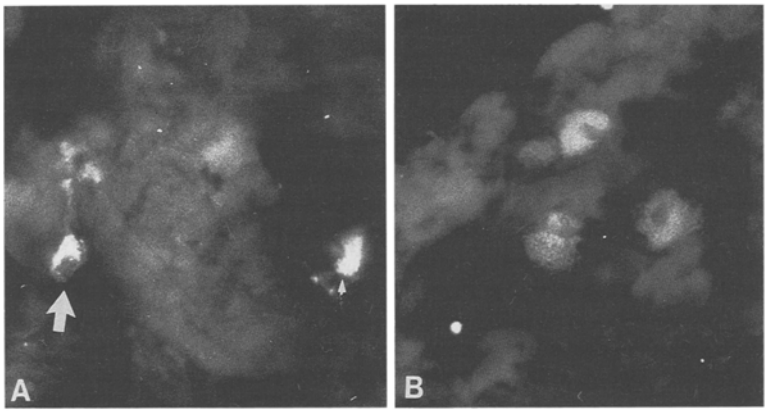

Fig. 2. A, B. NPY-immunoreactive SIF-like cells. **A** A unipolar SIF-like cell *(large arrow)*, and an NPY-immunoreactive bundle near a blood vessel *(arrowhead)*. **B** SIF-like cells after bladder distension. Original magnification ×125

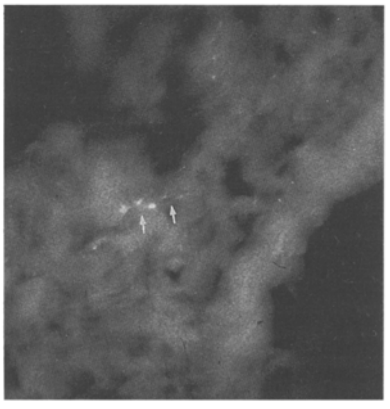

Fig. 3. NPY-immunoreactive nerve fibres in the detrusor muscle. Some NPY-immunoreactive nerves were observed 21 days after distension *(small arrows)*. Original magnification ×125

The rats were sacrificed 2 days (5 animals), 7 days (5 animals) or 21 days (8 animals) after distension. A further 5 adult female rats matched in age and weight with those subjected to bladder distension were used as controls to avoid the possible differences in innervation due to maturation and environmental factors. Whole-thickness biopsies (including all layers of the bladder) were taken from the wall of the dome and anterior body by a microscopic technique. They were then rinsed in 0.9% NaCl solution, covered with OCT compound (Miles, Elkhart, Indiana, USA), frozen in liquid nitrogen and stored at −70°C. The frozen samples were cut perpendicular to the bladder surface into cryostat sections of 10 μm thickness, and then dried for 1 h at room temperature. The sections were then mounted on glass slides coated with poly-L-lysine (molecular weight 350000), fixed in 0.4% benzoquinone in PBS at +4°C for 15 min, and subjected to modified indirect immunofluorescence [6]. The cryostat sections of the bladder muscle were incubated overnight at +4°C with polyclonal antibodies to NPY, dilution 1:2000, VIP, dilution 1:2000 and SP, dilution 1:2000. The sites of antigen-antibody binding were revealed by incubating with sheep anti-rabbit IgG conjugated to fluorescein isothiocyanate (FITC; Welcome Foundation, Dartford, UK) for 1 h at room temperature. The antisera were raised in New Zealand White rabbits and were obtained from Dr. J. M. Polak. Controls included preabsorption of the antisera with their respective antigens and the use of normal rabbit serum after PBS as the first layer and the fluorescein-conjugated second layer alone. The preparations were viewed using an Olympus microscope equipped for FITC fluorescence and phase contrast. Selected areas were photographed on TriX film (Kodak, Harrow, UK) for black-and-white photography and Ektachrome 400 (Kodak, Harrow, UK) for colour photography.

Results

Animal mortality

Mortality during the trial was 8%, occurring mostly during and after anaesthesia.

NPY immunoreactivity in the control *bladders*

NPY-immunoreactive nerves were only occasionally seen within the detrusor muscle, but there were some perivas-

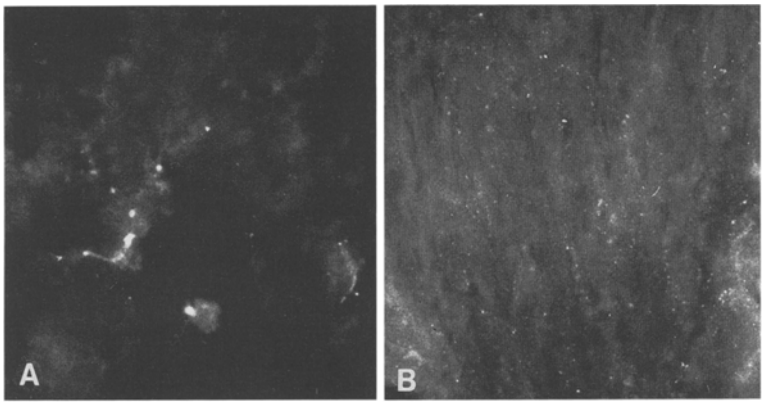

Fig. 4A, B. VIP-immunoreactive nerve fibres among the detrusor muscle. **A** A few strong VIP-immunoreactive fibres in control specimens. **B** Numerous VIP-immunoreactive fibres in a specimen taken 21 days after distension. Original magnification ×125

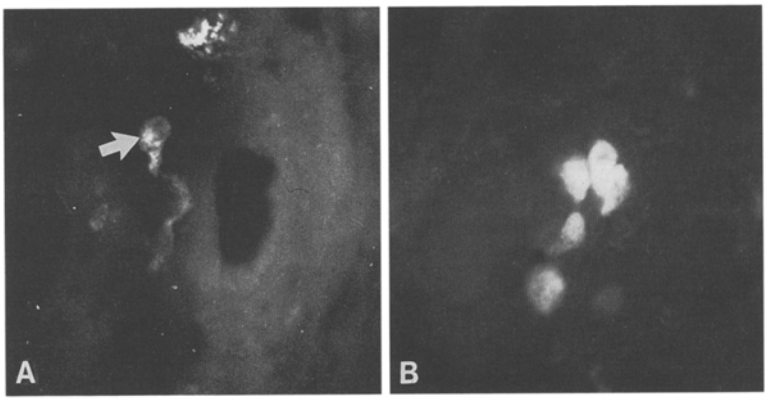

Fig. 5A, B. VIP-immunoreactive ganglion-like cells. **A** VIP-immunoreactive ganglion-like cell *(arrow)* in a specimen taken 7 days after distension. Original magnification ×100. **B** VIP-immunoreactive ganglion-like cells in a specimen taken 21 days after distension. Original magnification ×125

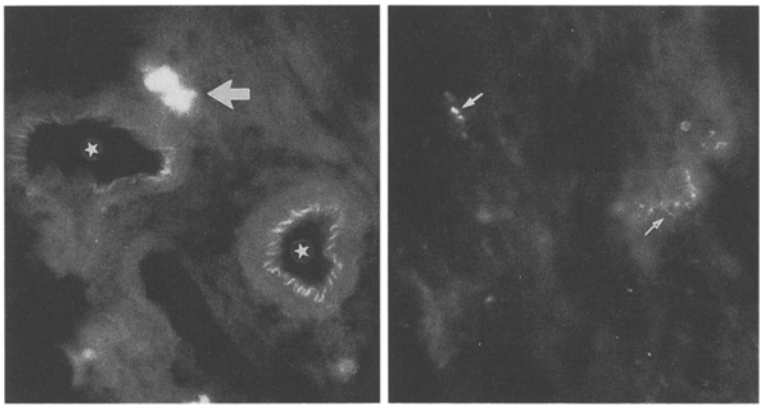

Fig. 6. A, B. SP-immunoreactivity. **A** Some SP-immunoreactive nerve fibres surrounding blood vessels (*) and perivascular bundles *(large arrow)* in control specimens. **B** An SP-immunoreactive nerve fibre *(small arrows)* within the detrusor muscle in a specimen taken 21 days after distension. Original magnification ×125

cular NPY-immunoreactive fibres (Fig. 1A). Some NPY-immunoreactive bundles were located near the blood vessels. NPY-reactive small, intensely fluorescing (SIF)-like cells were seen in a few specimens (Fig. 2A).

NPY immunoreactivity in the distended *bladders*

Depletion of NPY-containing nerves was observed 2 days after distension (Fig. 1B). Some SIF-like cells were seen (Fig. 2B). The fluorescence in the NPY-containing nerves among the bladder muscle and in perivascular nerves was increased 21 days after distension (Figs. 1C, 3).

VIP immunoreactivity in the control *bladders*

VIP-immunoreactive nerves were found surrounding the blood vessels, where thick VIP-immunoreactive bundles were seen. A few nerve fibres were seen running in the muscular layer, both inside and between the muscle fibres (Fig. 4A).

VIP immunoreactivity in the distended *bladders*

A marked decrease in the density and brightness of the VIP-immunoreactive nerves was observed 2 days after distension. Some VIP-reactive ganglion-like cells were seen in specimens taken 7 days and 21 days after

distension (Fig. 5A, B), and numerous small VIP-immunoreactive nerves were seen at the later point of these times (Fig. 4B).

SP immunoreactivity in the control *bladders*

SP-immunoreactive fibres were seen only in the nerve bundles and in perivascular plexus (Fig. 6A).

SP-immunoreactivity in the distended *bladders*

No difference could be seen between control and distended specimens 2 days after distension. Depletion of SP-immunoreactive nerves was seen 7 days after distension, but some regenerating ones were seen within the detrusor muscle by 21 days after distension (Fig. 6B), at which stage the intensity of fluorescence in the SP-containing nerves had increased.

Discussion

A marked, extensive disturbance in peptidergic innervation was observed in the urinary bladders of adult female rats after distension. The maximal diminution of VIP- and NPY-immunoreactive nerves was already apparent 2 days after distension, but the effect was reversible, and even stronger fluorescence was seen in the NPY-, VIP- and SP-immunoreactive nerves 21 days after distension than in the controls. A few NPY-immunoreactive SIF-like cells were seen in control and distended bladders.

VIP is a 28-amino-acid peptide with powerful effects, including smooth muscle relaxation, vasodilatation and stimulation of secretion [17], and the presence of nerves containing it has been reported in the urinary bladder in association with the detrusor muscle and surrounding the blood vessels [7]. This is in accordance with the findings in the present control specimens. VIP may play an important role in the control of bladder function, possibly acting by modifying the background activity of the detrusor muscle and keeping it relaxed during the filling phase [19]. The loss of some VIP nerves in the distended bladder may possibly be regarded as a feature of neuropathy, and may lead to an unrelaxed, irritable detrusor shortly after distension. The reappearance of VIP in increased amounts may then play a role as an inhibitor of spontaneous activity in the detrusor, as has often been seen after distension [9, 15].

NPY is a 36-amino-acid-peptide, which is thought to be a neuromodulator with a long-lasting action [1]. Nerve fibres containing NPY are richly distributed in the detrusor muscle, the pelvic ganglia and the walls of blood vessels, where the NPY-containing neurons are identical to adrenergic neurons [5, 12]. The other population of NPY-containing neurons, supplying the bladder muscle, may be non-adrenergic in nature [12]. Unlike VIP, NPY is a potent smooth muscle contractor [1], and it has been suggested that NPY and VIP released from perivascular plexuses may act as agonists and antagonists regulating local blood flow.

NPY immunoreactivity was also seen in this study in SIF-like cells, which are thought to play a role in interaxonal neurotransmission between cholinergic and adrenergic innervation [3]. Type I SIF cells are solitary and thought to act as interneurons, while type II SIF cells are found in clusters in the vicinity of blood vessels and are thought to act as paraneurons [24]. These paraneurons are also known to contain NPY [14]. The release of catecholamines or NPY from damaged type II SIF-like cells after distension could influence capillary permeability and the functioning of nearby principal neurons, i.e. parasympathetic nerves containing VIP.

Distribution of SP can be divided into two systems, one forming thick fibre bundles and the other running around the blood vessels [25]. Although SP is known to contract the rat urinary bladder in vitro, it most probably does not have a motor function in it [2]. It may influence the release of acetylcholine and noradrenaline, and is known to modulate the release of other neurotransmitters [8]. Because SP-immunoreactive nerves have disappeared after the application of capsaicin they are thought to have a sensory function in the rat urinary bladder [23].

We have previously observed transient cholinergic and adrenergic hypoinnervation in the rat urinary bladder after acute distension [21, 22], and in the present study nerves containing NPY and VIP were also transiently depleted after distension. This disturbance in the innervation may explain the prolonged voiding problems that often occur after acute urinary retention [20]. Increased SP activity may be involved in bladder instability, because it has been shown that no instability is found after depletion of the rat bladder of SP by capsaicin [2]. The transient therapeutic effect achieved by bladder overdistension is patients with painful bladder disease [4] may be thought to be at least partly related to depletion of SP-immunoreactive sensory nerves, and the reappearance of the symptoms may be related partly to regeneration of these nerves. The actual functional role of increased peptidergic innervation 21 days after distension needs further investigation, but we suggest that the increased SP activity in particular is related to neurogernic inflammation in the bladder wall.

In conclusion, acute overdistension of the rat urinary bladder causes a transient, almost complete depletion of the nerve fibres containing VIP, NPY and SP. The depletion of SP-immunoreactive sensory nerves may explain the transient benefit gained from distension therapy by patients with painful bladder disease. On the other hand, the extensive disturbance of the neural system involving cholinergic, adrenergic and neuropeptide-containing neurons may also partly explain the prolonged voiding problems often seen after overdistension of the bladder caused by urinary retention.

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