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ORIGINAL PAPER

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Changes in neuropeptide y tissue concentration in the wall of the rat urinary bladder after acute distension

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Abstract Neuropeptide Y (NPY) is known to be associated with the adrenergic system. The relationship among the late micturition disorders following acute urinary distension, the adrenergic system and NPY was investigated. A total of 90 rats were included in the study of which 30 acted as the control group. Acute urinary distension was created in 60 rats. The NPY concentration within their bladders was assessed by the use of radioimmunoassay (RIA) at 3 h after distension and subsequently on days 2, 7 and 21, then the third and sixth months. The NPY concentrations assessed in the third and sixth months were compared with the control group in the same age group. By means of the RIA method, a substantial decline of NPY concentration was observed at 2 days after distension, while the concentration started to increase after day 7 ($P = 0.003$). This increase continued until the twenty first day ($P = 0.004$). However, a significant decline was maintained when compared to the concentration before distension. In the third and sixth months, a significant decline were observed in the NPY concentration in comparison to the control group ($P = 0.004$ and $P = 0.005$, respectively). Early and late micturition disorders experienced after acute urinary distension may be the result of adrenergic denervation which may be related to NPY.

Keywords Urinary bladder · Rat · Acute · Distension · Neuropeptide Y · Micturition · Disorders

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Introduction

Early micturition disorders following urinary distension have been attributed to an increased muscarinic receptor response to humoral stimulation, due to denervation caused by distension. Neurotransmitters such as neuropeptide Y (NPY) may play an important role in this process. In addition, NPY concentration changes in severe bladder dysfunction pathologies [13, 19, 27].

Recent studies have indicated that NPY, as part of the peptidergic system, which is also apart of the nonadrenergic noncholinergic nervous system, can be intensively observed within the bladder by use of immunohistochemical methods [14, 31]. NPY was found localized around the adrenergic receptors and shown to produce its effect on the adrenergic receptors [10, 21, 23, 24].

NPY has also been observed in the human prostate, urethra, seminal vesicles and vas deferens [5, 16, 18]. However, its function in the suburogenital system is not yet clear, and there are contradictory findings in the studies carried out recently on this subject [1, 4, 7, 8, 32].

Following an acute distension period, early micturition disorders are usually observed. Despite the fact that NPY is basically related to early symptoms, an abnormal concentration 3 weeks after distension implies that it may also play a role in the occurrence of late symptoms. In this study, NPY concentration at the early and late periods following bladder distension was determined and its association with the micturition disorders investigated.

Materials and methods

During the study, 12-week-old female Sprague-Dawley rats weighting 200–250 g were used. Nine separate cages were prepared and ten rats were put into each cage. All ten rats put into the first seven cages survived over the planned period. However, due to the known high risk of mortality over longer periods, 20 rats were included in

both the 3 and 6 month groups. Fifteen of the rats in the third month and twelve in the sixth month survived. Ten rats from both of these groups were selected to be used in the study, giving equal group sizes throughout. The rats were provided with standard milk, fodder pellet and tap water. Rats were kept under a 12-h light 12-h dark cycle. They were randomly divided into the nine groups.

Ketamine anesthesia (35 mg/kg) was applied through intra-peritoneal injection. The bladder was catheterized with a Fogarty embolectomy catheter (3 F), the balloon filled with 0.05 ml water and then pulled into the bladder neck. The rats were given furosemide, 12 mg/kg body weight intramuscularly, and 4 ml Ringer solution intraperitoneally to induce maximum bladder distension for 3 h. Infection prophylaxis was achieved with an injection of cefuroxime, 30 mg/kg per body weight. After distension for 3 h, the bladders were emptied and the rats were allowed to recover. Buprenorfine was given subcutaneously at 0.1 mg/kg body weight when the animals appeared to be in pain. They were then watched carefully to check bladder emptying.

The rats were killed after 3 h, on days 2, 7 or 27, or at the third and sixth months after distension. A further 30 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. There were no age or weight differences between the 12-week-old, healthy rats and the rats in the experimental groups. Thus, 12-week-old rats were used as the control group for these groups (control 1). However, for the rats which were evaluated in the third and the sixth months, two different control groups were formed with matched age and weight (control 2, control 3).

Whole-thickness biopsies (including all layers of the bladder) were taken from the wall of the dome and anterior body. These were then examined by radioimmunoassay (RIA). Tissue for RIA was extracted by boiling in 0.5 M acetic acid (10% weight/volume) for 10 min. Aliquots (10 µl and 1 µl) of these extracts were then assayed in duplicate for NPY using antiserum YN 1. NPY-immunoreactivity was characterised by reserve phase high performance liquid chromatography (HPLC) using a C-18 μ Bondapak Column (Waters Associates) eluted with acetonitrile in water containing 0.2% trifluoroacatic acid. A linear gradient from 35 to 45% acetonitrile over 10 min was employed at a flow rate of 2 ml/min. Fractions of 1 ml were collected and subsequently assayed.

Results are expressed as pmol/g wet weight and values represent means ± SD. Statistical analyses were performed by determining the significance of the difference among the periods by means of the matched-pairs *t*-test, when one-way analysis of variance with repeated measures for each variable and the difference between periods were found to be significant.

This study was approved by the local ethics committee.

Results

No significant difference between NPY concentrations was observed in the wall of the dome and anterior body in the total study period ($P = 0.789$).

At 3 h after distension, the NPY concentration did not manifest any significant difference when compared to the control 1 group ($P = 0.598$). A clear decline was observed in the concentration 2 days later ($P = 0.001$).

At 7 days after distension, the decline in the NPY concentration was significant compared to control 1 ($P = 0.003$). A decline in the concentration was also significant compared to control 1on day 21 ($P = 0.004$).

NPY concentration was lower when compared to control 2 and control 3 in the third and sixth months ($P = 0.004$, $P = 0.005$, respectively). All values are summarized in Table 1.

There were significant declines in comparison with control 1 on days 2, 7 and 21 following distension. NPY concentration evidently declined 2 days after distension and increased from day 7 to day 21 following distension. However, the concentration observed on day 21 was relatively lower when compared to control 1. NPY concentrations observed in the third and sixth months were close to that of day 21, and there was no significant difference. However, the significant decline in the concentrations when compared to the control group was still present.

Discussion

Clinical observations have provided some information on the late stage of bladder distension in general. It has

Table 1 Mean value of NPY concentration on the wall of the dome and anterior body

	The wall of dome Mean ± SD (pmol/g)	Anterior body Mean ± SD) (pmol/g)	*P*
Control 1	21.83 ± 2.24	20.56 ± 2.18	
3 h after obstruction	21.34 ± 2.08	20.27 ± 2.09	0.598
2 days after obstruction	12.01 ± 1.29	11.98 ± 1.32	0.001
7 days after obstruction	14.27 ± 1.34	14.00 ± 1.30	0.003
21 days after obstruction	14.98 ± 1.22	14.57 ± 1.28	0.004
Control 2	22.04 ± 2.31	21.95 ± 2.30	
3 months after obstruction	15.33 ± 1.27	15.27 ± 1.25	0.004
Control 3	21.96 ± 2.32	21.75 ± 2.27	
6 months after obstruction	16.01 ± 1.13	15.89 ± 1.10	0.005

also been possible to conduct research on the physiological and morphological changes taking place as from the early period of distension using animal models [3, 20, 30]. The situation defined in animal models probably develops as a slower and longer process in humans. Research was carried out to define whether the changes occurring after the elimination of distension relapse or not. A prominent functional consequence of the bladder response to distension is over-activity. It is still controversial whether detrusor over-activity is a response of smooth muscle to distension or a result of the altered viscoelastic characteristics of the bladder wall. In various studies, it is stated that the over-activity develops due to the increased response of muscarinic receptors to the humoral stimulation because of partial denervation [14, 25, 30]. Such an increased stimulation may be related to cholinergic or adrenergic agents such as NPY, somatostatin, vasoactive intestinal polypeptide (VIP) or substance P (SP) [9, 11, 19]. In fact, this situation is a result of denervation supersensitivity. Elimination of over-activity by surgery applied to the bladder outlet is an important finding.

The existence of NPY, one of the peptides comprising the peptidergic component of the autonomous nervous system, in the human urethra, bladder, prostate, seminal vesicles and peripheral nerve fibers innervating the vas deferens, was determined using immunohistochemical methods [5, 16, 18].

NPY is comprised of 36 amino acids and is regarded as a cotransmitter of the sympathetic nervous system. It is unlikely that NPY functions as a neuromuscular transmitter in the urinary bladder [12]. In the rat bladder, it does have an excitatory action, causing an increase in the amplitude of irregular phasic contractions and increasing the tone; whether or not this represents a physiological process remains to be determined [2, 15]. More probably, NPY functions as a neuromodulator. In the rat detrusor, NPY inhibits cholinergic excitation, but in the rat urethra it inhibits adrenergic excitation [28, 29]. In the detrusor muscle of the guinea-pig's urinary bladder, it causes a prejunctional inhibition of purinergic transmission, and in the rabbit urethra it causes a prejunctional inhibitor of cholinergic transmission [22].

In human organs, NPY binding sites have been localized autoradiographically on smooth muscle cells of the bladder neck and urethra, and in both of these regions there is a dense NPY innervation, but a physiological role is yet to be determined [17].

Changes in the concentration and spread of NPY have been investigated in clinical and experimental studies. When an increase in NPY concentration was observed in bladder neck dyssynergia in the smooth muscle and the bladder base, there was a small decline in NPY concentration in the sub-motor lesions [6]. There was an increase in NPY concentration accompanying the increase in bladder sympathetic activation in interstitial cystitis [13]. It has also been reported that there was a decline in the total level and spread, together with VIP, calcitonine gene related peptide and SP, as regards obstructed bladders. Since NPYs close relationship with the adrenergic system has been identified, the relationship between early micturition disorders resulting from distension and this peptide has been widely studied [19.

Catecholamine depletion observed after distension starts at the tenth hour and reaches its maximum level on the second day. Keeping its high level until days 5–6, it returns to a normal level on day 21. NPY spread at bladder after distension declines on the second day and turns back to basic levels on day 21. Such a decline probably depends on temporary adrenergic hypoinnervation [4, 14]

Some patients experience micturition disorders following distension even at the late period. This implies that such problems may also be associated with the adrenergic system and NPY [19]. To understand the period required for adrenergic denervation, it is necessary to carry out studies on NPY at the later stages after distension. In our study, NPY concentrations were determined in adult female rat bladder after distension on days 2, 7, 21 and in the third and sixth months. NPY concentration showed a decline on day 2 and started to increase on day 7. This increase continued until day 21. However, it never reached basal levels. No change of NPY concentration was observed in the third and sixth months when compared to day 21, i.e. the concentration had not reached the level of pre-distension by the sixth month. This implies that adrenergic denervation still exists.

We concluded that micturition disorders continuing at the later stages following acute urinary distension may be associated with NPY and thus with the adrenergic system.

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