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Effect of distension on adrenergic innervation of the rat urinary bladder

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Summary. The effect of distension on adrenergic innervation was investigated in the rat urinary bladder. Bladders were distended for 3 h by forced diuresis and balloon obstruction, and specimens were taken from the bladder dome, body and neck for the demonstration of glyoxylic acid-induced fluorescence of catecholamines. Depletion of catecholamines started after 10 h and was almost complete after 2 days. The fluorescence had recovered part way after 5–7 days and was practically normal after 21 days. Small, intensely fluorescent (SIF) cells in the ganglia continued to leak catecholamines throughout the 21-day study period. The primary clinical success of distension therapy for the treatment of unstable bladder may be at least partly due to a reversible disturbance in the function of the adrenergic nerves, which have an excitatory alpha-adrenergic dominance in such cases, but the persistent leakage from SIF cells raises the question of whether distension causes prolonged disturbances in bladder function.

Key words: Urinary bladder – Overdistension – Adrenergic innervation – SIF cells – Glyoxylic acid – Rat

Adrenergic innervation of the urinary bladder has been studied by specific histochemical and pharmacological methods in various species, including man, the greatest density being found in the trigone and bladder neck [2, 6, 7, 10, 16]. Many of the adrenergic nerves innervate vessels. Cholinergic transmission is almost exclusively responsible for the contractile power in patients with normal detrusor function [1], whereas adrenergic transmission is inhibitory, i. e. beta-adrenergic [2, 16]. Parasympathetically denervated or idiopathically unstable bladders, on the other hand, show an increased density of excitatory alpha-adrenergic receptors [16, 18].

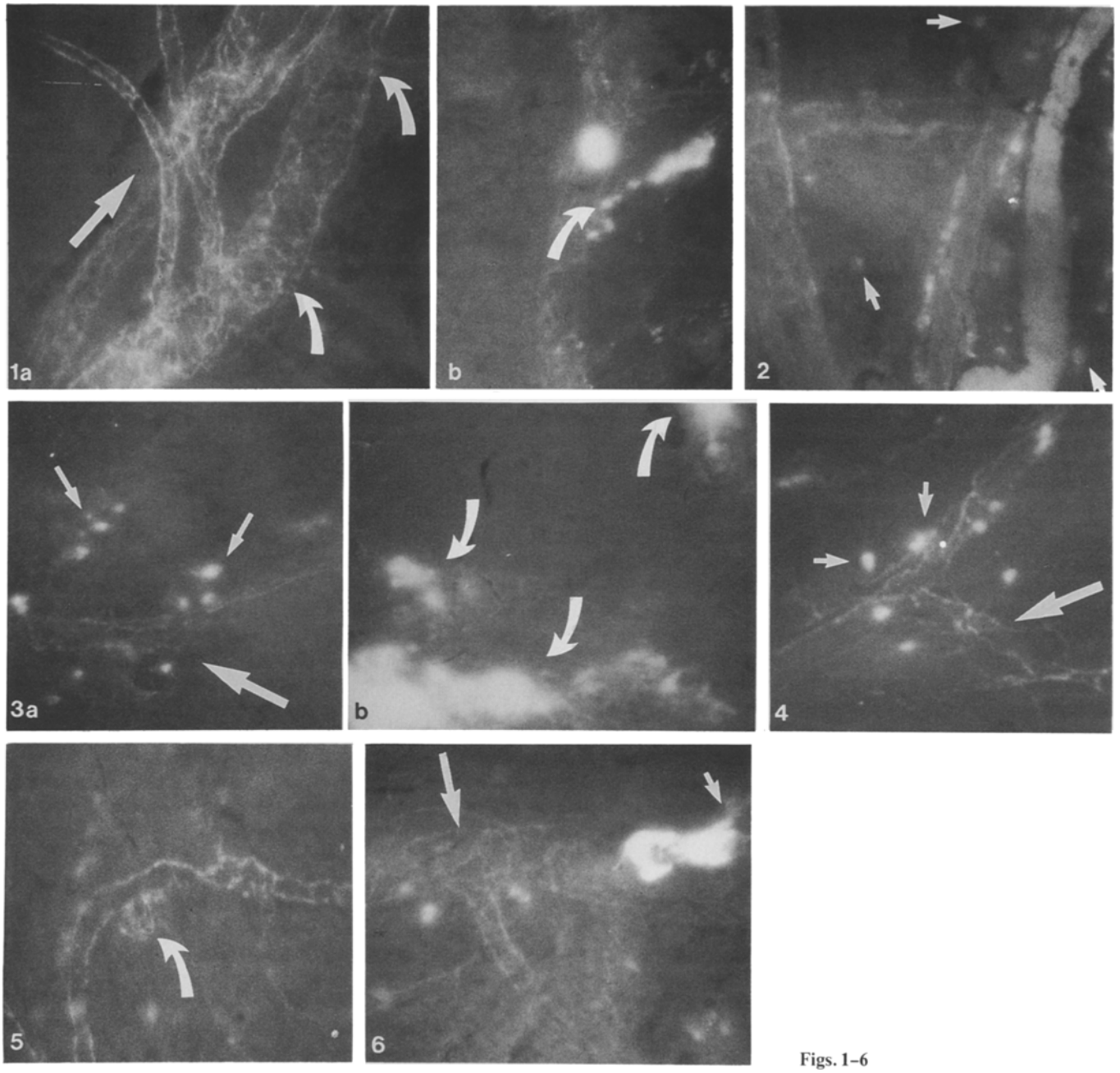
Overdistension of the bladder is caused by urinary retention, but it can also be used as a method for treating an unstable bladder or interstitial cystitis, although a rapid reappearance of the symptoms is often observed after initial success after distension therapy for an unstable bladder [5, 12]. On the other hand, prolonged micturition problems are often encountered after long-term overdistension caused by urinary retention [19]. No research has been carried out to date on the damage that may be caused to the adrenergic innervation of the bladder by overdistension.

The aim of the present work was to investigate the effect of bladder distension on its adrenergic innervation. The glyoxylic acid-induced fluorescence method [20] was used for specific demonstration of the adrenergic nerves.

Materials and methods

Sixty-five 3-month-old female albino rats of the Sprague-Dawley strain weighing 240–260 g were used. They had been bred at 22–24° C, three to four animals per cage. The light cycle was 12 h : 12 h. There was an adequate supply of tap water, and commercially-supplied pellets were available in the cages. The rats were anesthetized with pentobarbitone sodium, 35 mg/kg body weight i. p., and the bladder was catheterized with a 3-F Fogarty (Baxter, Santa Ana, USA) arterial embolectomy catheter (12-080-3F), the balloon being filled with 0.05 ml water and then pulled to the bladder neck. The rats were then given furosemide, 12 mg/kg body weight i. m. and Ringer solution, 12 ml/kg body weight i. p. to induce maximal bladder distension for 3 h and also cefuroxime 30 mg/kg body weight for infection prophylaxis. After distension for 3 h the bladder of each animal was emptied and the rat was roused. Buprenorfine 0.1–0.3 mg/kg body weight was given s. c. if the animals seemed to be in pain. They were then watched carefully to check bladder emptying. Ten rats served as controls without distension. Using the same anesthesia as described previously, full-thickness biopsies were taken from the wall of the bladder dome, anterior body and neck at 10 h, 2 days, 5 days, 7 days, and 21 days after the distension.

The specimens were immersed for 5 min in an ice-cold, freshly made solution of 1.0 g glyoxylic acid monohydrate (Fluka AG, Buchs SG, Switzerland) in 50 ml 0.1 *N* maleate buffer (pH adjusted to 7.0 by adding 1 *N* NaOH), and then stretched in this solution, dried with blotting paper and stretched onto microscope slides, dried in a hot current of air from a hair dryer and subsequently heated in an oven at +100° for 6 min. The pieces were then embedded in Entellan (Merck, Darmstadt, FRG) and examined and photographed with a Leitz Ortholux microscope equipped with fluorescence and transmitted light devices. The primary filter combi-



Figs. 1–6

nation employed for fluorescence consisted of BG 38, BG 3, TAL 405 and UG 1 (Schott & Gen. Mainz, FRG) filters. The secondary filter was of the Leiz K 470 type.

Results

Mortality among the rats during the trial was 15%, deaths occurring during the distension anesthesia and immediately afterwards. The bladders were flaccid and thin-walled until day 7 after distension, and small hemorrhages were found up to day 2. By day 21 no differences from controls were noted in the structure of the bladder wall.

Fluorescence microscopy of the normal bladder

Bluish-green fluorescence from adrenergic nerves with varicosities was seen around arteries and arterioles, representing single nerves or forming a network. Some occasional tiny, probably non-terminal, adrenergic nerves without varicosities were also observed running in small bundles. The adrenergic innervation was dense in the bladder neck and trigone and less dense in the dome. Yellow fluorescent mast cells were found scattered in the bladder wall, usually along the vessels. Some clusters of small, intensively fluorescent (SIF) cells (probably SIF cells of autonomic ganglia) were found in the dome, body and neck of the bladder (Fig. 1).

Fig. 1a, b. Fluorescence photomicrographs of stretch preparations from the normal urinary bladder body demonstrating glyoxylic acid-induced fluorescence of catecholamines. **a** Adrenergic nerves with varicosities in a network around arterioles (*arrows*). ×25 **b** A cluster of small, intensely fluorescent (SIF) cells (*arrow*). ×25

Fig. 2. Fluorescence photomicrograph of a stretch preparation from the bladder body 10 h after distension, demonstrating partial disappearance of glyoxylic acid-induced catecholamine fluorescence. The quantity of mast cells seems to have increased and most of them are degranulating (*arrows*). ×25

Fig. 3a, b. Fluorescence photomicrographs of stretch preparations from the bladder wall 2 days after distension, showing glyoxylic acid-induced fluorescence of catecholamines. **a** The catecholamine fluorescence from the adrenergic nerves has mostly disappeared (*large arrow*). Many degranulating mast cells can be seen (*small arrows*) (trigonum). ×25 **b** SIF cells (*arrows*) have burst open (bladder body). ×25

Fig. 4. Fluorescence photomicrograph of glyoxylic acid-induced fluorescence of catecholamines in a stretch preparation from the bladder trigonum 5 days after distension. A reappearance of some adrenergic nerves can be seen, showing partial re-formation of a network (*large arrow*). The quantity of mast cells seems to have increased further, and some of them are degranulating (*small arrows*). ×25

Fig. 5. Fluorescence photomicrograph of a stretch preparation showing glyoxylic acid-induced fluorescence of catecholamines in the bladder body 7 days after distension. Reappearance of some adrenergic nerves is seen, with a growth cone indicating regeneration (*arrow*). ×25

Fig. 6. Fluorescence photomicrograph of a stretch preparation showing glyoxylic acid-induced fluorescence of catecholamines in the bladder body 21 days after distension. A return to an almost normal appearance of the nerve network around the arteries and arterioles is observed (*large arrow*). The fluorescence of the SIF cells still suggests some leakage of catecholamines (*small arrow*). ×25

Fluorescence microscopy after bladder distension

Ten hours after distension the bluish-green fluorescence in the adrenergic nerves was reduced in places, varying from almost total depletion of catecholamines to partial disappearance, whereas the fluorescence intensity in some non-terminal fibers appeared to have increased. The number of mast cells seemed to be greater, and degranulating mast cells were observed. The SIF cells appeared upon fluorescence microscopy to have exploded, leading to obvious leakage of catecholamines, which is interpreted as an indication of cell injury (Fig. 2).

Two days after distension a major disappearance of the catecholamine fluorescence from the adrenergic nerves was evident, and only occasional single fluorescent nerves were observed. The number of mast cells seemed to have increased further, and these were scattered all over the bladder wall and were often degranulating. The SIF cells appeared torn on fluorescence microscopy (Fig. 3).

Five days after distension a reappearance of some pale catecholamine fluorescence was observed in some adrenergic nerves, and a very mild reticular structure was observed around the arteries and arterioles. The nerves were still devoid of catecholamine fluorescence in places, however. The mast cells again seemed to have increased in number, and some were degranulating. The SIF cells appeared to be leaking (Fig. 4). A similar picture was seen at 7 days, except that growth cones were more often observed in the adrenergic nerves and very mild fluorescence in the non-terminal fibers (Fig. 5).

Twenty-one days after distension the catecholamine fluorescence in the adrenergic nerves was almost normal, showing a reappearance of the nerve network around the arteries and arterioles. Non-terminal adrenergic nerves were also seen. Some degranulating mast cells were still found, but the cells were usually located in a more normal manner along the blood vessels. The catecholamine fluorescence in the SIF cells showed that they were still leaking, as they had been 2, 5 and 7 days after the distension (Fig. 6).

Discussion

Overdistension of the bladder was found in the present study to cause transient damage to its adrenergic innervation and disturbance of longer duration to the SIF cells in the bladder wall.

Specific histochemical demonstration of catecholamines has been possible since the development of the formaldehyde-induced fluorescence method [8, 9], and more recently the use of glyoxylic acid has been found to be informative [14, 21]. The specificity of these methods, in which catecholamines are converted to highly fluorescent quinolines, is well established [3, 11, 14, 20, 21], and the simplicity and reproducibility of the glyoxylic acid fluorescence (GIF) method makes it suitable for use with surgical [13] and experimental surgical specimens [21]. Because of the high sensitivity of the GIF method, the changes in amount and intensity observed in the fluorescent structures can be assumed to reflect true changes in the catecholamine content of the adrenergic nerves to some extent.

It has been suggested previously that the basic reason for bladder instability may be an increase in the density of excitatory alpha-adrenergic receptors in the body of the bladder [18]. The depletion of catecholamines from the adrenergic nerves of the bladder after overdistension, as found here, offers a possible explanation for the clinically well known effect of overdistension in cases of unstable bladder [5], in which the primary success of this therapy may be due to catecholamine depletion. The rapid recovery of the catecholamine fluorescence in the adrenergic nerves of the bladder wall within 3 weeks of distension may also explain the rather rapid reappearance of symptoms often observed after distension therapy [5, 12].

Small, intensively fluorescent cells are found in autonomic ganglia [17] and also in ganglia of the bladder wall [15]. It is suggested that they may function as interneurons or local endocrine cells [17]. An interneuron function has also been suggested for SIF cells in the bladder wall [15], and they obviously play an important role in cross-

connections between the sympathetic and parasympathetic neurons at the ganglionic level [4]. The catecholamine fluorescence observed here in the SIF cells of the bladder wall showed them to have burst rapidly after distension, and some leakage of catecholamines was still evident 21 days later. This leakage from the SIF cells could result in prolonged disturbances in the coordination of nervous functions, and could explain the prolonged micturition problems observed after overdistension caused by postoperative urinary retention [19].

The present results indicate that prolonged distention causes a reversible depletion of catecholamine fluorescence in the adrenergic nerves of the bladder wall. It is suggested that the primary success of distension therapy may be due to this transient nerve damage.

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References

1. Andersson K-E (1986) Clinical relevance of some findings in neuro-anatomy and neurophysiology of the lower urinary tract. Clin Sci 70 [Suppl 14]:21s
2. Andersson K-E, Sjögren C (1982) Aspects of physiology and pharmacology of the bladder and urethra. Prog Neurobiol 19:71
3. Corrodi H, Johnsson G (1967) The formaldehyde fluorescence method for the histochemical demonstration of biogenic monoamines. A review on the methodology. J Histochem Cytochem 15:65
4. de Groat WC, Saum WR (1972) Sympathetic inhibition of the urinary bladder and of pelvic ganglionic transmission in the cat. J Physiol 220:297
5. Dunn M, Smith JC, Ardran GM (1974) Prolonged bladder distension as a treatment of urgency and urge incontinence. Br J Urol 46:645
6. Ek A, Alm P, Andersson K-E, Persson CGA (1977) Adrenergic and cholinergic nerves of the human urethra and urinary bladder. A histochemical study. Acta Physiol Scand 99:345
7. Elbadawi A (1982) Neuromorphologic basis of vesicourethral function: I. Histochemistry, ultrastructure and function of intrinsic nerves of the bladder and urethra. Neurourol Urodyn 1:3
8. Eränkö O (1955) Distribution of fluorescing islets, adrenalin and non-adrenalin in the adrenal medulla of the hamster. Acta Endocrinol 18:174
9. Eränkö O (1967) The practical histochemical demonstration of catecholamines by formaldehyde-induced fluorescence. J R Microsc Soc 87:259
10. Gosling JA, Chilton CP (1984) The anatomy of the bladder, urethra and pelvic floor. In: Mundy AR, Stephenson TP, Wein AJ (eds) Urodynamics. Principles, practice and application. Churchill Livingstone, Edinburgh, p 3
11. Jonsson G (1967) The formaldehyde fluorescence method for histochemical demonstration of biogenic monoamines. Thesis, Stockholm
12. Jörgensen L, Mortensen SO, Colstrup H, Andersen JT (1985) Bladder distension in the management of detrusor instability. Scand J Urol Nephrol 19:101
13. Kyösola K, Waris T, Penttilä O, Ahonen A, Penttilä A, Mattila T, Järvinen A (1985) Multi-score estimation of catecholamine fluorescence for clinical purposes. Acta Histochem 76:65
14. Lindvall O, Björklund A (1974) The glyoxylic acid fluorescence histochemical method: a detailed account of the methodology for the visualization of central catecholamine neurons. Histochemistry 39:97
15. Mundy AR (1984) Clinical physiology of the bladder, urethra and pelvic floor. In: Mundy AR, Stephenson TP, Wein AJ (eds) Urodynamics. Principles, practice and application. Churchill Livingstone, Edinburgh, p 14
16. Norlen L (1976) The autonomous bladder. A clinical and experimental study. Scand J Urol Nephrol Suppl 36:1
17. Papka RE, Traurig HH, Klenn P (1987) Paracervical ganglia of the female rat: histochemistry and immunohistochemistry of neurons, SIF cells, and nerve terminals. Am J Anat 179:243
18. Restorick JM, Mundy AR (1989) The density of cholinergic and alpha and beta adrenergic receptors in the normal and hyperreflexic human detrusor. BR J Urol 63:32
19. Tammela T, Kontturi M, Lukkarinen O (1986) Postoperative urinary retention: II. Micturition problems after the first catheterization. Scand J Urol Nephrol 20:257
20. Waris T, Partanen S (1975) Demonstration of catecholamines in peripheral adrenergic nerves in stretch preparations with fluorescence induced by aqueous solution of glyoxylic acid. Histochemistry 41:369
21. Waris T, Liesi P, Panula P, Partanen S (1982) The resistance of glyoxylic acid induced catecholamine fluorescence to sodium borohydride reduction. Acta Histochem 71:161

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