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Effect of acute distension on cholinergic innervation of the rat urinary bladder

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Summary. The effect of short-term urinary bladder distension on its cholinergic innervation was studied in Sprague-Dawley rats. Distension was induced for 3 h by forced diuresis and balloon outlet obstruction, and whole thick biopsy specimens were taken from the dome and lateral side of the anterior body 2, 7 and 21 days afterwards. The acetylcholinesterase (AChE) method was used to demonstrate the cholinergic nerves in the distended bladder wall. Cholinergic hypoinnervation was observed 7 days after the distension, persisting up to 21 days, although AChE-reactive nerves were then observed to be more numerous. The distribution of hypoinnervation was uneven, being more marked in the lateral side of the anterior body than in the dome. The distribution of AChE-reactive nerves varied even in the same biopsies, with areas of total hypoinnervation occurring next to areas of slightly diminished innervation. This was especially true 21 days after distension. The findings indicate transient damage to the cholinergic innervation, which may in turn explain the prolonged voiding difficulties often seen after catheterization of an overdistended bladder in a patient with urinary retention. The short-lasting effect of bladder dilatation therapy used to treat detrusor instability or interstitial cystitis may be due to the fairly rapid regeneration of cholinergic innervation.

Key words: Urinary bladder – Overdistension – Cholinergic Hypoinnervation – Acetylcholinesterase – Rat

The contractile force of the urinary bladder is mostly controlled by its cholinergic innervation [1]. A rich uniform acetylcholinesterase (AChE)-positive nerve plexus can be seen in the bladder wall extending between the smooth cells and the AChE-positive nerve fibres in each muscle bundle throughout all regions of the bladder [12, 17]. Fibres with AChE also appear in the submucosa, the adventitia and the outer layers of most blood vessels. AChE fibres are slightly more numerous in the base of the bladder than in the body [17]. The cholinergic and adrenergic nerves run in close contact in the bladder wall [3, 17], probably modulating each other's functions [8].

The term cholinergic hypoinnervation is used to describe an obvious decrease in the density of histochemically demonstrable nerves. Cholinergic hypoinnervation has been seen in neurogenic bladders [4], obstructed bladders [6] and hyper-reflexic bladders [20]. We have recently developed a model for achieving maximal bladder distension for 3 h combined with balloon obstruction and have used it to observe transient depletion of adrenergic innervation and a more long-lasting injury to the small intensively fluorescent (SIF) cells [22].

The aim of the present work was to investigate the effect of acute distension of the female rat urinary bladder on its chlinergic innervation. AChE activity [11, 19] was used as an indicator of cholinergic innervation.

Materials and methods

Fifty-nine 3-month-old female albino rats of the Sprague-Dawley strain weighing 240–290 g were used. They had been bred at 22–24°C in cages of three to four animals each with a light cycle of 12 h light/12 h dark. There was an adequate supply of tap water and commercially produced pellets available in the cages.

The animals were anaesthetized with pentobarbitone sodium, 35 mg/kg body weight, injected intraperitoneally. Outflow occlusion was achieved using a Fogarty (Baxter, Santa Ana, USA) embolectomy catheter (12-080-3F), the balloon filled with 0.05 ml water and then pulled into the bladder neck. Distension was then induced with an intramuscular injection of furosemide, 12 mg/kg body weight, and an intraperitoneal injection of 4 ml Ringer solution. A large, very tense bladder could easily be detected by palpation approximately 30 min after the catheterization and injections. This overdistension was maintained for 3 h, after which the bladder was emptied and the rat was allowed to recover. The rats were also given cefuroxime, 30 mg/kg body weight, as prophylaxis against infection. Buprenorphine was given intracutaneously, 0.1–0.3 mg/kg body weight, if the animal seemed to have pain after overdistension. The rats were monitored carefully to check bladder emptying.

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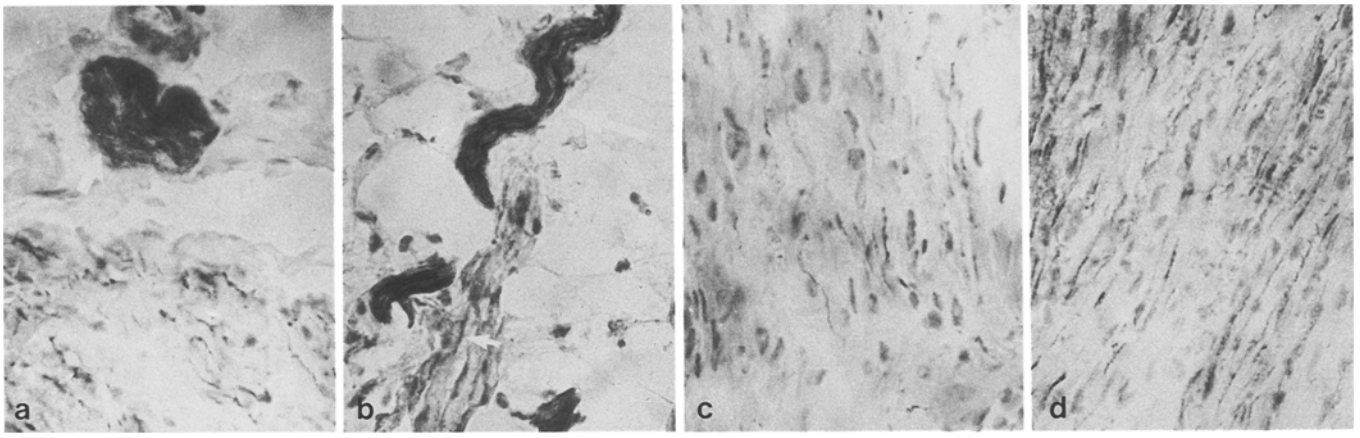

Fig. 1 a–d. Cholinergic innervation of the bladder dome. *a* Control dome. Cholinergic plexus *(small arrow)* and cholinergic axon bundles *(large arrow). b* Two days after distension. The bladder is very fragile. No difference in the density of cholinergic innervation can be seen between the control dome and this experimental specimen. Cholinergic plexus *(small arrow)* and cholinergic axon bundles *(large arrow) c* Seven days after distension. Marked hypoinnervation. *d* Twenty-one days after distension. Cholinergic innervation is denser than after 7 days. ×400

The animals were sacrificed 2 days (9 animals), 7 days (10 animals) and 21 days (10 animals) after overdistension. A further 10 animals matched for age and weight with those subjected to bladder distension were used as controls. Whole thick biopsy specimens(including all layers of the bladder) were taken from the wall of the dome and the left lateral side of the anterior body by a microscopic technique. These were rinsed in 0.9% NaCl solution, frozen in liquid nitrogen and stored at −70°C.

The frozen samples were cut perpendicularly to the bladder surface into cryostat sections 10 μm in thickness, which were then dried for 1 h at room temperature. AChE activity was demonstrated by the method of Karnovsky and Roots [11] modified by Meier-Ruge et al. [19]. The pH of the incubation solution was adjusted to 6.0 before incubation.

The samples were incubated for 30 min at 37°C. Inhibition of tissue pseudo-cholinesterase was achieved using 4 mM iso-OMPA in the incubation medium. The specificity of the enzymatic staining was tested by omitting the substrate from the incubation medium. Haematoxylin was used as a counterstain. After washing in distilled water, the samples were dehydrated over alcohol and xylene and covered with Eukitt (Oriola, Helsinki, Finland).

Multiple randomly chosen microscopic fields in the biopsy specimens from the dome and anterior body at different intervals after distension (2 days, 7 days and 21 days) were examined by two independent viewers and photographed at the same magnification. Coded biopsies were used to study whether there were any differences between the control and distended bladders. Changes were assessed by comparing the number of nerves per unit area in the microscopic fields with photographs on matching sites of the bladder (control dome versus distended dome, lateral side of anterior body in control bladder versus lateral side of anterior body in distended bladder) taken at the same magnification. A Leitz light microscope was used. Agfapan APX 25 film was chosen for the black-and-white pictures. The original magnification was ×400.

Results

Mortality during the trial was 5%, occurring mostly at the end of anaesthesia or immediately after distension. None of the distended bladders ruptured during the trial.

The distended bladders were more flaccid in appearance than the controls, and small haemorrhages were observed 2 days after distension.

Distribution of AChE-reactive innervation in control specimens

A rich, uniform AChE-positive nerve plexus was observed extending between the smooth muscle cells, and single AChE-positive terminals were seen in the vicinity of some

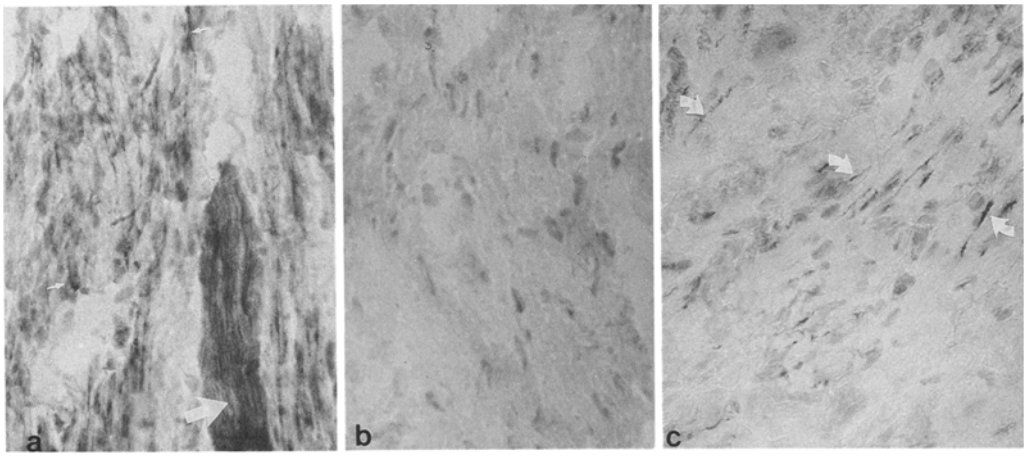

Fig. 2 a–c. Cholinergic innervation of the bladder anterior body. *a* Control anterior body. Cholinergic plexus *(small arrow)* and cholinergic axon bundles *(large arrow). b* Seven days after distension. Cholinergic nerve fibres cannot be seen. *c* Twenty-one days after distension. Some cholinergic fibres are reappearing *(large arrows).* ×400

blood vessels and in the mucosa without any obvious structural relationship (Figs. 1A, 2A). Easily recognizable AChE-positive axon bundles were found amongst the muscle cells. (Fig. 1A).

Distribution of cholinergic innervation in the distended urinary bladders

The urinary bladder specimens appeared to be fragile and the sections consisted of almost torn pieces representing different elements of the urinary bladder tissue. AChE staining in the axon bundles and the plexus between the muscle cells 2 days after distension was unchanged compared with the controls (Fig. 1B).

The AChE-reactive nerve plexus between the muscle cells was markedly reduced and unevenly distributed 7 days after overdistension. This hypoinnervation appeared to be more marked in the anterior body (Fig. 2B). AChE activity was still reduced 21 days after distension (Figs. 1D, 2C) and the distribution of the cholinergic plexus between the muscle cells was uneven. Areas of strong hypoinnervation and areas of almost normal innervation were observed even in the same biopsy specimen. The anterior body appeared to be more severely damaged than the dome, but the difference in AChE activity between the dome and the lateral anterior body had diminished slightly (Figs. 1D, 2C).

Discussion

Acute overdistension of the rat detrusor was found to cause cholinergic hypoinnervation. There appeared to be a difference in the distribution of cholinergic nerves between the dome (Fig. 1C) and the anterior body (Fig. 2B), the dome recovering from distension more rapidly.

The AChE method of Karnovsky and Roots [11, 19] is one of the thiocholine methods that have been shown to be reliable for demonstrating cholinesterases histochemically in the presence of a specific inhibitor [13]. The histochemical method for the visualization of AChE-positive nerves probably gives an accurate reflection of cholinergic innervation in the bladder [4]. The AChE reaction has been found previously to be reliable for the demonstration of denervation-induced changes [9]. The present specimens from distended bladders could be distinguished from the control bladder specimens with a high degree of accuracy in double-blind microscopic analysis, the predominant structural finding in them being a greatly reduced density and total number of AChE-positive nerves.

The mechanism of cholinergic nerve degeneration after bladder distension still remains speculative. There is probably some direct mechanical injury due to overstretching of the axons, and this may partly explain the slowness of cholinergic re-innervation; there may also be nerve injury due to inadequate blood supply to the bladder wall and its nerves [2, 14].

It has been shown that intact efferent sympathetic pathways are essential for eventual cholinergic re-innervation in cholinergicaly denervated bladders [9]. We have previously observed damage to adrenergic innervation and SIF cells after distension, which caused a transient degeneration of the adrenergic nerves [22]. Anatomically, it has been shown that interactions occur between adrenergic and cholinergic systems at the terminal and preterminal levels [16]. Cholinergic transmission in ganglia is thought to be modulated by adrenergic terminals, which are derived from SIF cells. The fact that the cholinergic innervation remains somewhat degenerated, even though almost normal adrenergic innervation can accompany damaged SIF cells, leads us to suggest that not only an adequate blood supply but also a fully intact adrenergic innervation is important for evenly distributed cholinergic innervation. This is also supported by the observation that adrenergic hypoinnervation was found 2 days after the same kind of distensions [22] but cholinergic hypoinnervation only at 7 days.

Harrison et al. [10] found that partial outflow obstruction caused damage to the cholinergic innervation in the rabbit urinary bladder. Detrusor muscle strips from obstructed bladders showed both a reduction in sensitivity to nerve-mediated stimulation and a reduction in the maximum force of contraction that resulted from such stimulation. This finding was combined with a striking degree of supersensitivity to acetylcholine. Levin et al. [15] found that short-term obstruction of the rabbit urinary bladder resulted in a transient reduction in contractile response and that the ability of the bladder to empty remained partly impaired. Ultrastructural studies of the effects of short-term partial bladder outlet obstruction on the rabbit detrusor have shown partially reversible neuromuscular changes in the detrusor [6]. All these results indicate at least transient damage to the urinary bladder innervation after obstruction, with which our findings agree.

Gosling et al. [7], studying the effect of bladder distension, found that the arrangement and distribution of the cholinergic nerves were unaffected by the distension. Their different results may partly be explained by the different experimental animal and differences in the experimental procedures.

Our experimental model simulates the clinical situation of urinary retention, where degeneration of the cholinergic nerves may partly explain the prolonged micturition problems often experienced after catheterization of the retention [21]. Our results can also explain why bladder dilatation therapy, used to treat bladder instability or interstitial cystitis with the aim of reducing neural activity, has only a transient effect because of the ability of the innervation to regenerate.

In conclusion, acute distension causes cholinergic hypoinnervation, which may partly be attributable to the direct axonal injury and partly to the ischaemic damage. The slowness of cholinergic re-innervation by comparison with adrenergic re-innervation may indicate the importance of an intact sympathetic pathway for re-innervation. The acute distension results in cholinergic hypoinnervation, which could lead to bladder dysfunction.

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