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The influence of pelvic nerve transection on the neuromuscular system of the canine urinary bladder

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Summary. We previously reported that bladder overdistension led to denervation and subsequent supersensitivity of the detrusor muscle to acetylcholine. Therefore, to exclude the influence of bladder overdistension, we produced a low-pressure bladder in female mongrel dogs using an indwelling urethral catheter, and performed pelvic neurectomy (decentralization). We examined the effects of decentralization on the neuromuscular system of the bladder. The contraction response levels of bladder strips in dogs 1 and 2 weeks after neurectomy was low, and significantly different from that of bladder strips in the control group. The dose-response curves of dogs 4 and 8 weeks after neurectomy showed a shift to the left when compared to those of the control group, indicating a significant increase in sensitivity of the bladder strips. Many cholinergic terminal and varicosity profiles had a normal ultrastructure in all of the groups subjected to neurectomy, while some had degenerating profiles representing clear axoplasm without any recognizable organelles. Microphotographs of bladders obtained from dogs with spontaneous catheter loss showed degenerating axons, which were observed more frequently than in bladders kept empty with indwelling urethral catheters. Micturition in the dogs with spontaneous catheter loss was achieved by overflow incontinence without catheterization. These findings suggest that post-synaptic nerve degeneration may be augmented by impairment of micturition, followed by decentralization. Our observations also suggest that post-synaptic nerve degeneration (denervation) plays an important role in the increased sensitivity of the detrusor muscle to acetylcholine in the parasympathetically decentralized urinary bladder, whether denervation is due to trans-synaptic degeneration or impairment of micturition.

Key words: Decentralized bladder – Low-pressure bladder – Trans-synaptic degeneration – Denervation supersensitivity

The preganglionic axons of sacral parasympathetics leave as part of the ventral roots of the sacral segments. These fibres join more distally to the pelvic nerve, which is located on the lateral wall of the distal portion of the rectum. Hypogastric and pelvic nerves on either side meet and branch to form the pelvic plexus, which lies on the lateral wall of the rectum, internal genital organs and bladder. Pelvic nerves receive preganglionic input from cholinergic axons. In the parasympathetic system, ganglia are generally located in or near innervated organs. Small autonomic ganglia are present throughout all regions of the bladder wall. Therefore, in radical pelvic surgery or in experimental neurectomy in animal models, we have performed parasympathetic decentralization of the bladder. We previously examined the effects of overdistension on the neuromuscular system of canine urinary bladder as a model of infravesical obstruction in terms of histological changes and pharmacological responses [10]. Bladder overdistension caused by lower urinary tract obstruction may lead to nerve degeneration (denervation) and subsequent supersensitivity of the detrusor muscle to acetylcholine through a decrease in the blood supply to the bladder [11]. Therefore, to exclude the influence of bladder overdistension on detrusor muscle, we experimentally produced a low-pressure bladder in female mongrel dogs using indwelling urethral catheters, and performed pelvic neurectomy (decentralization). We examined the effects of decentralization on the neuromuscular system of the bladder with respect to histological structure and pharmacological reactions.

Materials and methods

Adult female mongrel dogs (10–12 kg) were used. The following surgical procedures were performed under general anaesthesia induced by sodium thiopental injection. The bladder, uterus and rectum were exposed through a midline abdominal incision. The pelvic nerve trunk was found to expand into a plexus which also received the hypogastric nerves. The pelvic nerve trunk was cut bilaterally at both its origin and point of branching. Silk ligations were applied on both nerve endings to prevent nerve regeneration.

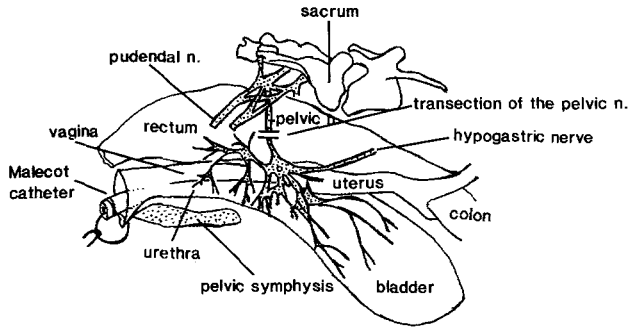

Fig. 1. Diagram of nerve supply to the pelvic viscera. The pelvic nerve trunk was cut bilaterally. An indwelling urethral catheter was placed in the bladder and cut into short lengths. The cut end was anchored to the external urethral meatus

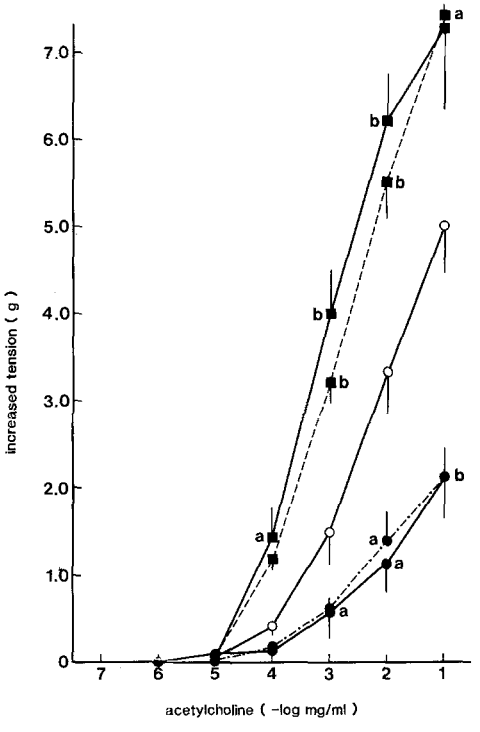

Fig. 2. Dose-response curve of bladder strips with acetylcholine following the bilateral transection of the pelvic nerve. Values represent means ± SE. ○—○, Control ($n=9$); ●—·—·—●, 1 week after transection ($n=5$); ●—●, 2 weeks after transection ($n=5$); ■— — —■, 4 weeks after transection; ($n=5$); ■—■ 8 weeks after transection ($n=5$). Probability: a <0.05, compared to control group; b <0.01 compared to control group

To keep the pressure in the bladder low, an indwelling urethral catheter (Malecot catheter, BARD, Murray Hill, USA) was placed in the bladder. The catheter was cut into short lengths, and the cut end was anchored by a silk suture to the external urethral meatus, preventing the catheter from withdrawing post-operatively (Fig. 1). In dogs subjected to decentralization, the bladders were continuously emptied by this method. The dogs were sacrificed 1, 2, 4 and 8 weeks after decentralization, and the bladders were exposed and excised through a midline abdominal incision.

The sensitivity of isolated bladder strips to acetylcholine was determined using an isometric in vitro technique. Bladder strips, 10×3 mm im size, were obtained from the anterior wall of the dome of the bladder. Freshly dissected bladder strips were placed in an isolated 50 ml organ bath containing Tyrode's solution. The solution was maintained at 37°C and aerated with 95% $O_2^{\ddagger}$ 5% CO_2. Contractions of the bladder strips were recorded isometrically with a force-displacement transducer connected to a thermal-array recorder (Nihon-Koden Kogyo, Tokyo, Japan). Approximately 0.5 g of resting tension was applied to the strips, and 1 h was allowed for equilibration before the strips were exposed to acetylcholine. Acetylcholine was applied for 1 min and washed out thoroughly. The strips were washed two to three times with fresh medium before exposure to the next dose of acetylcholine. For the construction of the dose-response curve, the peak tension development after each dose was regarded as the response.

The bladder specimens obtained from the anterior wall of the dome were studied using an electron microscope. The specimens were minced for immersion fixation in 2.5% glutaraldehyde for 1 h. Post-fixation was completed in 2% osmium tetroxide in cacodylate sodium buffer at pH 7.3 for 1 h. After fixation, the tissue pieces were dehydrated in ascending concentrations of ethyl alcohol and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate. A Philips EM-300 electron microscope was used to examine the sections.

Nine control dogs underwent sham operations consisting of abdominal incision without decentralization, with indwelling urethral catheters placed in the bladders. The dogs were sacrificed 1 week ($n=3$), 2 weeks ($n=3$) and 4 weeks ($n=3$) after the sham operation, and the bladders were examined as described above.

Statistical analysis was performed using Student's t-test for unpaired samples. Data were considered significant at the $P < 0.05$ level.

Results

The responses to acetylcholine of the bladder strips obtained from the three control groups, 1, 2 and 4 weeks respectively after sham operation, did not show significant differences. The dose-response curves of the three control groups were therefore displayed together. Figure 2 shows the increased tension of the bladder strips following bilateral transection of the pelvic nerve (pelvic neurectomy) as a function of increasing acetylcholine dosage, as compared to the control group. The contraction response levels of the bladder strips in the dogs 1 and 2 weeks after neurectomy were low, and significantly different from those of bladder strips in the control group at acetylcholine concentrations $> 10^{-3}$ mg/ml. It was noted, however, that the response level was significantly elevated 4 weeks after pelvic neurectomy, subsequently rising to a higher level 8 weeks after pelvic neurectomy. The dose-response curves of these groups showed a shift to the left when compared to those of the control group, indicating a significant increase in sensitivity of the bladder strips 4 and 8 weeks after pelvic neurectomy.

Electron micrographs of a cross-section of preterminal axon bundles within the bladder wall of the control group after sham operation showed a normal appearance of unmyelinated nerve fibres enclosed by Schwann cell processes, containing neurotubules, neurofilaments, and small clear and large granular vesicles. Cholinergic axon terminals, packed with small agranular vesicles and some mitochondria, showed no degenerating findings in the control group. Although many cholinergic terminal and varicosity profiles showed a normal ultrastructure in all of

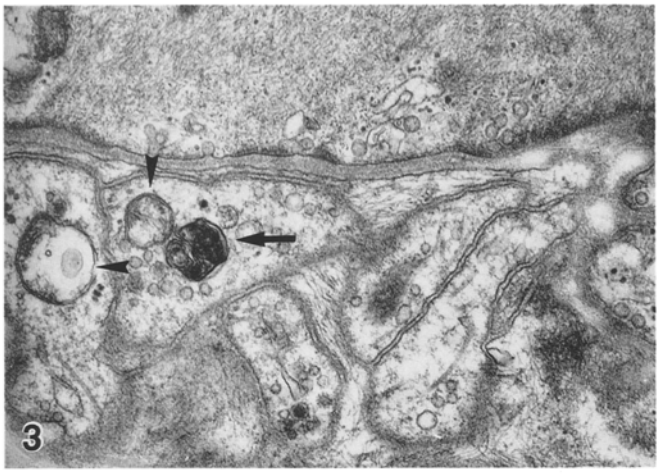

Fig. 3. Electron micrograph of a cross-section of nerve bundles within the bladder wall 1 week after bilateral transection of the pelvic nerve. Note the normal-appearing axons enclosed by Schwann cell processes. The matrix of some intra-axonal mitochondria showed an increase in electron density *(arrow)*, and swelling with disruption of others *(arrowheads)*. ×15,000

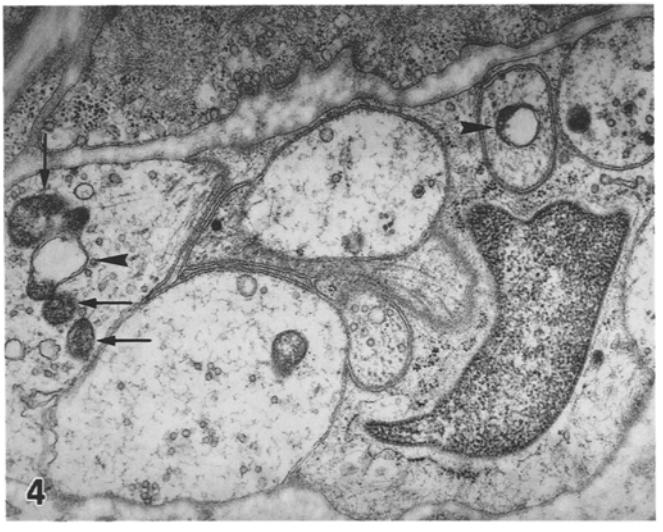

Fig. 4. A cross-section of nerve bundles in the control group. Note the normal-appearing axons with a slight increase in matrix density of some mitochondria *(arrows)* and disruption of others *(arrowheads)*. ×12,000

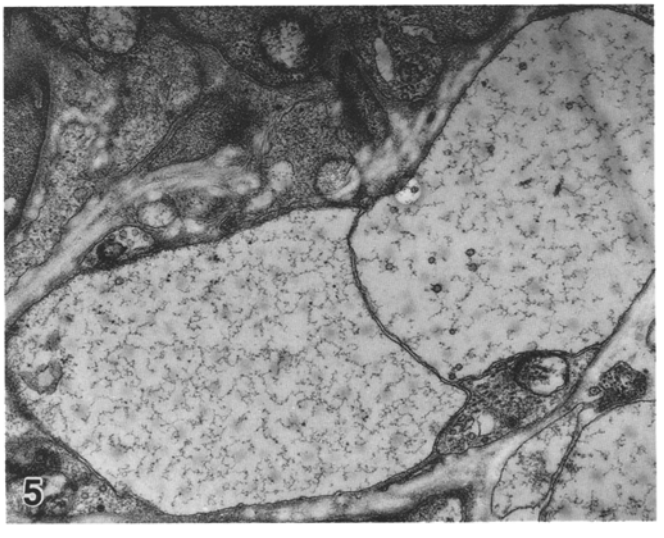

Fig. 5. Two weeks after bilateral transection of the pelvic nerve; a case of catheter loss. Note the degenerating axons with a clear axoplasm without any recognizable organelles. ×12,000

the groups subjected to neurectomy, some had flocculent osmiophilic bodies of high electron density representing degenerating axon profiles within the cytoplasm of the Schwann cell. Others had degenerating profiles representing clear axoplasm without any recognizable organelles, enclosed together with axons of normal appearance by the Schwann cell processes. Many terminal and varicosity profiles had a normal appearance, while some had minimal changes such as increased matrix density of some mitochondria, swelling with disruption of others, appearance of lysosomal dense bodies, and partial depletion of small clear vesicles in the axoplasm (Fig. 3). In this study these changes could be seen in dogs 1 or 2 weeks after neurectomy. However, since some changes like early degeneration within the bladder wall of the control group were found (Fig. 4), the possibility that these minimal changes might be artefacts resulting from the preparation of bladder specimens for electron microscopic examination could not be excluded.

Dogs with spontaneous catheter loss were excluded from this study. All these dogs developed post-operative retention of urine, which intermittently leaked from the urethral meatus. Micturition was achieved by overflow incontinence without catheterization. Electron microscopic examination of the bladders was performed in these dogs. The microphotographs of bladders obtained from these dogs showed degenerating axons having a clear axoplasm without any recognizable organelles, which were observed more frequently than in the bladders kept empty with indwelling urethral catheters (Fig. 5).

Discussion

Morphological studies of the decentralized feline bladder following unilateral or bilateral sacral ventral rhizotomy have been carried out ultrastructurally by Elbadawi and associates [1, 3]. They presented the first example of transsynaptic degeneration in an autonomically innervated mammalian smooth muscle system, using a unilateral decentralization model which did not result in any disturbance of micturition, vesical distension, or hypertrophy of the vesicourethral muscularis [1]. In this examination of the decentralized canine bladder many axon terminal and varicosity profiles had an almost normal appearance, while some showed minimal changes such as deformity or increased matrix density of mitochondria, and partial depletion of small clear vesicles. These changes are recognized to be early degenerating findings in cholinergic axons [1]. However, since some changes like early degeneration within the bladder wall

were also found in the control group, it was thought that these minimal changes might be artefacts resulting from the preparation of bladder specimens for electron microscopic examination. Degenerating axons of the bladder walls obtained from dogs with catheter loss were observed more frequently than in the bladders kept empty with indwelling urethral catheters. These findings suggest that post-synaptic nerve degeneration (denervation) may be augmented by impairment of micturition, followed by decentralization. In experimental animal models of bilateral decentralization, overdistended and high-pressure bladders appear post-operatively and should be emptied manually. This procedure results in an increase in the wall tension of the bladder and may lead to denervation.

In previous papers, we reported that overdistension and increased wall tension of the bladder caused by infravesical obstruction resulted in nerve degeneration with subsequent supersensitivity of the detrusor muscle to acetylcholine in dogs and human subjects, which was thought to be denervation supersensitivity [10–12]. Speakman and associates [7] also reached the conclusion that denervation supersensitivity occurred as a consequence of obstruction, using the pig as an animal model.

Concerning the effects of denervation and decentralization on the response of the smooth muscle of the guinea-pig vas deferens, Westfall and associates [8] found that denervation increased the maximal contractile response to norepinephrine, acetylcholine and histamine, whereas decentralization, which induced post-synaptic supersensitivity, did not. Mattiasson and associates [6] demonstrated that detrusor hypertrophy and an increased sensitivity to carbachol were found in cats subjected to parasympathetic decentralization, while no hypertrophy developed and no change in the EC_{50} value for carbachol was found if urinary diversion preceded the parasympathetic decentralization. Using bilaterally decentralized feline bladders, Malkowicz and associates [5] reported that non-hypertrophied bladder tissue did not exhibit supersensitivity to bethanechol provided that manual bladder decompression was performed twice daily. In the present investigation an indwelling urethral catheter was applied to exclude the denervation induced by overdistension of the bladder. The dose-response curves of the groups 4 and 8 weeks after decentralization showed a shift to the left and an increase in the maximal contractile response to acetylcholine, indicating the possibility of trans-synaptic nerve degeneration (denervation) caused by decentralization. However, during operation there is a risk of post-synaptic nerve impairment and/or blood vessel injury, which may induce a shift in the dose-response curve. It was found that a decrease in blood supply to the bladder might lead to post-synaptic nerve degeneration and subsequent supersensitivity of the detrusor muscle to acetylcholine [9]. Mattiasson and associates [6] suggested that bladder hypertrophy secondary to neurogenic lesions induced the increased sensitivity to carbachol in the parasympathetically decentralized urinary bladder, while bladder hypertrophy was a major but perhaps not the only factor associated with the increased response to carbachol. However, there is evidence that increased maximal response of smooth muscle is the result of enhanced synchronization of contraction by virtue of improved smooth muscle cell coupling in denervated tissue [2, 4]. These observations suggest that post-synaptic nerve degeneration (denervation) other than bladder hypertrophy plays an important role in the increased sensitivity of detrusor muscle to acetylcholine in the parasympathetically decentralized urinary bladder, whether denervation is due to trans-synaptic degeneration or impairment of micturition.

Finally, the results of this study suggest that high pressure adversely affects the bladder during the management of patients with neurogenic detrusor disfunction.

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