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Reinnervation of the Urinary Bladder after Microsurgical Reconstruction of Transsected Caudal Fibres

An Experimental Study in Pigs

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Summary. Reinnervation of the urinary bladder after transsection of cauda equina fibres and their microsurgical reconstruction was studied. In five pigs a partial reconstruction with end-to-end suture of the transsected roots was performed inside the dura mater spinalis; four of these, which had a denervated bladder after the transsection, reestablished the micturition reflex 6 months after the reconstructive operation. The results demonstrate that the reestablished micturition reflex was due to a functional regeneration of transsected and sutured motor fibres. Although a regeneration was proved in sensory as well as in motor neurons by morphologic methods, it is doubtful whether the regenerated sensory fibres took part in the reestablishment of the micturition reflex after contact with the spinal cord.

Key words: Cauda equina, Bladder innervation, Reconstruction, Electrical stimulation, Cystometry.

Introduction

Micturition is a spinal reflex with a centre in the parasympathetic components in the segments S_2–S_4, the afferent and efferent pathways of which run through the dorsal and ventral roots of the pelvic nerve. This spinal micturition reflex is controlled by higher centres in the medulla, basal ganglia and the cortex. Central afferent and efferent pathways of the sacral reflex are in or adjacent to the cortico-spinal tract, (funiculus lateralis and posterior).

The spinal micturition reflex remains intact after an injury to the cord above the caudal level. Voluntary control, however, is impossible. This condition results in the spinal reflex bladder, which empties suddenly and reflexly and at small volumes. In spinal cord injuries at the level of the centre of the bladder or in injuries to the peripheral afferent or efferent pathways the spinal reflex arc is discontinued. The atonic bladder empties incompletely only after uncoordinated contractions originating in the intramural ganglia at extremely high volumes (autonomic bladder) [2, 12, 13].

Reconstructive surgery is impossible when the spinal bladder centre is destroyed. In acute traumatic or neoplastic lesions of the cauda equina, however, there is, theoretically, a possibility to achieve satisfactory bladder function after surgical repair and functional recovery of the cauda equina fibres. Our group studied the possibility of spinal reinnervation in a preliminary investigation. It could be demonstrated morphologically that fibres of the cauda equina had the capacity to regenerate [9–11, 14]. Electron microscopic studies on the regeneration of nerve roots after intradural transsection and subsequent end-to-end suture demonstrated a good regenerative capacity in the motor fibres; results in the sensory fibres, however, were much less encouraging quantitatively, as well as qualitatively. It could not be demonstrated that the regenerating axons in the dorsal roots, after growing into the spinal cord, reestablished the spinal reflex pathways functionally. Regarding the minimal plasticity of the CNS such a recovery is improbable [3]. The objective of this study was to demonstrate the functional reinnervation of the bladder by means of the micturition reflex.

Materials and Methods

The investigation was carried out in three steps. In a preliminary study we determined the sacral segmental innervation of the porcine bladder. For this we prepared the sacral intradural roots surgically in three pigs under general endotracheal anaesthesia. After identification of the spinal nerves supplying the bladder from S_2 to S_4 the following denervation patterns via selective root transsections were established: 1) complete disruption of the sacral afferent component through bilateral transsection of the dorsal roots at S_2 to S_4; 2) complete disruption of the sacral efferent pathway through bilateral transsection of the ventral roots at S_2 to S_4; 3) sacral denervation of the bladder through transsection of the dorsal and ventral roots bilaterally at S_2 to S_4; 4) one-sided sacral denervation of the bladder

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Table 1. Types of microsurgical procedures

Type of lesion	Animal	Procedure	
		Roots transsected	Roots sutured
Intact control	1	–	–
	2	–	–
Sacral de-efferentiation	3	S_2, S_3, S_4 (m, bil.)	–
	4	S_2, S_3, S_4 (m, bil.)	S_2, S_3, S_4 (m, bil.)
Sacral de-afferentiation	5	S_2, S_3, S_4 (s, bil.)	–
Complete denervation	6	S_2, S_3, S_4 (m, s, bil.)	–
	7	S_2, S_3, S_4 (m, s, bil.)	S_2, S_3 (m, s, bil.)
	8	S_2, S_3, S_4 (m, s, bil.)	S_2, S_3 (m, s, ri)
	9	S_2, S_3, S_4 (m, s, bil.)	S_2 (m, s, bil.)
	10	S_2, S_3, S_4 (m, s, bil.)	S_2 (m, s, ri)
Unilateral denervation	11	S_2, S_3, S_4 (m, s, bil.)	S_2, S_3, S_4 (ri)

m = motor root, *s* = sensory root, *bil* = bilateral, *ri* = right side

through unilateral transsection of the dorsal and ventral roots at S_2 to S_4.

After recovery from the spinal shock, 3 weeks postoperatively, we determined the cystometric effects of the different types of lesion (details of the method will be dealt with in another paper).

The actual reinnervation study was carried out in six young pigs, weighing 30–40 kg. The animals underwent a laminectomy under general endotracheal anaesthesia. Transdural access to the sacral roots at S_2 to S_4 was established. Transsections were made in different patterns, as described above and the transsected roots were united again with an end-to-end suture immediately after transsection. (See Table 1 for details of the experimental procedures.) The bladders of the experimental animals were emptied by manual pressure by a caretaker twice daily during the time of atony. Regulations for the conduct of animal experiments established by the Dept. of Vet. Med. were observed. At the end of an observation period of up to 28 weeks postoperatively the animals underwent cystometry and were killed by exsanguination; two animals were used as intact controls. The sacral nerve roots were fixed in Karnowski's solution in situ and then prepared for light and electron microscopy.

Results

a) Results of Electrical Stimulation (Table 1)

In three experimental animals the sacral roots were stimulated bilaterally with an electrical stimulator under cystometric control with a bladder volume of approximately 300 cc. In all animals cystometry showed the highest increase in intravesical pressure during stimulation of the roots at S_2. The detrusor muscle showed less activity during stimulation of the roots S_{3-4} in all animals. Stimulation of the roots at L_6 and S_1 showed no increase in intravesical pressure.

b) Results of Transsections (Tables 2, 3 and 4)

In animals 3 and 4 the sacral motor roots were transsected (sacral de-efferentiation). Three weeks postoperatively both animals presented with an atonic bladder; the micturition reflex could not be elicited either through retrograde filling or through cold stimulation of the bladder mucosa.

In animal 5 the sensory pathways at S_{2-4} were disconnected (sacral de-afferentiation). After 3 weeks the micturition reflex could be elicited both through retrograde filling and instillation of small amounts of cold water, as demonstrated on cystometry.

In five animals a complete sacral denervation of the bladder was carried out (animals 6, 7, 8, 9, 10). All animals showed a complete abolition of the micturition reflex and an atonic bladder 3 weeks after transsection of the motor and sensory fibres at S_{2-4}.

Animal 11 was denervated only by unilateral transsection of the S_{2-4} roots. After 3 weeks a micturition reflex could be demonstrated on cystometry as in healthy animals.

c) Results of the Reinnervation Studies

In animal 4 cystometric control 3 weeks postoperatively showed an atonic bladder; 16 weeks after the suture of the transsected roots, however, a micturition reflex could again be demonstrated.

As a comparison, in animals 3 and 7 the sacral roots were not sutured after transsection; in these animals no micturition reflex could be demonstrated on cystometry after 12 and 17 weeks, respectively.

In animal 7 after a complete denervation through dissection of motor and sensory roots at S_{2-4} bilaterally, the sensory and motor components at S_{2-3} were sutured (exeption S_4). After a period of 14 weeks with an atonic bladder a micturition reflex was detected again on cystometry.

In animal 8 after complete denervation S_{2-3} roots were only sutured on the right side. The micturition reflex appeared again after 28 weeks; bladder capacity, however, remained increased in this case of unilateral innervation.

Table 2. Results of electrical stimulation of sacral roots

Stimulation of	Interversical pressure
S_1	–
S_2	3 + increase
S_3	1 + increase
S_4	1 + increase

Table 3. Transsections

Type of lesion	Animal	Micturition reflex after 3 weeks
Sacral de-efferentiation	3	neg.
	4	neg.
Sacral de-afferentiation	5	pos.
Complete denervation	6	neg.
	7	neg.
	8	neg.
	9	neg.
	10	neg.
	11	neg.

Table 4. Results of the last postoperative cystometric measurements

Animal	Week post. op.	Capacity (ml)	Micturition reflex	
			Upon filling	Upon cold stimulation
1	–	700	pos.	pos.
2	–	800	pos.	pos.
3	12	< 2,000	neg.	neg.
4	16	900	pos.	pos.
5	9	900	pos.	pos.
6	17	> 2,000	neg.	neg.
7	14	850	pos.	pos.
8	28	1,400	pos.	pos.
9	20	800	pos.	pos.
10	17	> 2,000	neg.	neg.
11	24	1,250	pos.	pos.

In animal 9 after complete sacral denervation only the roots S_2 were resutured. After a period of 20 weeks with atonic bladder the micturition reflex reappeared again on cystometry and the bladder capacity was not increased.

In animal 10 only the right-sided root at S_2 was resutured after a complete denervation. No micturition reflex could be elicited on cystometry after 17 weeks.

In animal 11 after unilateral denervation at S_{2-4} these three roots were sutured again. The left side was left intact. Micturition reflex reappeared after only 3 weeks, therefore the functional recovery of the sutured fibres could not be evaluated.

d) Results of the Morphologic Studies

It could be demonstrated in all sutured roots that the axons grew through the site of the sutures. Microscopic studies of the suture sites indicated that after equal lengths of time regeneration in the motor roots was superior to that in the sensory fibres.

Discussion

The porcine urinary bladder receives its nerve supply from the sacral segments of S_2 to S_4. Differing from humans, where the main nerve supply to the bladder runs through the S_3 roots, the porcine bladder displayed the most intense contractions by stimulation of S_2 roots [6, 7]. Stimulation of S_1 and lower lumbar roots did not result in bladder contractions. We did not stimulate higher lumbar or thoracic segments.

In complete sacral denervation of the bladder (bilateral transsection of both motor and sensory roots at S_2 to S_4) no micturition reflex could be elicited by filling the bladder. Thus it was demonstrated that, similarly to humans, sympathetic innervation of the bladder does not produce contractions of the detrusor muscle in the pig. In other mammals, however, e.g. in the rat, stimulation of the sympathetic outflow from the lower thoracic and upper lumbar segments results in contractions of the detrusor muscle [8].

In selective sacral de-efferentiation, after transsection of the motor fibres at S_2 to S_4 bilaterally, cystometric results are identical with those in complete denervation. Micturition reflex of the porcine bladder depends on the integrity of parasympathetic efferent fibres.

Selective sacral de-afferentiation resulted in an astonishing picture; in spite of complete interruption of the dorsal afferent supply at S_2 to S_4, after recovery from the shock period, a micturition reflex, with almost the same increase in intravesical pressure as in controls, could be demonstrated although bladder capacity was slightly increased. Based on this finding we suggest that there has to be a centripetal neural supply from the wall of the bladder to the sacral centre other than the sacral afferent pathways at S_{2-4}. There are two possible explanations: the one is that a small number of sensory fibres run through the ventral roots; the second is that some of the afferent impulses of the micturition reflex reach the spinal cord via the hypogastric plexus and the thoraco-lumbar roots. Our opinion is, as the studies on regeneration indicate, that the latter explanation is more probable (animals 4, 7, 8, 9) [4, 15–17].

Unilateral transsection of both dorsal and ventral roots at S_2 to S_4 had no influence on the micturition reflex; this corresponds to the effects of unilateral denervation in humans [5, 7].

In a second series the same types of cystometrically verifiable lesions were produced but the transsected fibres were reconstructed in a microsurgical procedure immediately. The functional lesion was determined with cystometry

three weeks after the procedure. After 4–7 months, when regeneration of the lesion between sacral roots and the bladder of approximately 10 cm length could be expected (anticipating an axonal speed of regeneration of 1 mm per day), a second cystometry was done [13].

In animals with a sacral de-efferentiation there was a restitution of the micturition reflex after a period of 4–7 months, as demonstrated on cystometry; therefore, one can assume that the motor axons regenerated functionally. In all animals bladder capacity (volume at which the micturition reflex is activated) increased from the normal 300–400 cc to 800–1,400 cc. This did not apply to animal 3, in which after complete sacral de-efferentiation only the root S_2 was reconstructed unilaterally; no micturition reflex could be demonstrated after 17 weeks.

In animals with sacral de-afferentiation, 3 weeks after the operation a micturition reflex could again be demonstrated, which persisted during the whole period of observation (4–6 months). Although not significant statistically given the small numbers it is important to note that the baseline tone of the bladder (pressure at minimal volume) was considerably reduced compared to animals with intact sacral afferent and reinnervated efferent pathways.

In the completely denervated bladders, 4–7 months after microsurgical reconstruction of the transsected motor and sensory fibres, the micturition reflex could again be demonstrated on cystometry. Based on the above observations we conclude that the micturition reflex lost after complete denervation and de-efferentiation can be reinstituted through functional regeneration of the transsected and reconstructed segmental roots. We can only assume a functional reinnervation of the efferent axons, however, since a simple de-afferentation did not result in the elimination of the micturition reflex. Therefore cystometric studies of bladder function cannot prove that the morphologically demonstrated regeneration in the dorsal roots leads to a functional recovery.

Based on the morphologically and functionally demonstrated regeneration we suggest the practicability of surgical reconstruction of injured motor fibres of the intradural roots. Moreover these experiments prove that after proximal axotomy – 2–3 cm distal to the cell body – a regeneration can be expected. Our experience suggests that this procedure may be of value clinically, for instance following de-efferentiation or *denervation* of the bladder caused by trauma or tumour.

An effective treatment for this type of lesion has not been developed so far. Methods of conservative therapy such as emtying the bladder by Crede's manoevre, self-catheterisation, use of an appliance are all frustrating and have a severe limitations in terms of ascending infections, which may be life-threatening. Experiments with electrotherapy have not demonstrated any long-term success. Trial of surgical therapy is indicated in this situation.

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