

Limitations in using rabbit bladders in electrophysiological and urodynamic experiments

P. ZERHAU¹, Z. MACKERLE¹, M. HUSAR¹, E. BRICHTOVA¹, D. SOCHURKOVA², E. GÖPFERT³, M. FALDYNA³

¹Clinic of Paediatric Surgery, Orthopaedics and Traumatology, University Hospital and Masaryk University Brno, Czech Republic

²Clinic of Neurosurgery, University Hospital of St. Anna and Masaryk University Brno, Czech Republic

³Veterinary Research Institute Brno, Czech Republic

ABSTRACT: The aim of this study was to explore the possibility of using rabbit bladder as a model for experimental detrusor electrostimulation research. In a study of urinary bladder activity induced through electrostimulation of the ventral roots, the functional and morphological parameters of the rabbit detrusor were investigated. Under general anaesthesia, open electrostimulation of ventral spinal roots leading towards the detrusor (usually S₂, S₃) was performed in 20 rabbits. Detrusor response was recorded by repeated electromyography and cystometry in two groups: animals with naturally concentrated urine content (Group A, eight rabbits) and animals after flushing and filling the bladder with saline (Group B, 12 rabbits). Histological examination of bladder wall was performed in both groups. The measured values were compared to one another as well as with data from the veterinary and human literature. The histological specimens were compared with histological specimens of human bladder. The reaction of detrusor fibres was detectable by electromyography in all cases. Elevation of intravesical pressure as a consequence of detrusor contraction was more difficult to detect, as this depends more on the density of the intravesical content. The pressure rise in Group B had a higher amplitude – up to 15 cm H₂O versus 5 cm H₂O in the first group ($P = 0.00046$). Histological examination of bladder wall from the two groups of rabbits showed no differences. In comparison with the bladder wall in humans, the only differences found were significantly thinner detrusor layers relative to the overall thickness of bladder wall. It is possible to use rabbit bladder for research into experimentally electrostimulation-induced activity of the detrusor or for experimental detrusor reinnervation research. It is necessary, however, to take into account certain limits – the lower contractility of the bladder wall and the need for qualitative control of bladder content. The present results also suggest that the physiological micturition of rabbits is probably more dependent on abdominal pressure than in humans.

Keywords: electrostimulation; rabbit detrusor; intravesical pressure; micturition; abdominal pressure

The lower urinary tract consists of the bladder and urethra. The shape, size and position of the bladder depend upon the amount of urine it contains. The bladder's volume increases several fold while filling. The histological structure of the bladder wall in rabbits and humans has been described as identical. Its external surface is mostly covered

by the peritoneum. The bladder's muscle is divided into three layers with different fibre orientations: the external layer with a longitudinal arrangement, the middle layer with a circular arrangement, and the internal layer with a longitudinal arrangement. The internal surface consists of irregularly folded mucosa (Konig and Liebich 2005, Maxie 2008).

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The detrusor muscle is relaxed during the filling phase, is contracted during the voiding phase, and ensures evacuation of the bladder in conjunction with the sphincter mechanism. Abdominal pressure is not involved in micturition in healthy humans. In animals, the function of abdominal pressure is not adequately described in the available literature. Innervation of the bladder is partly sympathetic, facilitated by transmissions from the hypogastric nerves relaxing the bladder muscle, and partly parasympathetic, mediated by transmissions from the pelvic plexus contracting the detrusor. In humans, sympathetic nerve fibres originate from spinal cord segments Th₁₀–L₂. Parasympathetic innervation in humans originates from the sacral spinal cord segments (segments S₂–S₄) and in rabbits mostly from segments S₂–S₃ (Pikov and McCreery 2004).

Urine composition and density is greatly influenced by the composition of the diet (especially by the calcium and phosphorus contents), type of housing and overall condition (Harcourt-Brown 2002). If calcium carbonate deposits are present, so-called “sludgy urine” is formed and urination becomes difficult. This situation is commonly encountered in laboratory animals kept in cages. In humans this phenomenon does not occur under physiological circumstances.

The aim of the present work is to summarise the experience to date with experimentally induced electrophysiological and contractile activity of the rabbit detrusor and simultaneously to examine the suitability of a rabbit model at two qualitatively different bladder loads.

MATERIAL AND METHODS

Between February 2013 and January 2014, under general anaesthesia (introduction of ketamine 25 mg/kg *i.m.* + xylazine 0.4mg/kg *i.m.*, analgesic butorphanol 0.3 mg/kg *i.m.* in a single injection at the start of the surgery and anaesthetic propofol 15mg/kg *i.v.* continuously throughout the operation), a group of 20 rabbits (17 males, three females) weighing between 2.8 and 4.9 kg was operated on. First, the bladder was entered through a lower midline laparotomy with the rabbits in a supine position. A bipolar stripped electrode (AU1X4P, Cardion, Brno, Czech Republic) was positioned in the bladder and a CH 10 catheter (Porges, AH 5010, Paris, France) was inserted through the urethra to measure intravesical pressure (P_{ves}). At the same

time, a small specimen of the bladder wall was resected for histological examination (H&E staining, zoom 2.5×). After the abdominal wall was closed, a sagittal incision over the lumbosacral trunk exposed the spine with the rabbits in a prone position. Laminectomy and identification of anterior roots in L₄–S₃ segments were performed.

After EMG detection of the ventral roots leading towards the detrusor, electrostimulation with a bipolar hook electrode (Renix International, Sialkot, Pakistan) was performed (amperage 0.5–3.0 mA, 0.05–0.200 ms pulse width, 5 or 15 Hz frequency of repetitive stimulation, 4 s duration of stimulation).

In each animal, all the ventral (motor) roots that provide innervation to the detrusor were probed. The root whose stimulation evoked the largest response was selected for recordings (usually S₂).

Two groups of animals were stimulated: animals with naturally concentrated urine content (30–40 ml of “sludgy urine”, Group A, eight rabbits) and animals after flushing and filling the bladder with 30–40 ml of saline heated to 35 °C (Group B, 12 rabbits). The urinary bladder volume was chosen partly based on the determined mean amount of natural content and partly based on data in the literature (Kontani et al. 2006; Chou et al. 2007; Gomez et al. 2011). No substance was used that could affect the muscle response during root stimulation.

The response of the detrusor was monitored by electromyography (TruTrace EMG, Alien technic, Hronov, Czech Republic), using a bipolar stripped electrode (AU1X4P, Cardion, Brno, Czech Republic), and by cystometric (CTM) measurement of intravesical pressure (UD 5500 unit from Dantec, Skovlunde, Denmark) (Figure 1). The total

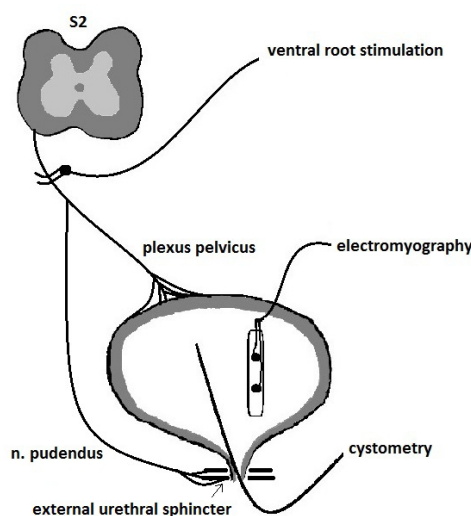


Figure 1. Scheme of electrodes and catheter placement

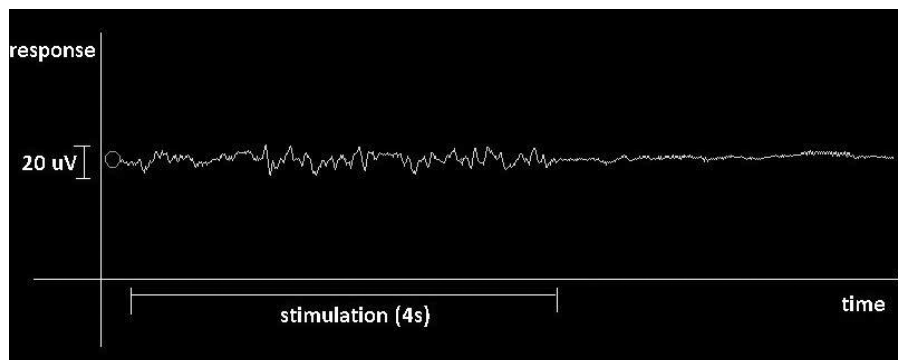


Figure 2. Group A: Electromyography of detrusor after anterior spinal root (S2) stimulation (0.5 mA current intensity, 0.05 ms pulse width, 5 Hz frequency of repetitive stimulation, 4 s duration of stimulation, response to amplitude up to 20 uV)

duration of the experiment (anaesthesia + surgery) ranged from 75–155 min (mean 110 min). Detrusor stimulation was performed repeatedly for 4 s. The measured EMG and CTM values were compared to one another (using mean, median, Student's *t*-test) and compared with data in the literature on experiments performed in animal and human subjects. The histological specimens of rabbit bladder (four from each group) were compared with histological biopsy specimens of human bladder (Institute of Pathology and Anatomy, the University Hospital Brno).

Animal experiments complied with Act No. 246/92 Sb. and were approved by the Branch Commission for Animal Welfare of the Ministry of Agriculture of the Czech Republic (No. 22-2012; Reg. No. 1400).

RESULTS

The reaction of detrusor fibres was detectable by electromyography in both animal groups with com-

parable values. However, intravesical pressure values differed between the two groups. During stimulation, significantly higher P_{ves} values were measured in group B, which contained physiological saline. In animals with natural bladder content ("sludgy urine"), the reaction was absent or only minimal.

Group A

Figure 2 presents EMG records under stimulation for all eight animals (filled with 30–40 ml of sludgy urine) with amplitude of evoked potentials up to 20 uV. Figure 3 and Table 1 show the CTM record in stimulation without elevation of P_{ves} in five cases (62.5%) and with minimal elevation of P_{ves} up to 5 cm H₂O (mean 1.5, median 0) in three cases (37.5%).

Group B

Figure 4 shows EMG records under stimulation for all 12 rabbits (filled with 30–40 ml of physiologi-

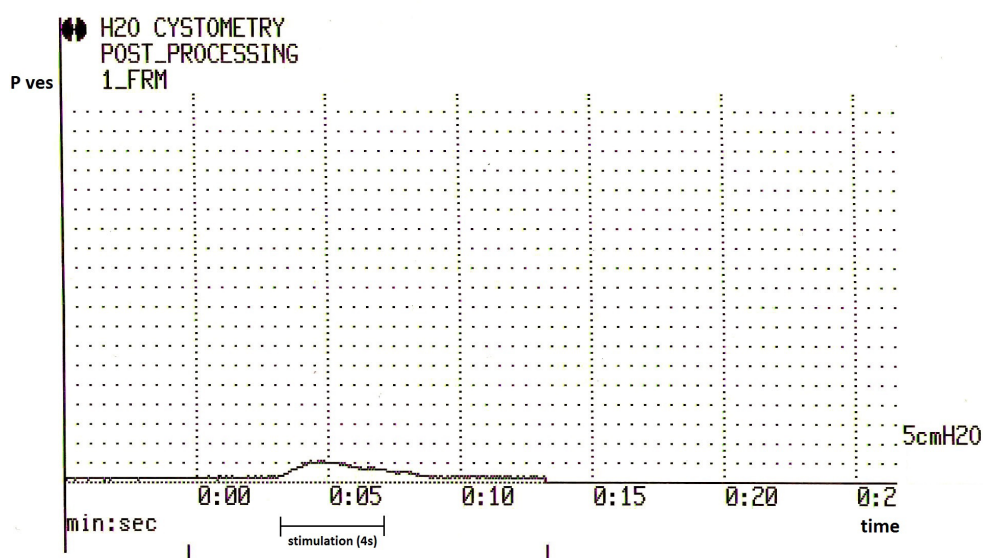


Figure 3. Group A: Cystometry, increase in P_{ves} up to 5 cm H₂O after stimulation

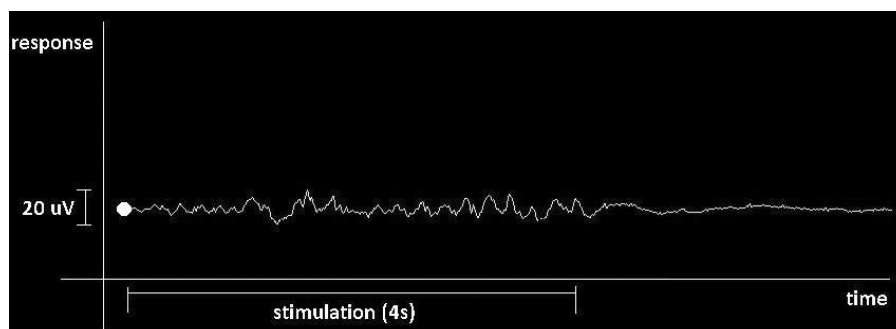


Figure 4. Group B: Electromyography of detrusor muscle after anterior spinal root (S2) stimulation (0.5 mA current intensity, 0.05 ms pulse width, 5 Hz frequency of repetitive stimulation, 4 s duration of stimulation, response to amplitude up to 20 uV)

cal saline) under conditions comparable with those for Group A (amplitude up to 20 uV). Figure 5 and Table 1 present the CTM record under stimulation without elevation of P_{ves} in two cases (17%) and with a rise of P_{ves} to as high as 15 cm H₂O (mean 9.9, median 12) in 10 cases (83%). The measured values of P_{ves} in groups A and B differed with a significance level of $P = 0.00046$.

Histological examination of bladder wall (Figure 6) taken from the two groups of rabbits showed no differences. In comparison with the bladder wall in humans (Figure 7), the only differences found were significantly thinner detrusor layers relative to the overall thickness of the bladder wall. The macroscopic “membranous” pattern of the rabbit bladder wall is documented in Figure 8.

DISCUSSION

For the experimental study of induced detrusor activity in rabbit bladder via the ventral roots – pelvic plexus – the detrusor is essential for determin-

ing how realistic it is to monitor this activity. As is clear from our experience, monitoring using electromyography is easier and the pressure response depends on several factors.

The reaction of detrusor fibres (EMG monitoring) was detectable in all cases, whether the bladders were naturally filled with denser urine as in Group A (having sludgy urine as is often found in rabbits kept in hutches) or after the bladders were flushed and filled with saline, as in Group B.

Elevation of intravesical pressure as a consequence of detrusor contraction (cystometric monitoring of P_{ves}) is more difficult to detect. This depends more on the density of the intravesical content. A minimal increase in intravesical pressure was observed in only three of eight (38%) animals in Group A (with sludgy urine), while pressure elevation was observed in 10 of 12 (83%) rabbits in Group B (with saline). Furthermore, the pressure rise had a higher amplitude – up to 15 cm H₂O versus 5 cm H₂O in the first group ($P = 0.00046$). Nevertheless, none of the rabbits in either group released urine during the experiments.

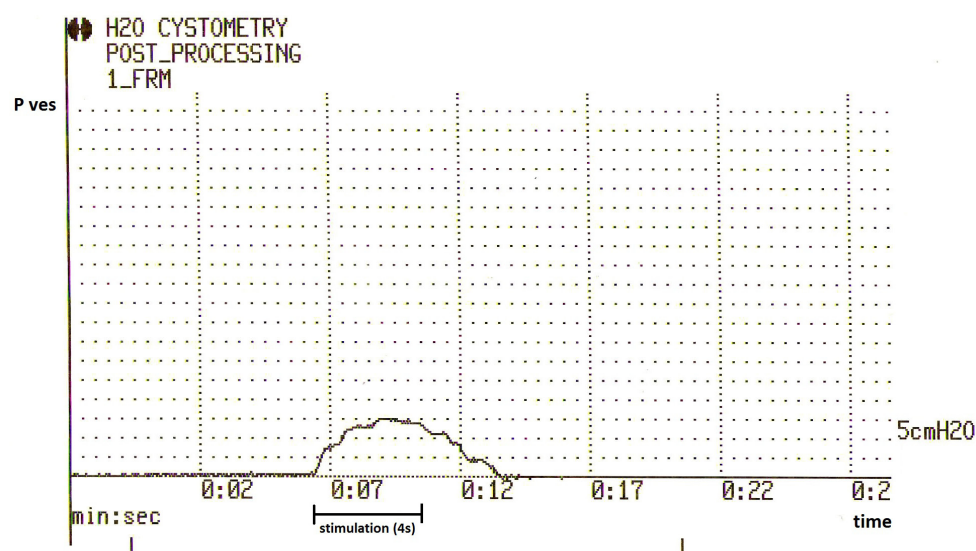


Figure 5. Group B: Cystometry, increase in P_{ves} up to 15 cm H₂O after stimulation

Table 1. Intravesical pressure (P_{ves}) values for both groups of experimental animals

Group A	1	2	3	4	5	6	7	8						Mean	Median
P_{ves} H ₂ O	0	0	0	0	0	5	3	4						1.5	0
Group B	1	2	3	4	5	6	7	8	9	10	11	12			
P_{ves} H ₂ O	0	0	12	10	15	14	7	12	15	13	13	8		9.9	12

Our experience has shown that in experimental studies it is necessary to account for the different absolute value of intravesical pressure in rabbits and humans. The strength of human detrusor contraction as evaluated by P_{ves} can be as high as 50 cm H₂O (Dai and Xiao 2005; Xiao 2006) or even 80 to 90 cm H₂O (Dolezel et al. 2002; Martens and Heesakkers 2011; Creasey and Craggs 2012). The values of P_{ves} in rabbits (with comparable parameters of stimulating potentials) are significantly lower – in our experiments up to 5 cm H₂O for Group A and up to 15 cm H₂O for Group B. There are no data in the available literature of comparable experiments concerning these animals. Pharmacologically modified P_{ves} values in rabbits have been described to reach up to 20 cm H₂O (Kontani et al. 2006; Chou et al. 2007), although no parameter for comparison at different bladder filling densities is described. Lin (Lin et al. 2005) also described P_{ves} values of under 20 cm H₂O for rabbits in a study of the influence of ischaemia on the bladder, although the study focused on pressure in overfilled bladders achieved under entirely different circumstances.

The different P_{ves} values in rabbits and humans (including children of comparable weight) raise an associated question: to what extent does detrusor

contraction itself influence micturition intravesical pressure and what role does abdominal pressure play? Although the voiding phase and the role of individual muscle systems have been described in detail for human medicine (Krhut et al. 2005; Yoshimura and Chancellor 2012), this issue has not yet been addressed within the available literature for veterinary medicine. One of the reasons for this is certainly an absence of open cooperation in monitoring the subject, which is essential for proper urodynamic examination.

The lower detrusor pressure during micturition in rabbits compared to that in humans can be indirectly inferred, too, from the macroscopic and histological structures of the bladder wall. Rabbit bladder is thin-walled, and when filled it resembles the gall bladder in humans. The muscle width in the histological examination supports this observation. For purposes of studying evoked potentials and detrusor pressure responses, abdominal pressure nevertheless plays no essential role.

We conclude that the rabbit model can be used in experimental studies of detrusor muscle activity by electrostimulation of the spinal roots. It is nevertheless necessary to recognise certain limitations,

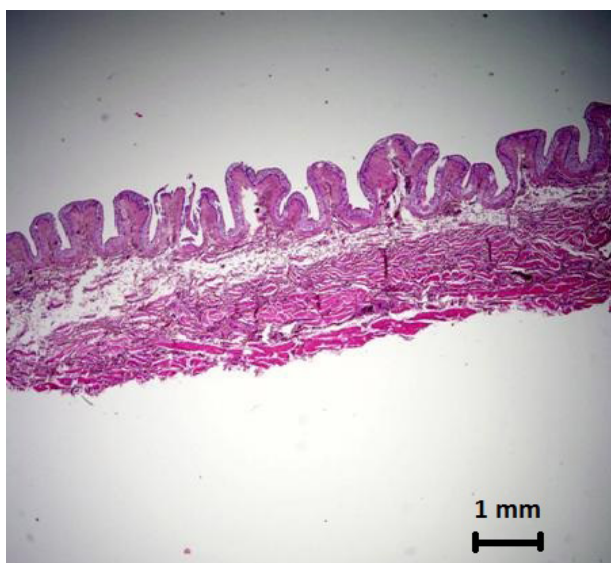


Figure 6. Histogram of rabbit bladder wall; H&E staining, magnification 2.5×

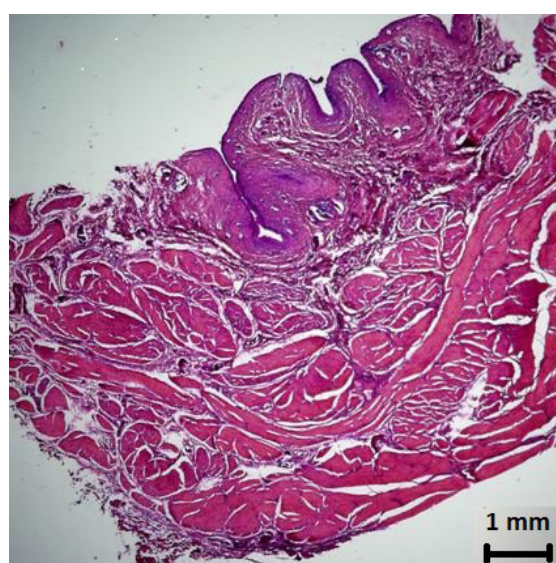


Figure 7. Histogram of human bladder wall, biopsy; H&E staining, magnification 2.5×

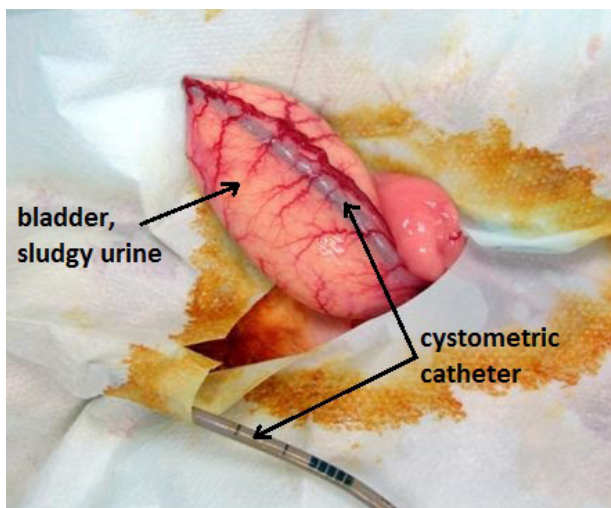


Figure 8. Intraoperative photo of rabbit bladder, “sludgy urine” and catheter for cystometry inside

namely, the lower contractility of the bladder wall and the need for qualitative control of the bladder content.

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Corresponding Author:

Pavel Zerhau, University Hospital and Masaryk University Brno, Clinic of Pediatric surgery, orthopedics and traumatology, Cernopolni 9, 625 00 Brno, Czech Republic
Tel. +420 532 234 360, E-mail: pzerhau@fnbrno.cz; pavel.zerhau@tiscali.cz