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Urological Research

Urodynamic evaluation and electrical and pharmacologic neurostimulation

The rat model

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Summary. We introduce a rat model that allows simultaneous or independent recording of bladder and sphincteric activity. Via a polyethylene tube inserted into the bladder dome, bladder pressure is measured in response to constant intravesical saline perfusion. The electrical activity of the intra-abdominal urethra (a well-defined striated muscular tube which, in the rat, constitutes the external urethral sphincter) is recorded simultaneously with an electromyography needle electrode. Thus, precise statements can be made about detrusor/sphincter interrelationships. Changes in urodynamic parameters with the anesthetics urethane, methoxyflurane (Metofane), and thiobutabarbital sodium (Inactin) were investigated. High-frequency oscillations in intraluminal bladder pressure could be demonstrated during micturition cycles only in rats anesthetized with urethane or Metofane. As this high-frequency activity is generated by the striated muscle of the intra-abdominal urethra, the external sphincter of the rat is the force behind urine expulsion. The anesthetic Inactin combined with a low intravesical perfusion rate attenuated spontaneous bladder and sphincteric activity and abolished micturition cycles. This rat model can provide accurate and reproducible measurements of urodynamic changes in response to electrical stimulation of the pelvic and pudendal nerves and pharmacologic stimulation with neuropeptides at the lumbosacral spinal cord level. We recommend using this model with urethane or Metofane for physiologic studies of micturition and with Inactin for meticulous neuropharmacologic and electrostimulatory evaluation of urodynamic parameters.

Key words: Electromyography – Electrostimulation – Micturition – Pharmacostimulation – Rat model – Urodynamics

Since 1971, many acute and chronic studies in animals have been undertaken to define the most efficient and practical route for bladder stimulation [15–19]. For these experiments the dog was found to be the most suitable animal model. In addition, both dogs and monkeys have been used effectively as models for erection induced by electrostimulation [6, 7] and pharmacologic agents [1, 3].

We have recently developed a new animal model in which we use the rat for acute urodynamic studies in combination with either electrical or pharmacologic neurostimulation. The rat offers multiple advantages such as convenient size, availability, affordability, genetically well-defined stock, ease of care, and postoperative disease resistance [12], and has proven to be an excellent choice for basic urodynamic studies.

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Materials and methods

Surgical preparation

A total of 86 male albino rats (Wistar Charles River strain), weighing between 450 and 600 g, were used. They were anesthetized either with thiobutabarbital sodium (Inactin, Byk; 100 mg/kg, i.p.) ($n = 51$) or with urethane (510 mg/kg, s.c.) plus methoxyflurane (Metofane, Pitman-Moore; 0.5 ml on a tampon as an inhalant) ($n = 35$), depending on the study.

After a tracheostomy with placement of polyethylene tubing (Intramedic, PE-200) to facilitate respiration, PE-50 tubing was inserted into the right common carotid artery for blood pressure monitoring and PE-90 tubing into the left external jugular vein for saline infusion and drug administration. The urinary bladder was exposed through a ventral midline incision and bladder pressure was monitored by inserting a PE-50 catheter into the bladder dome. The PE-50 catheter was attached via a T-connector to both a perfusion pump and a pressure transducer. To prevent exsiccation, the abdominal cavity was filled with warm mineral oil (37°C) which, in addition, is an excellent thermal and electrical insulator. Body temperature was maintained between 37°C and 38°C throughout the experiment with an operator-controlled copper heating plate.

A delicate bipolar electromyography (EMG) needle-electrode (length 30 mm, diameter 0.3 mm; Dantec) was placed suprapubically at the midline onto the surface of the intra-abdominal urethra, which, in male rats, is considered the external urethral sphincter (Fig. 1). Needle placement was controlled by the audio output of the EMG amplifier. As the electrode touched the striated muscle of the

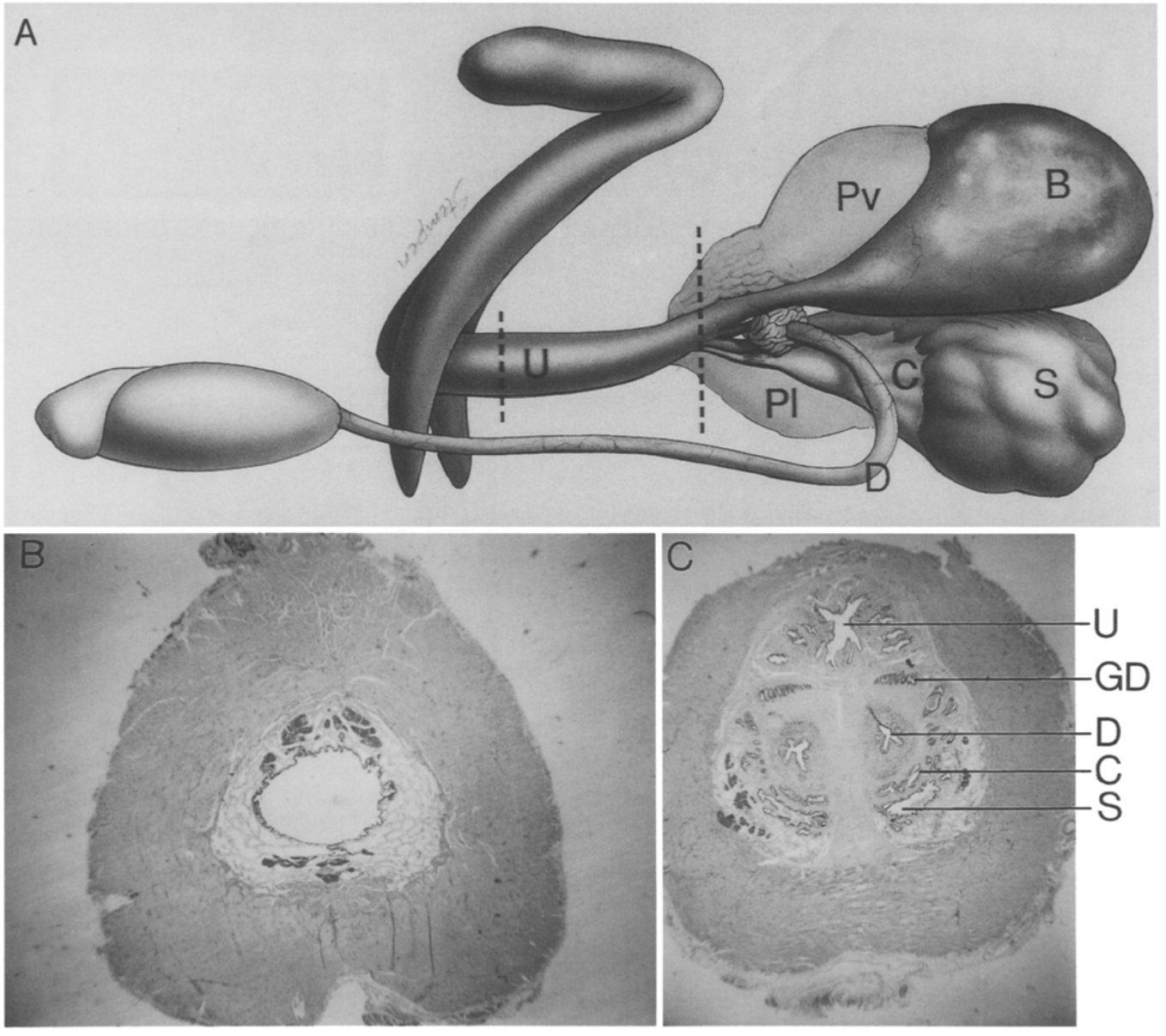


Fig. 1. **A** The intra-abdominal urethra of the male rat and its relationship to the urinary bladder and sex glands (viewed from the left; the left ventral lobe of the prostate gland and the left ureter are not depicted). *B,* Urinary bladder; *C,* coagulating gland; *D,* ductus deferens (*left*) with its terminal gland; *Pv,* right ventral lobe of prostate gland; *Pl,* left lateral lobe of prostate gland; *S,* seminal vesicle; *U,* intra-abdominal urethra. **B** Cross-section through the distal part of the intra-abdominal urethra (see *left broken line* in **A**). The strong urethral wall can be distinguished as an outer layer with longitudinally oriented striated muscle fibers and an inner layer with more oblique or circular striated muscle cells. H & E, ×5. **C** Cross-section through the proximal part of the intra-abdominal urethra at the level where the bladder infundibulim and the sex glands converge (see *right broken line* in **A**). *U,* Urethra with periurethral ducts of the ventral lobes of the prostate; *GD,* duct of the gland of the ductus deferens; *D,* ductus deferens; *C,* main duct of the coagulating gland; *S,* main duct of the seminal vesicle. H & E, ×5

intra-abdominal urethra, the sound of distantly firing motor units could be heard. Once the needle was correctly situated, a tonic interference pattern was observed.

Blood pressure, bladder pressure, and EMG activities were registered on a Gould Recorder 2400, and the pressures measured via Gould Statham P 23 ID transducers. In the 0.1 Hz–3.0 Hz range, the EMG potentials were preamplified with a Tektronix amplifier with a storage oscilloscope for visual observation and a loudspeaker for audio monitoring. The completed experimental set-up was electrically shielded with a Faraday cage.

Electrical neurostimulation

As in the technique used for pudendal nerve electrode implants in humans [15], paravertebral access was chosen at the S1–4 level for electrostimulation of either the pudendal or the pelvic nerve. It was important to stimulate the nerves in question as far from their effector organs as possible to avoid artifacts owing to current spread.

The sciatic nerve was easily located (Fig. 2) at the distal end of the 3-cm paravertebral incision. Used as a landmark, the sciatic nerve was followed up to the sacroiliac articulation; at this level the nerve is

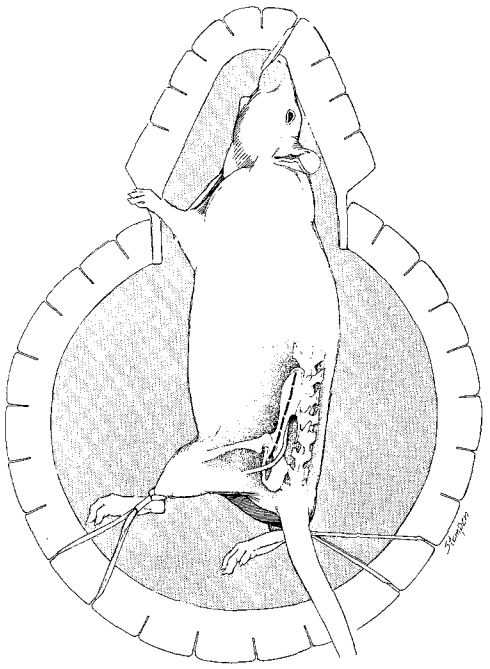


Fig. 2. At the distal end of a 3-cm paravertebral skin cut from S1 to S4 (*broken line*), the sciatic nerve serves as a landmark to locate the lumbosacral trunk and the L6–S1 trunk

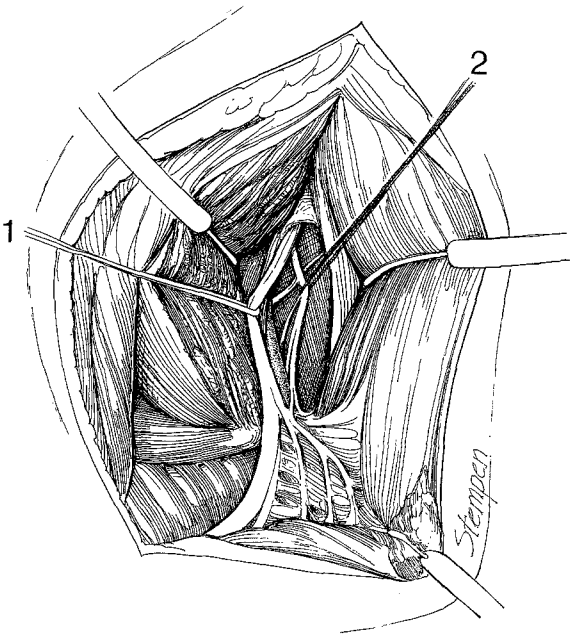


Fig. 3. Close-up of the surgical field shows the lumbosacral (*1*) and L6–S1 (*2*) trunks. The internal iliac vein has been removed

the lumbosacral trunk L3–5 (Fig. 3) [11]. After mobilization and retraction of this trunk, the L6–S1 trunk, identified as the pudendal nerve in the rat [2, 11], was still covered by the large internal iliac vein. In most instances the first branch off the L6–S1 trunk was the parasympathetic pelvic nerve, but in others the pelvic nerve had no link and arose directly from S1 [2]. To gain access to both pudendal and pelvic nerves, the internal iliac vein had to be ligated proximally and distally and the intervening segment removed.

For electrical stimulation, a specially designed, miniaturized bipolar fork electrode was used. At its tip two tungsten wires (Medwire, diameter 0.127 mm) were separated by a distance of 1 mm. The electrode was connected to an Avery transmitter giving square-wave pulses ranging from 50–200 ms, voltage up to 14 V, 1 kΩ load; frequency up to 200 Hz. Stimulation was performed with the aid of an operating microscope (up to ×30).

Pharmacologic stimulation

A bilateral laminectomy at the T13–L1 junction was performed. Intrathecal tubing (Intramedic, PE-10), previously stretched to one third of its original diameter, was passed caudally through a slit in the dura and positioned so that the inner tip lay at L1 (in the rat L1 corresponds to the L5, L6, and S1 spinal cord segments).

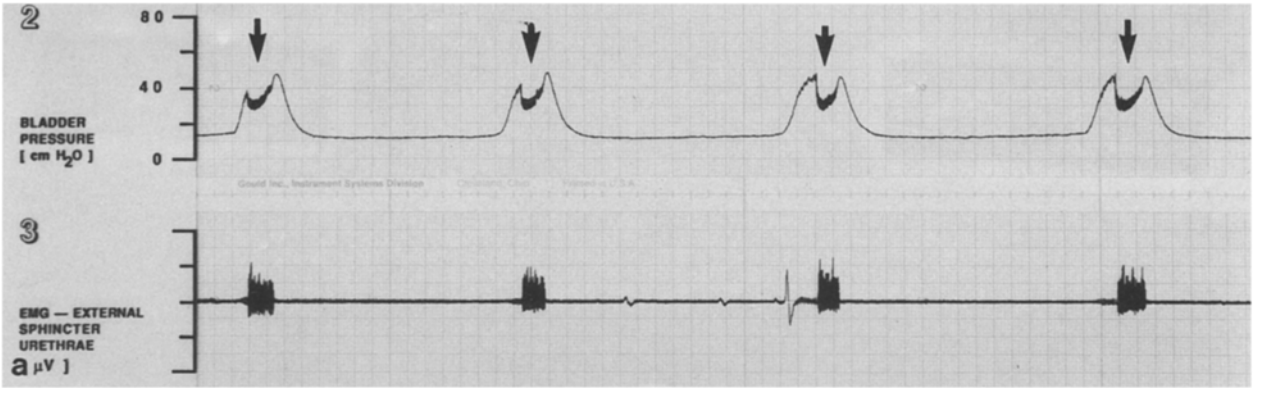
The afferents and efferents to the urinary bladder travel via the pelvic nerves. The efferents originate from the preganglionic neurons of the sacral parasympathetic nucleus located within the L6–S1 level of the spinal cord [12, 13]. The motor innervation to the striated external urethral sphincter is carried by the pudendal nerve, which has been reported to arise from segments L5–L6 [11] and L6–S1 [2, 4, 13] of the spinal cord.

The validity of this method for correct positioning of the inner tip of the tubing was confirmed in preliminary experiments and verified by necropsy. We developed a functional test for correct positioning: intrathecal administration of 1% lidocaine via the subdural tube (see Results). After fixing the catheter with adhesive silicone, the skin was sutured and the animal placed in the supine position. The operating table was lowered caudally 45°.

The following neuropeptides (from Peninsula Laboratories, Belmont, Calif., USA) were used: substance P (SP, molecular weight (M_r) 1347.80); vasoactive intestinal polypeptide (VIP, M_r 3325.68); peptide histidine isoleucine (PHI, M_r 3011.24). They were delivered intrathecally by means of a Hamilton syringe over a period of 10–15 s at a dosage of 10 µg per 10 µl artificial cerebrospinal fluid (CSF) (Elliott's B Solution, LyphoMed), followed by flushing with 10 µl artificial CSF (catheter volume was approximately 7 µl). Control injections of 10 µl pure artificial CSF were done in every experiment about 60 min before and after neuropeptide administration. The rats underwent serial cystometrograms by constant perfusion (21.6 µl/min) of saline (37°C) via the PE-50 catheter in the bladder dome. Blood and bladder pressure and sphincteric activity were measured before, during, and after bladder filling. These measurements were repeated before and after intrathecal administration of the neuropeptide in question.

Results

The response pattern of both bladder and sphincteric activity was decisively influenced by the anesthetic used. With urethane, the animal was only lightly anesthetized, which could easily be verified by evocation of the lick reflex with drops of water in the rat's mouth. If the animal swallowed (and started moving), we administered methoxyflurane by inhalation for a few seconds until the lick reflex disappeared. Approximately 15 min later, a series of cystometrograms could be obtained continuously without artifacts owing to movement. In this state reproducible voiding cycles were registered in the 35 rats so studied.



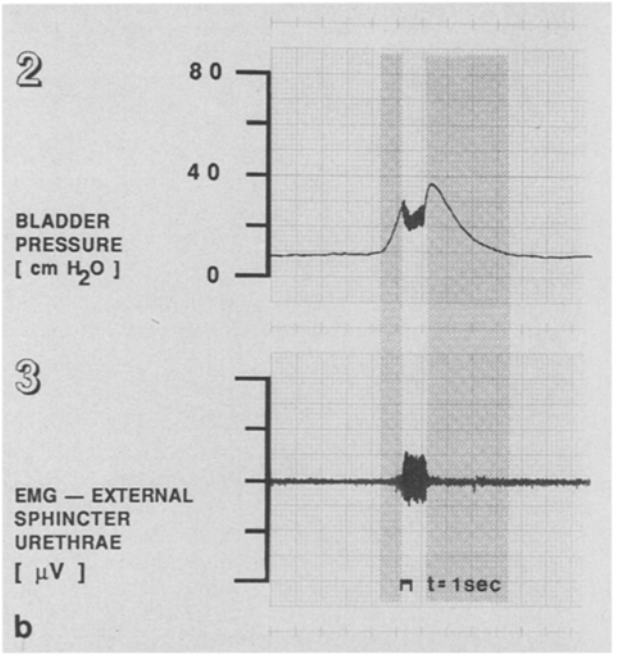
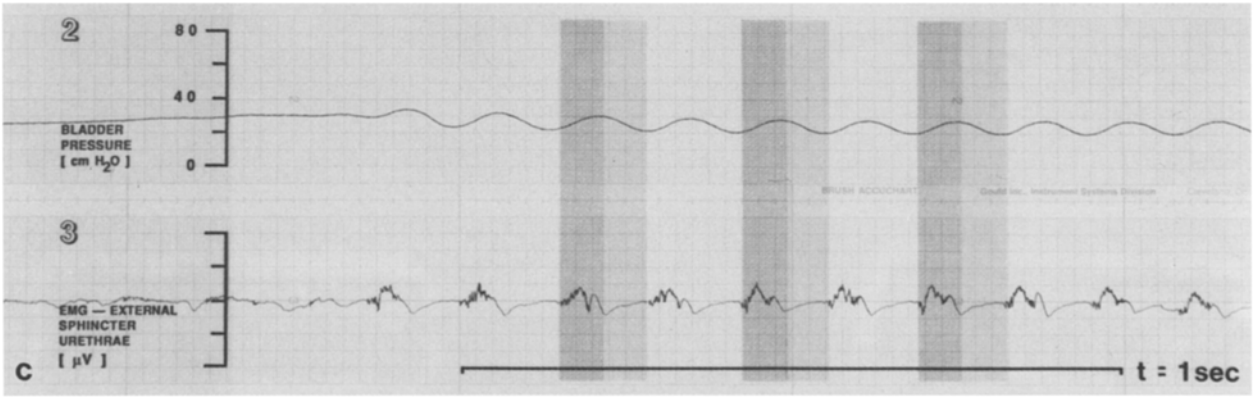


Fig. 4. a Cystometogram (0.25 ml/min) in a urethane-anesthetized rat; recording speed 1 mm/s. Each of the four micturition cycles is characterized by a series of high-frequency oscillations in intraluminal bladder pressure (*arrows*) which interrupt the smooth bladder contraction waves. These oscillations correspond to the high-amplitude/high-frequency EMG activity of the external urethral sphincter during which micturition occurs *EMG*, 1 unit = 1000 μV). **b** A higher recording speed (2 mm/s) in the specimen in **a**. Each micturition cycle is framed by a smooth rise and fall in bladder pressure (smooth components; *gray bars*) (*t*, 1 s; *EMG*, 1 unit = 1000 μV). **c** Recording speed 200 mm/s. High-amplitude/high-frequency EMG activity of the external urethral sphincter and its relation to the high-frequency oscillations in intraluminal bladder pressure during a portion of a 14-s micturition cycle. Each spike complex (*dark gray bar*) is followed by a resting interval (*light gray bar*); each lasts 66 ms and is paralleled by a wave-like bladder pressure change. Micturition takes place exclusively during the high-amplitude/high-frequency EMG activity of the external urethral sphincter, and urine appears in small spurts in a manner similar to ejaculation (*EMG*, 1 unit = 1000 μV)



Cystometrograms (0.25 ml/min) performed in urethane-anesthetized rats showed that the volume-evoked micturition reflex terminated in a voiding cycle characterized by a series of high-frequency oscillations in intraluminal bladder pressure (IPHFO; the abbreviation established by the authors of the first report [10]. Figure 4 shows the correlation between these and high-amplitude/high-frequency EMG activity of the striated muscular wall of the intra-abdominal urethra (external sphincter). The increase in bladder pressure combined with an increase in sphincteric activity might be misinterpreted as detrusor/sphincter dyssynergia, but instead represented the true micturition pattern in male rats. Typically each micturition cycle started with an increase in bladder pressure (smooth component), which finally triggered the high-frequency contractions (7–9 Hz) of the external urethral sphincter (striated component) (Fig. 4). With the rat's abdomen open these urethral contractions were

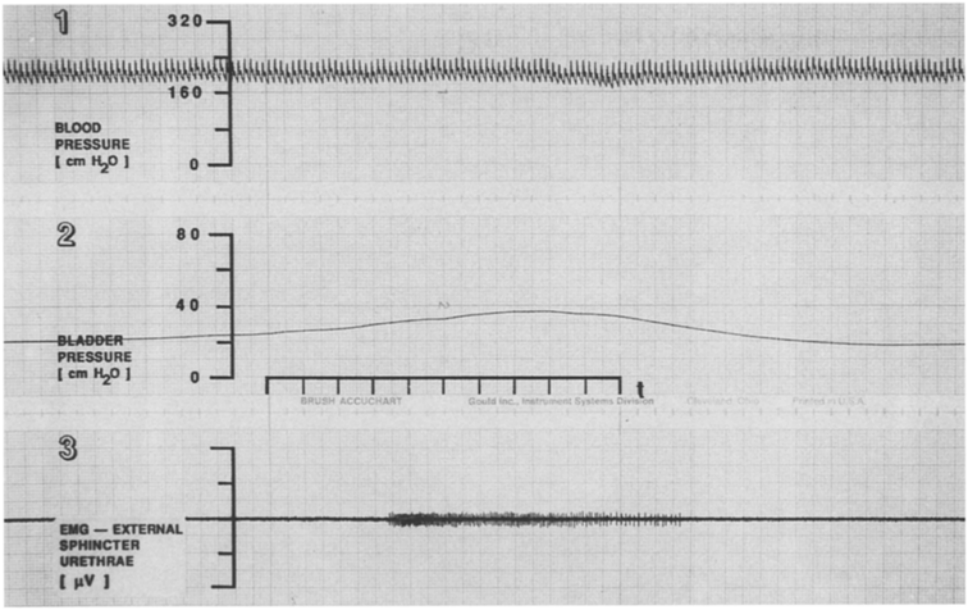


Fig. 5. Cystometogram (21.6 μl/min) in an Inactin-anesthetized rat; recording speed 10 mm/s. The typical pattern of a detrusor contraction accompanied by burst of sphincteric EMG spikes of higher amplitude is seen. Although the amplitude of the detrusor contraction in this animal was atypically high (usually < 10 cm H_2O), no micturition occurred (*t*, 1 unit = 1 s; *EMG*, 1 unit = 500 μV)

easily visible, and at a slow recording speed (1 mm/s) they were seen as high-amplitude spikes (500–1000 μV) in the EMG tracing. Small spurts of urine were emitted serially only during these sphincteric contractions, in a manner very similar to ejaculation. The emissions were so powerful that the penis recoiled and spurted drops of urine a distance of 15 cm. The micturition cycle was completed by a return in bladder pressure to baseline (smooth component) (Fig. 4b). Recordings at fast paper speeds (100 or 200 mm/s) revealed that the high-amplitude EMG interval was composed of individual spike complexes followed by a resting interval (Fig. 4c). Each complex consisted of several spikes in an amplitude range from 50 to 1000 μV and was related to a rolling increase in bladder pressure (2–10 cm H_2O) which decreased during the resting interval (Fig. 4c). As smooth muscle is not able to contract and relax at a frequency of 7–9 Hz, the high-frequency oscillations in intraluminal bladder pressure most probably reflect the transmitted pressure changes of the propulsive urethral activity.

The synchronization between the initializing tonic detrusor contraction and the succeeding phasic sphincter contractions was very sensitive to anesthetics. Inhalation of Metofane for 5 s reduced the frequency of sphincteric contractions during the high-frequency/high-amplitude interval to 5 Hz or even less. This reduction in frequency only, without visible changes in the spike complexes themselves, led to a reversible inability to urinate. When urethane was given in a dosage higher than 510 mg/kg body weight ($n = 10$), 40% of animals failed to evince reproducible micturition cycles.

Inactin administered intraperitoneally at a dose of 100 mg/kg body weight provided mild but sufficient anesthesia for about 12 h. Inactin was combined with an intravesical perfusion of saline at 21.6 μl/min, which approximates the rate of spontaneous urine formation in the rat and (at 1.3 ml/h) simulated a less than half-maximal hourly diuresis value [9]. As do other barbiturates, Inactin exerted an inhibitory effect on bladder and sphincteric activity that led to a blockade of the volume-evoked micturition reflex and to overflow incontinence. As a result no specific statement concerning micturition was possible. However, Inactin seemed to be the anesthetic of choice to avoid confusion between spontaneous detrusor contractions and electrically or pharmacologically generated contractions.

Typical patterns in bladder pressure and sphincteric activity obtained under Inactin anesthesia are shown in Figs. 5 and 6. When the bladder was beginning to fill in response to intravesical perfusion, the detrusor presented a series of contraction waves with amplitudes usually less than 10 cm H_2O. These contractions were accompanied by periods of sphincteric EMG spikes with increased amplitude (Fig. 5). When the bladder reached its maximal capacity, the bladder contractions became weaker and the sphincteric EMG displayed continuous firing of high-amplitude spikes (Fig. 6).

In the rats prepared for intrathecal administration of neuropeptides, ($n = 39$ of the 51 Inactin-anesthetized animals), the correct location of the subdural tube was tested with intrathecal lidocaine (50 μl). As the motoneurons innervating the external urethral sphincter are located at L5–S1, lidocaine given at this level abolished reversibly the reflexogenic sphincteric EMG response generated by bladder distension (Fig. 6). With the subdural tube at a higher or lower spinal cord level the result was distinctly protracted and/or diminished.

Electrical neurostimulation

The pelvic nerve arises at the formation of the L6–S1 trunk (Figs. 3, 7). In almost all of the 12 rats that underwent electrostimulation, this nerve bundle consisted of three distinguishable fibers that we numbered cranially to caudally as P_1, P_2, and P_3. P_1 is the uninterrupted

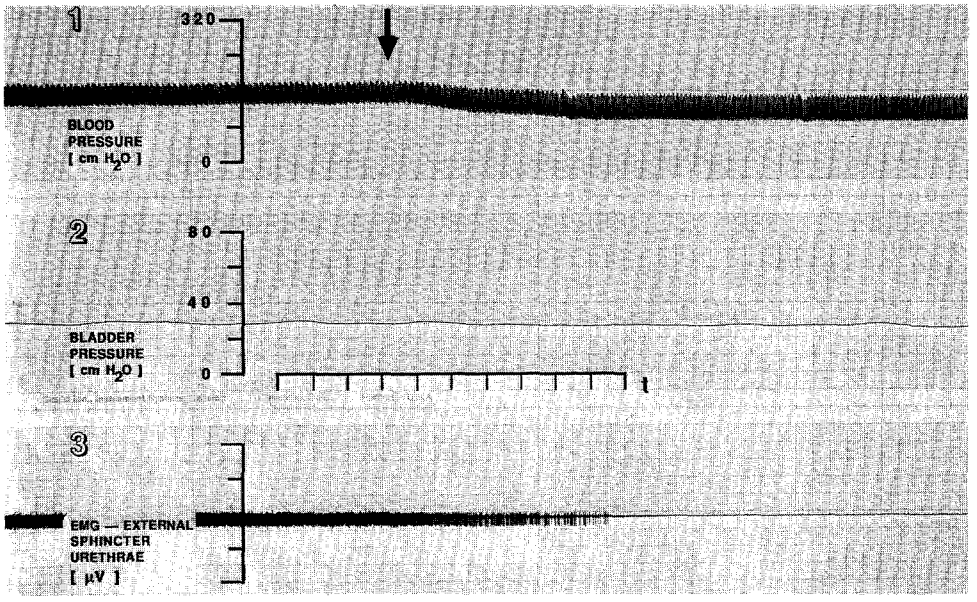


Fig. 6. Cystometogram (21.6 µl/min) in an Inactin-anesthetized rat; recording speed 2 mm/s. Lidocaine (50 µl of 1%) was given intrathecally when the bladder reached its maximal capacity (*arrow*) and the sphincteric EMG displayed a continuous firing. It abolished the sphincteric activity completely after about 30 s, confirming the correct placement of the intrathecal tube. The effect lasted for about 15 min (*t*, 1 unit = 5 s; *EMG*, 1 unit = 1000 µV)

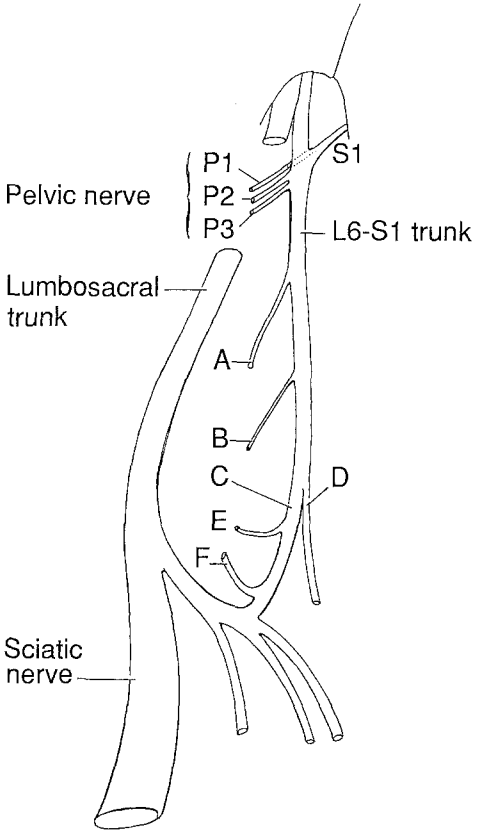


Fig. 7. Diagram of the lumbosacral trunk and the L6–S1 trunk and branches (*A–F*)

continuation of the spinal nerve S1 and has no physical connection to the L6–S1 trunk. P_2 and P_3 arise from the trunk directly. Occasionally the pelvic nerve is too delicate to separate into all of its components.

When all three fibers of the pelvic nerve were stimulated at the same time, the bladder pressure typically rose instantly (20–30 cm H_2O) and was sustained for the period of stimulation (Fig. 8). The maximal increase was usually obtained with 0.1–0.2 V; increasing the voltage did not necessarily increase pressure. Sphincteric activity was initially increased when all three fibers were stimulated, while the blood pressure showed a minor increase (Fig. 8) or usually no change at all.

Stimulation of either P_1 or P_2 augmented bladder pressure (≈20 cm H_2O) and sphincteric activity (Fig. 9). Post-stimulus suppression of the urethral sphincteric activity was reproducible only with stimulation of P_1 (Fig. 9). The bladder pressure response to simultaneous stimulation of P_1 and P_2 (Fig. 10) was almost as strong as that to stimulation of the complete pelvic nerve.

Along its connecting loop to the lumbosacral trunk, the L6–S1 trunk gives off a number of small branches (Fig. 3), which we named A to F cranially to caudally (Fig. 7). The number of branches was less variable than their level of origin.

Stimulation (0.1 V, 20 Hz) of the L6–S1 trunk directly distal to the pelvic nerve (Fig. 7) generated shaking of the tail and the ipsilateral hindlimb. Urethral sphincteric activity was distinctly increased – so much so that it was even possible to pick up its activity via the detrusor EMG (Fig. 10). Bladder pressure remained almost unchanged; minor undulations visible in the bladder pressure tracing were due to tail and hindlimb shaking (Fig. 10). The systemic blood pressure was consistently increased for the period of stimulation.

Test stimulation of the various branches arising from the L6–S1 trunk (the pudendal nerve in rats) revealed branches E and F to be the most selective in eliciting reasonable urethral sphincteric activity combined with the least effect on bladder pressure and tail/hindlimb movement (Fig. 8). However, blood pressure was increased constantly during stimulation, suggesting that these are not pure motor nerves.

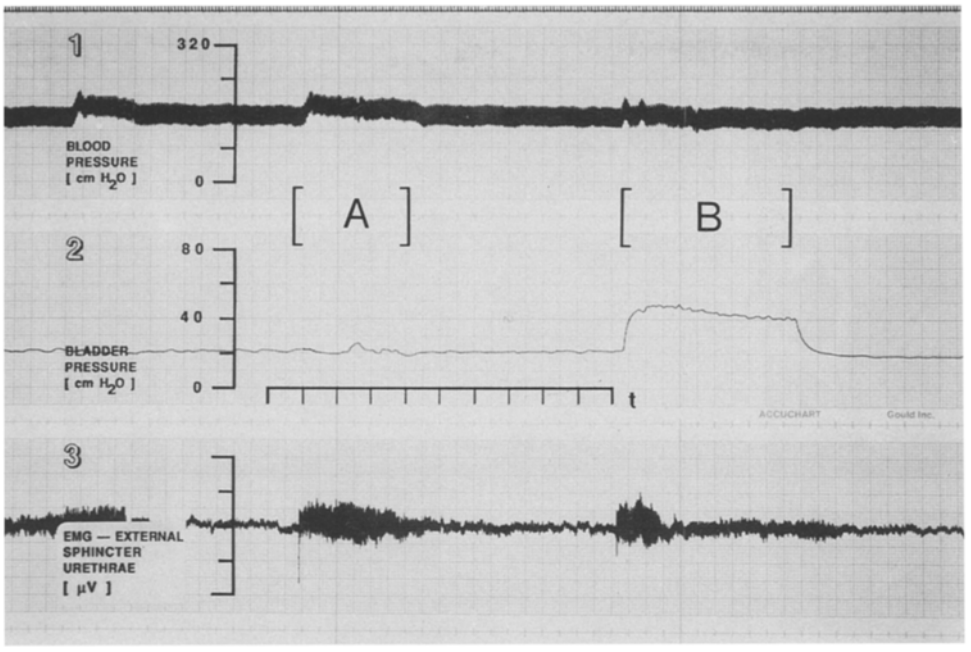


Fig. 8. A Electrostimulation of branch F of the L6–S1 trunk for 33 s; **B** electrostimulation of fibers P1, P2, and P3 of the pelvic nerve for 52 s (0.2 V, 20 Hz). Recording speed 1 mm/s (*t*, 1 unit = 10 s; *EMG*, 1 unit = 200 μV)

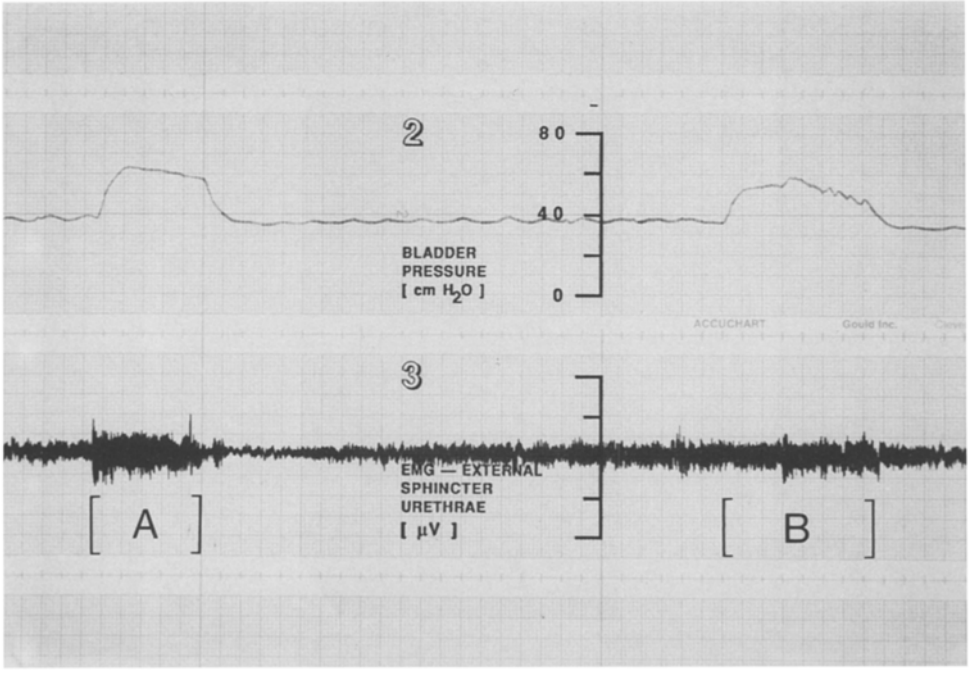


Fig. 9. A Electrostimulation of P1 for 27 s; **B** electrostimulation of P2 for 36 s (0.1 V, 20 Hz). Recording speed 1 mm/s (*EMG*, 1 unit = 200 μV)

Pharmacologic neurostimulation

The different ways in which the neuropeptides tested appear to act at the L1 level of the spinal cord will be summarized here. The intrathecal administration of 10 μg VIP caused a fall in bladder pressure of about 10 cm H_2O in 6 of 8 rats, even though their bladders were full and intravesical perfusion (21.6 μl/min) continued (Fig. 11). It took several minutes for VIP to exert its maximal effect, which sometimes lasted for more than 30 min. No significant change in urethral sphincteric activity was detectable. Particularly noteworthy is the fall in mean blood pressure combined with an increase in pressure amplitude in all animals.

The results obtained after intrathecal administration of 10 μg PHI showed a tendency (6 of 11 rats) toward suppression of the EMG activity of the external urethral sphincter for 20–30 s with a subsequent increase in activity (Fig. 12). Changes in blood and bladder pressure were minor, but frequently both appeared to increase slightly (Fig. 12). All parameters returned to baseline after about 15 min.

When 10 μg substance P was given, three different patterns of bladder pressure were generated: (1) an

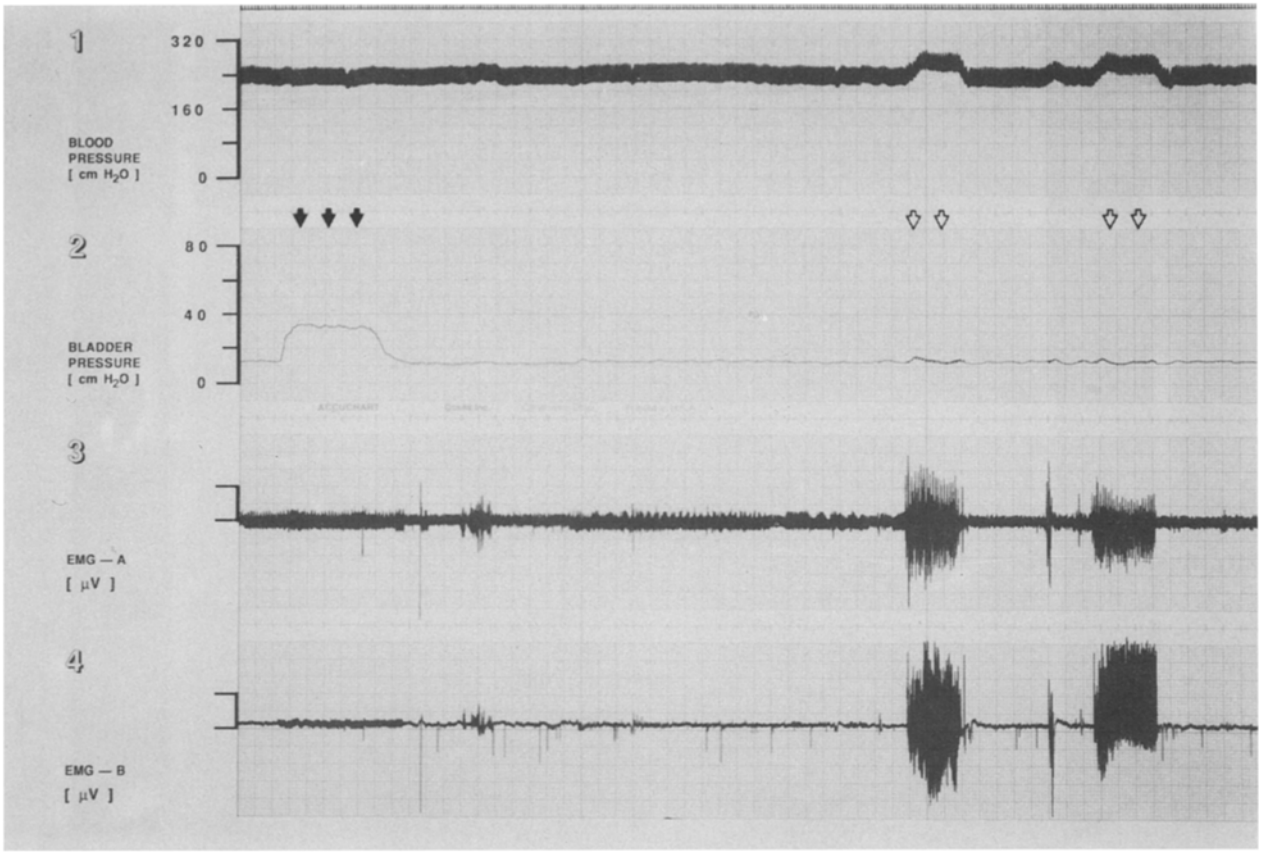


Fig. 10. *Filled arrows,* Simultaneous electrostimulation of P1 and P2 for 26 s. *Open arrows,* Electrostimulation of the L6–S1 trunk for 17 and 19 s. Note the increase in blood pressure during stimulation (0.1 V, 20 Hz). Recording speed 1 mm/s; *EMG-A,* urinary bladder; *EMG-B,* urethral sphincter; 1 unit = 100 μV

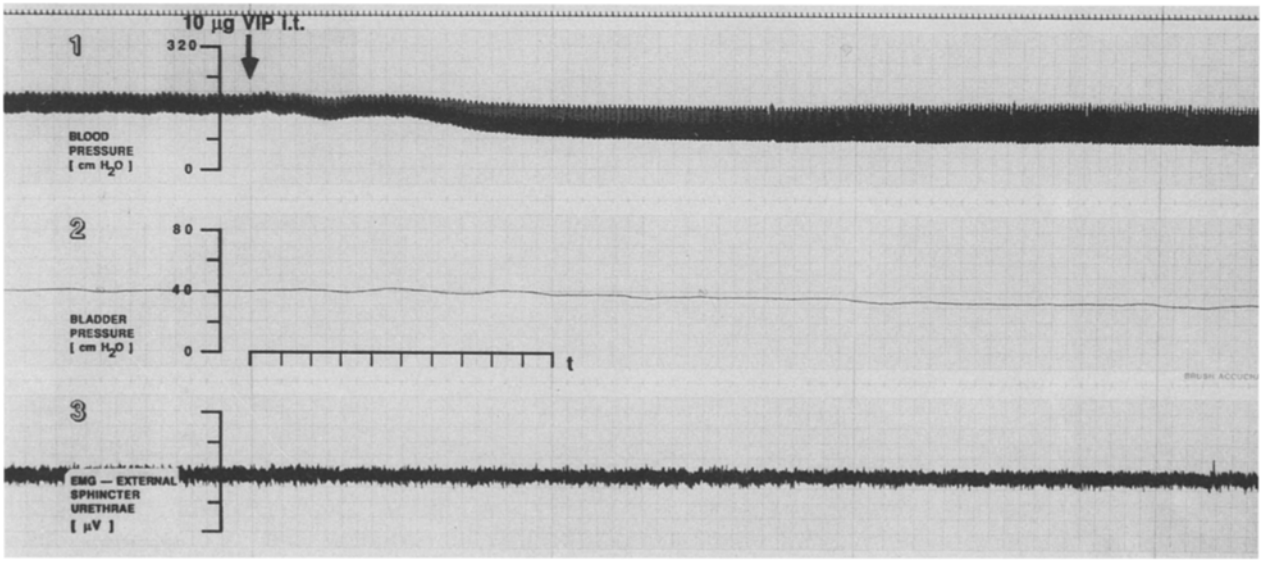


Fig. 11. Cystometogram (21.6 μl/min) in an Inactin-anesthetized rat in which intravesical perfusion continued after the bladder reached its maximal capacity. Approximately 170 s after intrathecal administration of 10 μg vasoactive intestinal polypeptide (VIP), bladder pressure dropped about 10 cm H_2O. Sphincteric activity remained unchanged. Note the fall in blood pressure with an increase in pressure amplitude (recording speed 1 mm/s; *t,* 1 unit = 5 s; *EMG,* 1 unit = 1000 μV)

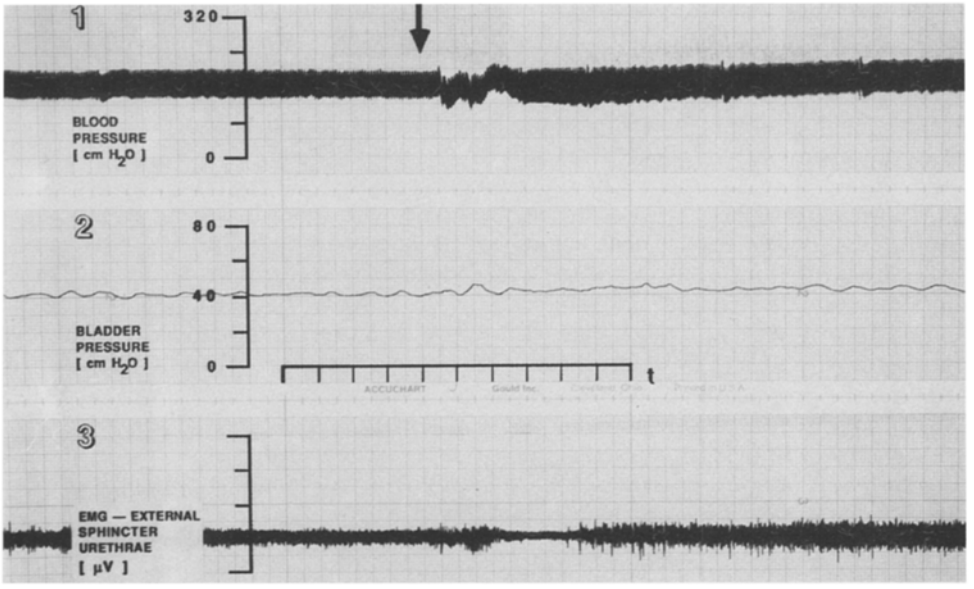


Fig. 12. Cystometogram (21.6 μl/min) in an Inactin-anesthetized rat in which intravesical perfusion continued after maximal bladder capacity had been reached. Twenty seconds after intrathecal administration of 10 μg peptide histidine isoleucine (PHI; *arrow*), sphincteric activity decreased significatly for 25 s but doubled thereafter (recording speed 1 mm/s; *t*, 1 unit = 10 s; *EMG*, 1 unit = 1000 μV)

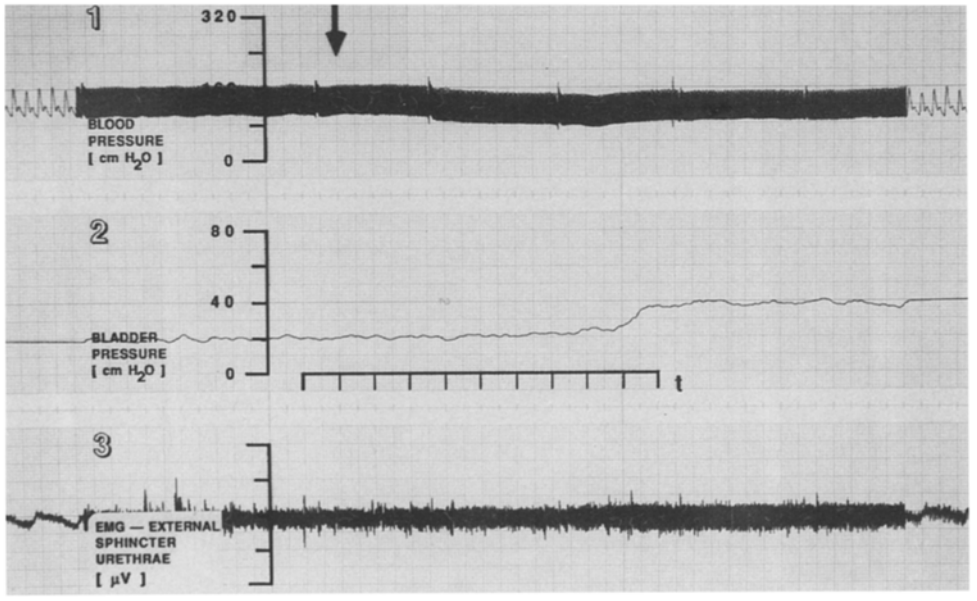


Fig. 13. Substance P (10 μg) was administered intrathecally (*arrow*) when the bladder reached approximately 75% of its capacity and the intravesical perfusion was stopped. After a 70-s delay, bladder pressure began rising and sphincteric activity increased distinctly at the same time (recording speed 1 mm/s, *t*, 1 unit = 10 s; *EMG*, 1 unit = 1000 μV)

immediate, single, strong bladder contraction ($n=5$); (2) augmentation of the micturition reflex, as indicated by strong detrusor contractions in response to intravesical perfusion ($n=4$); and (3) a gradual increase in bladder pressure to an elevated plateau ($n=8$) (Fig. 13). The sphincteric activity in all three groups was consistently increased. Most rats showed increased blood pressure, while in only 5 of 17 did blood pressure drop. Unlike VIP and PHI, substance P provoked varying side effects such as hypersalivation, lacrimation, tracheobronchial constriction, and discomfort or pain behavior (evidenced as hindlimb movement).

Discussion

This animal model has the ability to measure bladder and sphincteric activity simultaneously in a standardized preparation, thus enabling precise statements about detrusor/sphincter interrelationships and their responses to pharmacologic and electrical influence. The conditions are ideal for selective recording via electromyography of the activity of the external urethral sphincter, as in male rats this is composed of the entire intra-abdominal urethra, which is a strong striated muscular tube [11]. Furthermore, the intra-abdominal urethra is well defined because rats lack a striated muscular pelvic floor. The EMG needle is merely placed onto the urethra's surface and need not penetrate its wall. In addition, the electrode is suspended from an elastic support that allows it to follow each of the high-frequency urethral contractions. Thus, exact electromyographic measurements are feasible without disrupting the function of the external urethral sphincter.

The outstanding work by Maggi et al. [8–10] related the high-frequency oscillations in intraluminal bladder

pressure (IPHFO) sensitive to D-tubocurarine to the activation of "some urethral/periurethral skeletal muscle which accelerates urine flow." They found that not only could D-tubocurarine abolish these oscillations, but sectioning of the pudendal nerve also resulted in incompetent urine emission even though sustained detrusor contractions were still present.

In our model we demonstrated that, in rats anesthetized with urethane, the actual propulsive power for micturition is generated by the skeletal muscle of the intra-abdominal urethra (external sphincter). Any detrusor contraction during a micturition cycle that triggers and outlasts the IPHFO phase serves two basic purposes: (1) as a force from behind guaranteeing that the propulsive contractions of the urethra transport urine only in the distal direction; and (2) as a guarantee that the wide intraluminal space of the distal two thirds of the intra-abdominal urethra is always filled with urine, which can thus be emitted in small spurts.

The fine-tuning of the detrusor/sphincter synergism was very sensitive to each of the anesthetics used (thiobutabarbital sodium, methoxyflurane, urethane), and micturition cycles could be replicated in all of the rats with a low dose of urethane combined with the easily controlled inhalant methoxyflurane.

As do other barbiturates [20], thiobutabarbital sodium (Inactin) exerts a central and directly inhibitory effect on bladder and sphincteric activity, which causes suppression of the volume-evoked micturition reflex and terminates in overflow incontinence. Combined with a constant, physiologic rate of intravesical perfusion, Inactin is useful for studies where the expected changes in urodynamic parameters (owing to pharmacologic or electrical stimulation) should not be confused with a spontaneous increase in activity. We believe that agents that may reduce detrusor activity (e.g., VIP intrathecally) should not be studied in Inactin-anesthetized animals, as its suppressive effect may occlude a weak response and render it undetectable. Even though Inactin diminishes bladder and sphincteric activity, the threshold for discomfort and pain perception in rats is still low (positive pinch reflex). This characteristic permits the investigator to monitor the potential for pain of intrathecal administration of a particular neuropeptide (e.g., substance P) or electrostimulation of a nervous structure (e.g. stimulation of the abdominal sympathetic trunk).

Even though the anatomy of the genitourinary tract in rats and their physiologic micturition patterns are quite different from those of primates, our study demonstrates that the rat is a suitable model for electrostimulation. A common observation made in quadriplegic humans who receive therapeutic electrostimulation of the pudendal nerve is an increase in blood pressure during stimulation; small pressure elevations are also observed in neurologically intact humans and are sometimes combined with pleasant vibratory sensations or sensations of warmth. Furthermore, pudendal nerve stimulation is successfully used to alleviate pain in patients with pelvic pain syndrome. In all rats pudendal nerve stimulation or its equivalent was paralleled by an increase in blood pressure, which may be explained by the high number of unmyelinated sensory fibers contributing to the pudendal nerve [5], and provoked no signs of discomfort. Thus, the rat can provide the model for sophisticated experimental work to understand the phenomena of blood pressure increase and pain relief during pudendal nerve stimulation in humans.

In a chronic rat model the significance of blood pressure increase as a yet unknown side effect of permanent neural electrostimulation could be investigated; blood pressure increase due to electroneuromodulation of the pudendal nerve may be of particular interest in patients who belong to a cardiovascular risk group. It is not understood whether the relief of pelvic pain due to electrostimulation of the pudendal nerve is yielded by the neural conducted activity itself or derived secondarily by systemic or intrathecal release of transmitters (neuropeptides, endorphins?). The rat model supplies the experimental requirements to examine these topics.

In recent years a variety of neuropeptides were discovered in the central and peripheral nervous system. Many of these are located in high concentrations in the dorsal horn of the spinal cord. Among the classical neurotransmitters (monoamines and amino acids), they act as neuromodulators [14]. It seems to be accepted that some of these (e.g. substance P) are intimately involved in afferent processing of sensory information such as nociception. Our experimental preparation described here permits study of the acute effects on bladder and sphincteric activity of the influence of neuropeptides on neural circuits at the lumbosacral spinal cord level (where control of bladder and sphincteric function originates). Particularly impressive are the results obtained with intrathecal substance P: a tonic rise in bladder pressure; an increase in sphincteric activity; a lowering of the threshold for the volume-evoked micturition reflex; and discomfort or pain behavior. Substance P may be involved at the spinal cord level in the pathophysiology of detrusor hyperactivity.

In conclusion, this new and versatile rat model yields accurate and reproducible measurements of urodynamic changes consequent to electrical and pharmacologic neurostimulation.

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