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Threshold for efferent bladder nerve firing in the rat

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Van Asselt, Els, Joost le Feber, and Ron van Mastrigt. Threshold for efferent bladder nerve firing in the rat. *Am. J. Physiol.* 276 (*Regulatory Integrative Comp. Physiol.* 45): R1819–R1824, 1999.—In this study, the mechanism involved in the initiation of voiding was investigated. Bladder pressure and bladder and urethral nerve activity were recorded in the anesthetized rat. Bladder nerve activity was resolved into afferent and efferent activity by means of a theoretical model. The beginning of an active bladder contraction was defined as the onset of bladder efferent firing at a certain time (t_0). From t_0 onward, bladder efferent activity increased linearly during δt seconds (rise time) to a maximum. The pressure at t_0 was 1.0 ± 0.4 kPa, the afferent nerve activity at t_0 was 2.0 ± 0.6 μV (53 ± 15% of maximum total nerve activity), and δt was 11 ± 13 s. Between contractions the afferent activity at t_0 was never exceeded. Urethral afferent nerve activity started at bladder pressures of 2.1 ± 1.1 kPa. Therefore, we concluded that urethral afferent nerve activity does not play a role in the initiation of bladder contractions; voiding contractions presumably are initiated by bladder afferent nerve activity exceeding a certain threshold.

micturition threshold

MANY of the mechanical properties of the lower urinary tract have been described (3), as well as the anatomy of the major innervating nerves (6, 10, 21). Our aim is to develop a model that describes the quantitative relationships between mechanical properties of the bladder and urethra and the activity in the innervating nerves.

In the rat, both spinal and supraspinal reflexes play a role in the micturition cycle (16, 17). It is generally assumed that the voiding reflex is triggered by bladder afferents, which originate in the bladder wall and run through the major pelvic ganglion and, via the pelvic nerve, into the spinal cord (20). Efferent fibers originate in the spinal cord, travel via the pelvic nerve, synapse in the major pelvic ganglion, and innervate the smooth muscle cells in the bladder wall (9). The role of the hypogastric nerve in the rat still remains obscure. The thin nerves between bladder and ganglion have been called postganglionic bladder nerves (17) and contain both afferent and efferent fibers. To study bladder afferent and efferent activity separately without damaging the nerves, we developed a theoretical model to differentiate between both directions of nerve traffic in these nerves (12). In the present study, the beginning of a contraction was defined as the onset of firing in efferent bladder nerves (t_0), and nerve signals that might be involved in the initiation of bladder contractions (bladder and urethral afferent) were investigated.

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MATERIALS AND METHODS

Male Wistar rats (n = 10, mean weight 434 ± 37 g) were anesthetized with urethan (1.2 g/kg) and placed on a heated undercover. An abdominal midline incision was made to gain access to the bladder, the proximal urethra, and the innervating nerves. The left vas deferens and testis were tied, and traction was put on the major pelvic ganglion and the underlying tissue to facilitate dissection. Postganglionic bladder nerves or urethral nerves on the left side of the animal were carefully dissected from the underlying tissue and marked with sutures. The animal was then placed in a frame. The abdominal wall was tied to the frame, and the abdominal cavity was filled with warm paraffin oil. Bladder pressure was measured through a 23-gauge needle inserted near the top of the bladder and connected to a pressure transducer and monitor (Statham SP1400). The bladder was filled with saline through the same needle with a pump (Hospal K10) at an infusion rate of 0.05 or 0.1 ml/min.

A bladder nerve or a urethral nerve was mounted on a bipolar platinum-iridium electrode consisting of two wires 0.5–1 mm apart. The recorded signal was amplified by an electromyogram amplifier (DISA 15C01; amplification range 5,000–50,000×, common mode rejection >100 dB, pass band 20–2,000 Hz). Pressure and nerve signals were recorded on tape (Racal tape recorder; frequency band 0–5,000 Hz) or read into a personal computer directly at sample rates of, respectively, 10 and 25,000 Hz with a specially developed program. The tape-recorded nerve signals were read into the computer as well. All nerve signals were filtered with a 100-Hz high-pass Bessel filter (Krohn-Hite model 3944). A Bessel filter was chosen because it is a linear phase filter and does not change the shape of the signal (4). All settings were adopted from previous work in which optimal conditions for recording, amplification, and filtering were determined (12). Overall, the nerve signal that was used for analysis was filtered with a pass band of 100–2,000 Hz, thus rejecting the 50-Hz power supply interference and allowing the measurement of spikes with pulse widths down to 0.5 ms. The mean value of the rectified nerve signal in 100-ms intervals was calculated as a measure for nerve activity (Fig. 1). During voiding, bladder pressure oscillations occurred that were caused by contractions of the urethral sphincter (14, 15, 18). The period during which the oscillations took place (Fig. 1; between t_1 and t_2) was excluded from analysis because the measurement of nerve activity during this period was inaccurate as a result of movement artifacts. The quality of the nerve recording was assessed by the signal-to-noise ratio (SNR). On the assumption that the minimum activity represented noise only and that noise and nerve activity add linearly, the SNR was estimated as the mean of the 10 highest nerve activity values minus the mean of the 10 lowest values divided by the mean of the 10 lowest values. In fact, the sum of noise and nerve activity is not linear, but it is the most simple assumption. Noise mainly contributes to the integrated value of the rectified nerve signal at low firing rates; at high firing rates the contribution is less (11). The real SNR values are thus a little higher than the ones calculated.

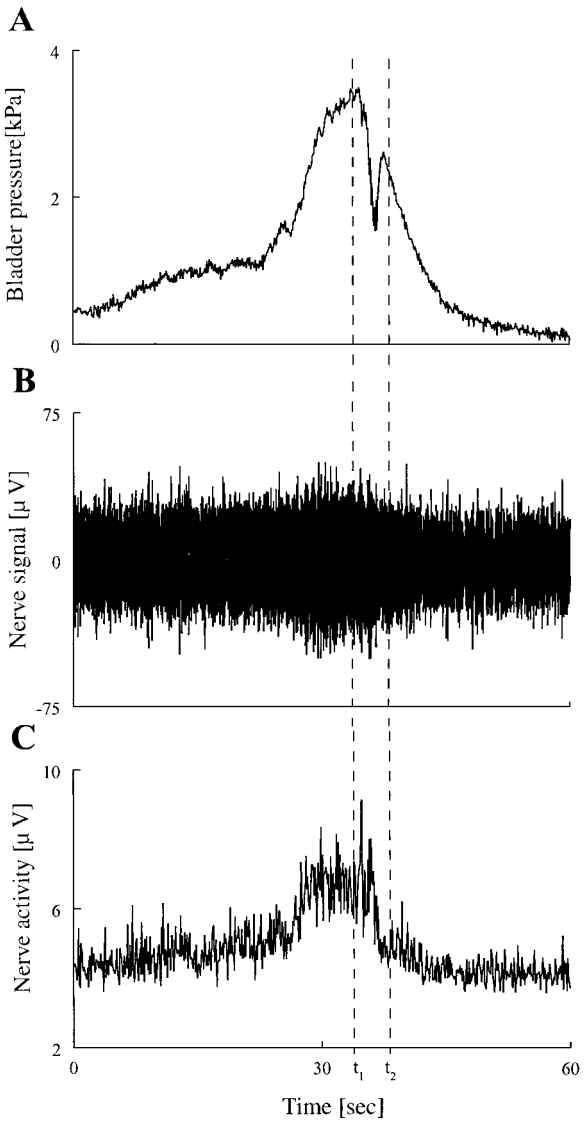

Fig. 1. Bladder pressure (*A*) and postganglionic bladder nerve signal (*B*) recorded during detrusor contraction. *C*: calculated total bladder nerve activity. Between t_1 and t_2 voiding took place. Pressure oscillations disturbed adequate measurement of nerve activity; therefore, this time period was omitted from analysis.

Animals that showed three or more contractions with an estimated SNR >1 were included in the present study. In a recorded signal with an SNR >1, action potentials could clearly be distinguished from background noise.

In a previous study, a bladder afferent-efferent model has been developed that allows the distinction of afferent and efferent bladder nerve activity in intact postganglionic bladder nerves (Fig. 2) (12). This model is based on the following assumptions: *1*) efferent bladder nerve activity is negligible during the pressure decline immediately after voiding, *2*) total bladder nerve activity is the linear sum of afferent and efferent activity, and *3*) the relation between bladder pressure and afferent bladder nerve activity can be described by a linear equation.

Because during the pressure decline after voiding all nerve activity was assumed to be afferent, the relationship between afferent activity and pressure could be determined. This relationship was then used to estimate the afferent activity during the pressure rise. Once afferent activity was known, efferent activity could be calculated by subtracting afferent activity from the measured total nerve activity.

In the present study, the initiation mechanism for efferent bladder nerve firing was investigated. Efferent activity during the period of pressure development was described using a bladder efferent model based on the following assumptions: *1*) the onset of a voiding contraction was defined at time (t) = t_0; *2*) efferent activity is 0 until t_0; and *3*) after t_0, efferent activity increases linearly during δt seconds (rise time) to a maximum value.

The pressure at t_0, the afferent nerve activity at t_0, and δt were determined in 30 contractions measured in six animals. The model was fitted to measured data by minimizing the mean squared error with a standard Simplex search method (Matlab; example in Fig. 3).

Additionally, the maximum bladder pressure, the maximum efferent bladder nerve activity, and the maximum total bladder nerve activity were determined. The maximum total activity was used for normalization; the afferent activity at t_0 and the maximum efferent activity were expressed as a percentage of the maximum total activity that allowed comparison between animals. The maximum total activity was calculated by taking the mean of 10 samples of total nerve activity just before oscillations started.

To determine if exceeding the afferent nerve activity at t_0 always resulted in a voiding contraction, long-lasting recordings including contractions and in-between rest periods were investigated. In total 107 min of recording, including 37 contractions from six animals, were analyzed.

Urethral nerve activity, measured during the pressure rise of bladder contractions, was described as a function of bladder pressure in separate experiments (4 animals, 10 contrac-

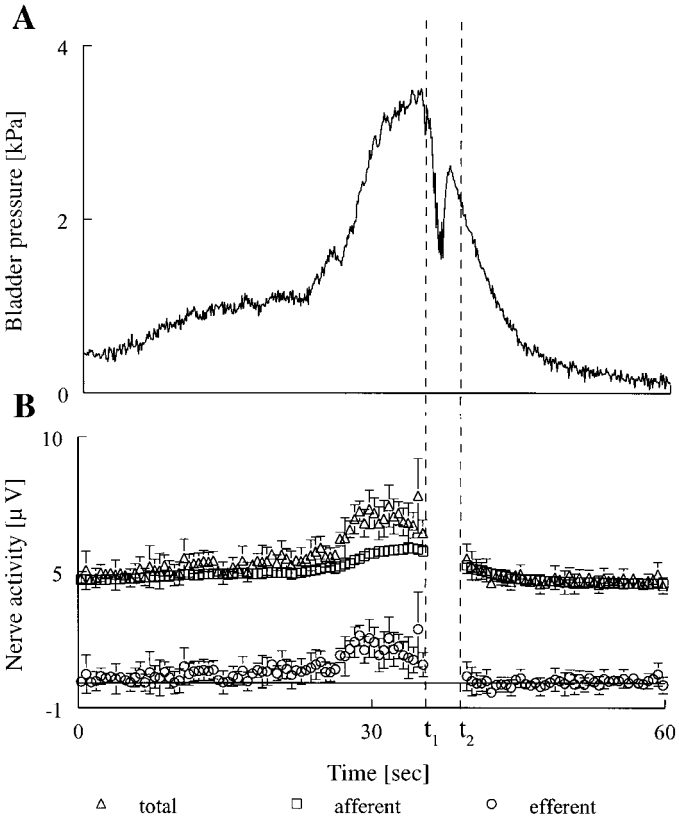

Fig. 2. Bladder pressure (*A*) and bladder nerve activity (*B*) during contraction. Total nerve activity (△) was resolved into afferent (□) and efferent (○) activity (means ± SD in 0.5-s intervals).

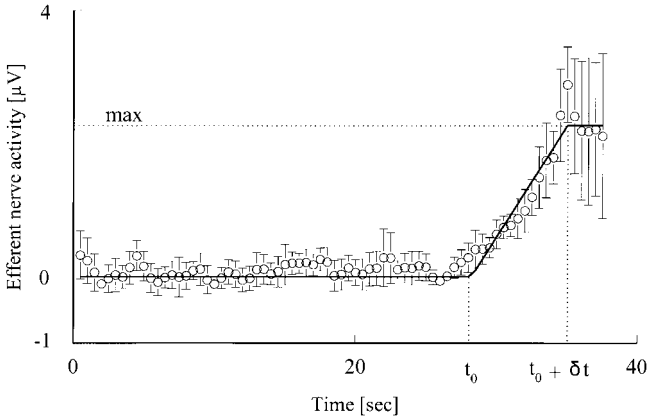

Fig. 3. Calculated (○) and fitted (solid line) efferent bladder nerve activity during pressure rise of bladder contraction (means ± SD in 0.5-s intervals). Efferent activity was assumed to be 0 until t_0, then increased linearly to maximum value in δt seconds. Fit error in this example was 5.8%.

tions). This urethral afferent model is based on the following assumptions: *1*) up to a certain bladder pressure, afferent urethral activity is constant; and *2*) above this bladder pressure, afferent urethral activity starts to increase linearly.

The analysis was restricted to the period during which the bladder pressure increased; the episode during which voiding and oscillations took place was not taken into account.

The bladder pressure at which urethral afferent firing started to increase was compared with the bladder pressure at t_0 to determine whether urethral afferents are involved in the initiation mechanism.

The reproducibility of the parameters was assessed by the coefficient of variation [%SD = (standard deviation/mean) × 100%]. To estimate the accuracy of the models, the mean difference between measured and estimated nerve activity was calculated as a percentage of the mean activity (relative fit error).

All data are presented as means ± SD. Parameter values were compared using the Mann-Whitney *U* test, Student's *t*-test, or one-way ANOVA.

Experiments were carried out as outlined in the "Erasmus University of Rotterdam Guidelines for the Care and Use of Laboratory Animals," which, in general, follows the NIH *Guide for the Care and Use of Laboratory Animals*.

RESULTS

The bladder afferent-efferent model necessary to distinguish afferent and efferent nerve activity was verified in previous experiments. Lesion experiments were described in which bladder pressure and nerve activity were recorded before and after a central cut of the bladder nerve. The remaining afferent activity was linearly related to the recorded pressure. Peripheral cutting and pelvic nerve stimulation showed that efferent activity was negligible during the pressure decline. Overall, the low fit error (6.7 ± 1.9%) prompted us to accept the model (12).

In this study, the beginning of an active contraction was defined as the onset of firing in efferent bladder nerves. The onset of pressure was not used because we have shown that the pressure starts to increase 0.8 s after the nerve activity starts (delay) (12). Furthermore, building up pressure takes time; the smooth muscle of the bladder wall itself is slow (time constant of pressure development was 3.4 s) (12).

Bladder nerve activity was measured during 30 contractions in six animals (mean weight 430 ± 32 g). The detrusor pressure at the onset of a voiding contraction (at t_0) was 1.0 ± 0.4 kPa, and the maximum pressure (just before voiding) was 3.2 ± 0.5 kPa. The mean afferent bladder nerve activity at t_0 was 2.0 ± 0.6 μV. The efferent nerve activity (just before oscillations occurred) was 1.2 ± 0.6 μV (Table 1). The efferent activity leveled off at a plateau in 23 of the 30 measurements (77%). In the other seven measurements, voiding started while the efferent activity was still increasing. In the latter cases, the efferent activity at the onset of voiding was taken as the maximum. The increase of efferent activity from 0 (at t_0) to a maximum lasted 11 ± 13 s. The average error made by fitting the bladder efferent model to the measured data was 17 ± 10% (Table 1) (example in Fig. 3).

To find out if the recorded nerve activity changed during the experiment, the relative nerve activity was plotted against time (Fig. 4). Nerve activity during the experiments was expressed as a percentage of the nerve activity at the beginning of the experiment ($t = 0$). It seemed to increase slightly with time, but the change was not significant (1-way ANOVA, $P > 0.05$).

Because absolute values of nerve activity depend on the coupling between nerve and electrode and because the coupling varied between animals, afferent activity at t_0 and maximum efferent activity were normalized. Maximum afferent, maximum efferent, and maximum total nerve activity showed coefficients of variation of, respectively, 18, 27, and 14%. Therefore, the maximum total activity was used to calculate the normalized nerve activities. The afferent activity at t_0 was 53 ± 15% of the maximum total activity, and the maximum efferent activity was 29 ± 17%. The coefficients of variation were, respectively, 11 and 22% (Table 1). The mean instilled volume at which the contractions occurred was 0.6 ± 0.2 ml.

In one animal, contractions occurred without filling the bladder; these 10 spontaneous contractions were

Table 1. *Values and reproducibility of pressure and nerve activity parameters and fit error*

	Mean ± SD	%SD
Bladder pressure at t_0, kPa	1.0 ± 0.4	15
Maximum bladder pressure, kPa	3.2 ± 0.5	7.7
Afferent bladder nerve activity at t_0, μV	2.0 ± 0.6	20
Maximum efferent bladder nerve activity, μV	1.2 ± 0.9	26
Rise time, s	11 ± 13	44
Normalized afferent bladder nerve activity at t_0, %	53 ± 15	11
Normalized maximum efferent bladder nerve activity, %	29 ± 17	22
Fit error, %	17 ± 10	

Values are means ± SD and reproducibility [coefficient of variation [%SD = (SD/mean) × 100%]] for 30 contractions of 6 animals. Mean ± SD and %SD were calculated for each animal and then averaged. Normalized values were obtained by dividing by maximum total nerve activity. t_0, Onset of bladder efferent activity; rise time, time during which efferent activity increased.

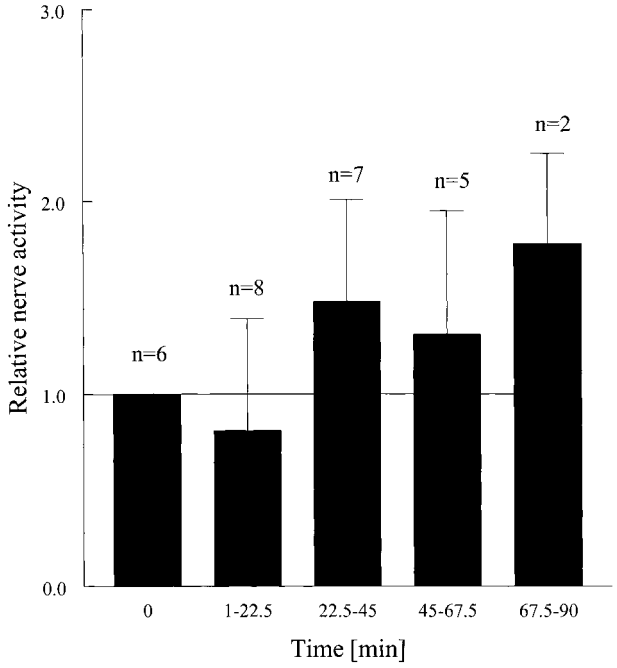

Fig. 4. Relative nerve activity as a function of time. For each of 6 animals, recorded nerve activity during experiment was expressed as percentage of activity at beginning (t = 0, nerve activity = 1). Bars represent means ± SD of all animals. Change was not significant (1-way ANOVA, $P > 0.005$).

compared with the 20 evoked contractions in the other animals. Comparison of spontaneous and evoked contractions showed significant differences: the spontaneous contractions were triggered at a lower pressure (0.4 ± 0.04 vs. 1.2 ± 0.3 kPa), reached a lower maximum pressure (2.6 ± 0.1 vs. 3.3 ± 0.5 kPa), and started at a lower level of normalized afferent activity (37 ± 4 vs. 56 ± 15%) (Mann Whitney test, $P < 0.001$). The slope of the linear relationship between pressure and afferent nerve activity, which is a measure for the sensitivity of afferent endings in the bladder wall, was significantly higher in the evoked than in the spontaneous contractions (1-way ANOVA, $P < 0.005$).

The analysis of the long-term recordings, in total 107 min, including 37 contractions and in-between rest periods, showed that the mean afferent activity ± SD was not exceeded in between contractions (example in Fig. 5). In one case, the actual voiding contraction was preceded by a small nonvoiding contraction (not shown).

Urethral nerve activity was recorded simultaneously with bladder pressure in 10 contractions from four animals (mean weight 440 ± 49 g). Fitting the urethral afferent model, assuming a constant value of nerve activity followed by a linear increase starting at a certain bladder pressure, resulted in a mean fit error of 6.0 ± 3.5% (example in Fig. 6). Afferent urethral activity was found to increase linearly with bladder pressures exceeding 2.5 ± 1.1 kPa. This pressure was significantly higher than the pressure at which efferent bladder nerves started to fire (1.0 ± 0.04 kPa) (t-test, $P < 0.01$).

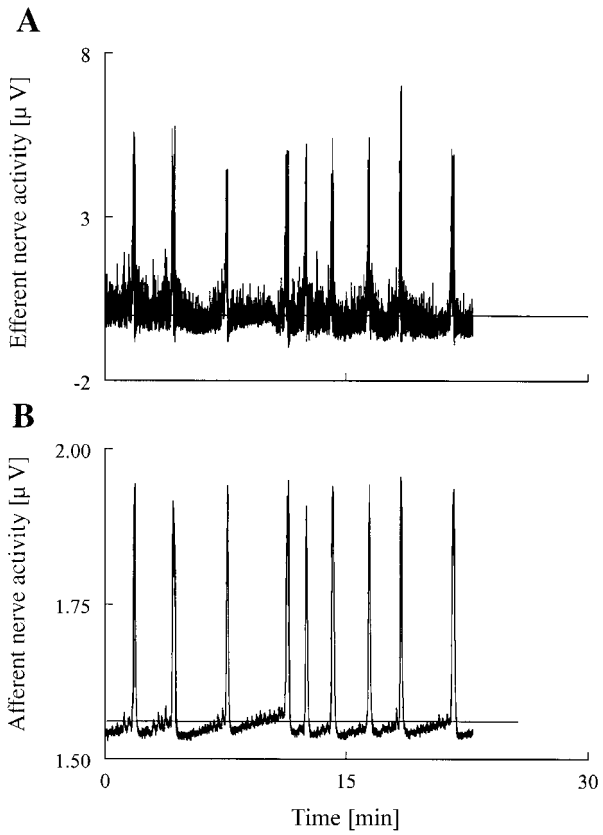

Fig. 5. Example of efferent bladder nerve activity (*A*) and afferent bladder nerve activity (*B*) during continuous recording (23-min period includes 9 spontaneous contractions). Solid line in *B* shows mean afferent bladder nerve activity at t_0 (1.56 μV) for all contractions included.

DISCUSSION

In the literature, bladder pressure thresholds have been defined as the trigger for efferent activity. The values presented, 0.74 ± 0.2 kPa (17), 0.67 ± 0.05 kPa (14), and 0.89 kPa (7) (male Wistar rats; filling rate, respectively, 0.052, 0.1, and 0.2 ml/min), are in the same range as the bladder pressure at t_0 in our study:

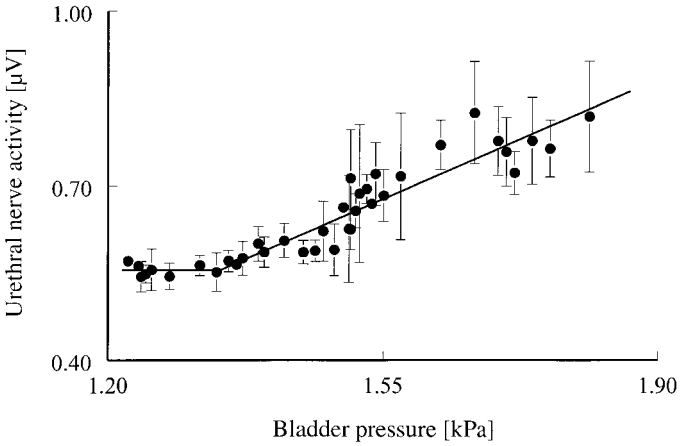

Fig. 6. Example of urethral afferent nerve activity as a function of bladder pressure (means ± SD in 0.3-s intervals). Straight line was fitted to increase. Fit error in this example was 5.3%.

1.0 ± 0.4 kPa. The volumes at which the contractions occurred, 0.59 ± 0.06 and 0.6 ± 0.4 ml (14, 17), are the same as the 0.6 ± 0.2 ml reported here. Micturition pressures of 3.4 ± 0.8 kPa (17) and 3.8 kPa (7) correspond well with the 3.2 ± 0.5 kPa found in this study.

Instead of defining the pressure at which the active contraction starts, we determined the level of bladder and urethral afferent nerve activity at the onset of efferent bladder nerve activity. Most work relating to bladder nerve activity has been done on afferent fibers. In the rat, a very low continuous basal activity is described when the bladder is empty, followed by a monotonic increase of activity in afferent pelvic nerve fibers in response to increasing pressures during bladder distension (22). Moss et al. (19) described hypogastric fibers with a low basal activity and a linear relationship between nerve activity and pressure. Pelvic afferents showed little or no activity when the bladder was empty but reacted to bladder filling promptly at a pressure threshold of 0.4–0.8 kPa (19).

Bahns et al. (1) describe ongoing activity in both hypogastric and pelvic bladder afferents and an increasing activity with increasing intravesical pressure up to 13.6 kPa in the cat. Several authors have made a distinction in the rat between low- and high-threshold afferent fibers. These fibers start to fire at, respectively, 0.76 ± 0.14 and 4.6 ± 0.34 kPa (22) and below or above 5.4 kPa (8). In the cat, the mean pressure threshold for hypogastric fibers was 1.96 ± 0.9 kPa (1) and 1.12 ± 0.27 kPa for pelvic fibers (2).

In our study, no distinction was made between hypogastric and pelvic fibers because we recorded activity between the major pelvic ganglion and the bladder. Furthermore, presumably only low-threshold fibers were recorded, because measured bladder pressures did not exceed 4.0 kPa.

Hosein and Griffiths (5) stated in their simulation model that bladder afferents initiate and urethral afferents sustain voiding contractions. In our study, bladder efferent activity could adequately be described by the bladder efferent model, assuming this activity to be 0 until a certain time, t_0, and then to increase linearly during δt seconds until a certain maximum. The afferent activity at t_0 (2.0 ± 0.6 μV) and the pressure at t_0 (1.0 ± 0.4 kPa) showed good reproducibility (respectively, 20 and 15%). It was thus concluded that efferent activity is triggered at an afferent activity of 2.0 ± 0.6 μV and then increases linearly. Efferent bladder nerve activity reached a maximum in 11 ± 13 s (δt). This corresponds well with the finding that the rise in pressure reaches its maximum within 3–10 s (15). When the efferent bladder model was applied to 30 contractions from six animals, the mean fit error was only 17 ± 10%, which shows that it describes the measured data well.

It is very difficult to compare nerve activities because the absolute values depend on the electrical coupling between nerve and electrode, and this varied between animals. The maximum total activity was used as a normalization standard. Afferent activity increased from 53% at t_0 to 71% just before voiding; efferent activity increased from 0 to 29%.

To verify if exceeding the value of the afferent activity at t_0 always resulted in voiding contractions, long-term periods were analyzed. Only in one case did exceeding the afferent bladder nerve activity at t_0 lead to a small nonvoiding contraction that preceded the actual voiding contraction. This is a well-known phenomenon; Maggi et al. (15) described ineffective micturition contractions preceding effective ones in 20% of the preparations. Overall, it can be concluded that there is a distinct value of afferent bladder nerve activity that reproduces well and when exceeded always leads to a contraction.

Comparison of evoked and spontaneous contractions showed significant differences in bladder pressure at t_0 and the normalized afferent activity at t_0. Apparently, the spontaneous contractions were triggered at a lower pressure and started at a lower level of afferent activity than contractions evoked by filling. The lower detrusor pressure threshold can be explained by either a higher sensitivity of afferent fibers (peripheral cause) or by bladder contractions triggered at a lower level of afferent bladder nerve activity (central cause). The results of our experiments contradict the first explanation because the slope of the linear relationship between pressure and afferent nerve activity, which is a measure for the sensitivity of afferents, was significantly lower in the spontaneous contractions. Therefore, the cause of the difference in threshold has to be found in the central nervous system, as suggested by Jiang and Lindström (7).

When afferent urethral nerve activity was recorded simultaneously with bladder pressure (13), it was found to increase at a bladder pressure exceeding 2.5 ± 1.1 kPa, which is significantly higher than the bladder pressure at t_0 (1.0 ± 0.4 kPa); thus efferent bladder activity starts at lower bladder pressures than afferent urethral activity. The absence of urethral afferent activity at low bladder pressures may be caused by a higher threshold pressure for urethral afferents but also by a closed bladder neck, which prevents urethral pressure from increasing with bladder pressure.

In summary, it was shown that efferent bladder nerve activity is not an all-or-nothing phenomenon but starts at a certain level of afferent activity and then increases to a maximum value in 77% of the cases. In 23% of the recorded voidings, a maximum efferent activity was not reached before voiding started.

The normalized afferent bladder nerve activity at t_0 (53% of the maximum total activity) reproduced well (%SD was 11%), and every time it was exceeded, a bladder contraction followed.

The detrusor pressure at which efferent bladder nerve activity started was 1.0 ± 0.4 kPa; urethral afferent activity started to increase at a detrusor pressure of 2.5 ± 1.1 kPa.

We therefore conclude that urethral afferent nerves do not play a role in the initiation of a bladder contrac-

tion and that there is a definite, reproducible threshold in afferent bladder nerve activity that has to be exceeded for efferent bladder nerves to start firing.

Perspectives

The aim of our work is to quantitatively describe the relationships between mechanical properties of the lower urinary tract and the activity of the innervating nerves. In rats, bladder and urethral pressure and urethral flow rate, as well as the activity of bladder and urethral nerves, are measured and quantitatively related. In the present study we have determined what triggers an active micturition contraction and which nerves are involved.

An overall model of the lower urinary tract, describing its components and the way in which they interact, will hopefully lead to the development of new or better diagnostic modalities and/or treatment options in urology.

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