Neurophysiological modeling of voiding in rats.

Neurofysiologische modelvorming van de urinelozing bij ratten.

Proefschrift

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1. Introduction.

INTRODUCTION

Like man, most animals need a regular supply of nutriments. Eating and drinking cater for this need, but not with 100% efficiency. A considerable part of the consumed goods are expelled from the body as defecation. Waste products from the blood are filtered by the kidneys and excreted as urine. This process continues throughout the day. In many situations, however, it would be very inconvenient to be expelling urine. Animals that leak urine continuously are more easily traced by preditors than the ones that don't. Besides this, for mankind it is socially intolerable at certain moments. Nature supplied a solution that enables storage of urine until a convenient moment for voiding: the bladder. Upon desire the bladder contracts and urine is expelled through the urethra, which, with the bladder, constitutes the lower urinary tract. Thus the bladder owner controls when and where to expel urine.

Occasionally the lower urinary tract does not perform properly, which may cause severe problems to the patient. The bladder may fail to contract or the resistance of the urethra may be too high to allow adequate bladder voiding. To be able to distinguish between these possible causes for dysfunction, the mechanical properties of the lower urinary tract have been investigated extensively in the last decades. This has resulted in mechanical models of the lower urinary tract. Also, new methods and techniques to diagnose dysfunction of bladder and/or urethra have been developed.

A frequently used technique called cystometry, involves measurement of bladder pressure and flow rate during voiding. To enable these measurements, the bladder is filled at a pace ~10 times higher than the natural renal excretion, while a pressure transducer inserted through the urethra into the bladder monitors the intravesical pressure. The flow rate is usually measured externally. The technique enables differentiation between low bladder contractility and a high urethral resistance. However, it does not address possible dysfunction of the innervation of bladder and urethra.

Efficient bladder voiding requires coordinated action of the bladder and the urethra. In the storage phase the bladder pressure is maintained low while the urethral muscles are contracted strongly enough to prevent leakage. In the voiding phase the bladder contracts and the urethra relaxes. This proces is called micturition. Both contractions and relaxations are controlled by the central nervous system. Nerve fibers run between the spinal cord and the bladder and the urethra. Two types of nerve activity play a role in the micturition reflex. Afferent nerve signals originate within the organ and provide the central nervous system with information on the physical state of the bladder and urethra. Efferent nerve signals are sent by the central nervous system to the bladder or the urethra to evoke contraction or relaxation of muscle tissue.

In this thesis the existing mechanical models have been extended with neurophysiological relations between nerve activity controlling the lower urinary tract and mechanical variables such as bladder pressure and urethral pressure and flow. Detailed knowledge of the nervous control system of the lower urinary tract may enable the development of new diagnostic methods or treatment of neurogenic dysfunction. In paraplegics, for example, the control of the lower urinary tract could perhaps be taken over by a computer that uses afferent nerve activity as input, if all the nerve signals involved were known in sufficient detail.

Basically any neurophysiological model of the lower urinary tract consists of three steps. In the first step measures for bladder fullness and urethral flow are transformed into afferent nerve signals, which are sent to the central nervous system. We investigated the relations between mechanical variables of the lower urinary tract and the afferent nerve signals. Using these signals, the central nervous system coordinates the functioning of the bladder and urethra.

The second step describes the reaction of the central nervous system to the received afferent nerve signals. This may involve efferent activity to the bladder, to the urethra, or to both. In this thesis we investigated the efferent nerve signals to the bladder in response to afferent activity from the bladder and the proximal urethra.
In the third step efferent activity to the bladder or the urethra results in contraction or relaxation of muscle fibers. We determined how the bladder and the urethra respond to efferent nerve signals from the central nervous system.

The obtained relations were based on rat experiments, and are not necessarily valid for the human urinary tract. However, there are large similarities between the human and rat bladder and urethral innervation, and it seems likely that the majority of the results in this thesis may be extrapolated to the human system.

Nervous control of both the human and rat lower urinary tract is accomplished through three major nerves: the pelvic nerve, the hypogastric nerve and the pudendal nerve. The bladder is innervated by the pelvic and hypogastric nerves and the urethra probably by all three.
One chapter, however, is dedicated to a phenomenon that is observed in rats only. During voiding the rat urethral sphincter contracts 6-10 times per second. This seems contradictory to the desired relaxation of the urethra in the voiding phase. However, it appeared that these oscillatory urethral contractions result in an effective urethral relaxation, which is maximum at ~10 contractions per second. In patients treated with neuromodulation the pudendal nerve is electrically stimulated, usually at this frequency. Although this treatment is meant to suppress instable bladder contractions, one of the yet unexplained side effects is urethral relaxation.
Thus, even this specific rat behaviour may provide insight into yet not understood principles of human urethral relaxation.

Although not all aspects involved in the micturition reflex are investigated in the study presented in this thesis, it brings computerized control over the lower urinary tract much closer.

2. Neurophysiological modeling of voiding in rats: bladder pressure and postganglionic bladder nerve activity.

Joost le Feber, Els van Asselt and Ron van Mastrigt
Am. J. Physiol. 272 (Regulatory Integrative Comp. Physiol. 41) R413-R421, 1997.

ABSTRACT

A model was developed that describes the relations between bladder pressure and both efferent and afferent nerve activity in post ganglionic bladder nerves in urethane-anesthetized rats. Nerve activity was calculated as the 100 msec time integral of the rectified nerve signal. Afferent and efferent nerve signals were measured separately by crushing the nerve proximally or distally. A linear relation was found between bladder pressure and afferent nerve activity (fit error < 7%) and the relation between bladder pressure and efferent nerve activity was described by a low-pass filter (fit error < 9%). In most experiments combined (efferent and afferent) nerve activity was measured. Absence of efferent nerve activity was assumed during the pressure decrease immediately after voiding. From this episode the afferent nerve activity was estimated for the entire measurement. Efferent nerve activity was then estimated assuming linear addition in the bidirectionally conducting nerves. The model described the measured data very well (fit error < 7%) and the model parameters showed a good reproducibility in each rat (SD was ~ 30% of the mean).

Key words: Detrusor contraction; quantitative model; efferent and afferent nerve activity

INTRODUCTION

Many of the mechanical properties of bladder and urethra have been investigated and described (5). Based on these mechanical properties clinical methods have been developed to examine patients who suffer from voiding disorders. Using these methods different causes for voiding complaints, more specifically low bladder contractility or high urethral resistance, can be discriminated (11,12). A model that combines mechanical and (neuro)physiological properties might enable the development of diagnostic methods for the further differentiation between myogenic and neurogenic dysfunction of the lower urinary tract. Other researchers qualitatively investigated the innervation of the bladder and the urethra (2,3,9,13,16,17). Based on their results, the relations between bladder pressure and both efferent (NA_{eff}) and afferent nerve activity (NA_{aff}) can be drawn schematically, as in Figure 1. However, the exact mechanisms by which the afferent fibers reflect the current status of the bladder and by which the efferent fibers control bladder contractions are yet unknown (Fig. 1). A model that describes quantitative relations between mechanical properties of the lower urinary tract and nerve signals has to our knowledge not yet been published. As a first step towards such a complete model, in this study the activity of rat postganglionic bladder nerves during the micturition cycle was investigated. The aim of our study was thus to find quantitative expressions for H_1, H_2 and H_3 in Figure 1, using a black box model. One of the major problems that arose was the bi-directional conductance of postganglionic bladder nerves, so that a combination of efferent and afferent nerve activity was measured. The relation between

the measured nerve activity (NA_{tot}) and efferent and afferent nerve activity was symbolised in a separate transfer function in Figure 1 (H_3).

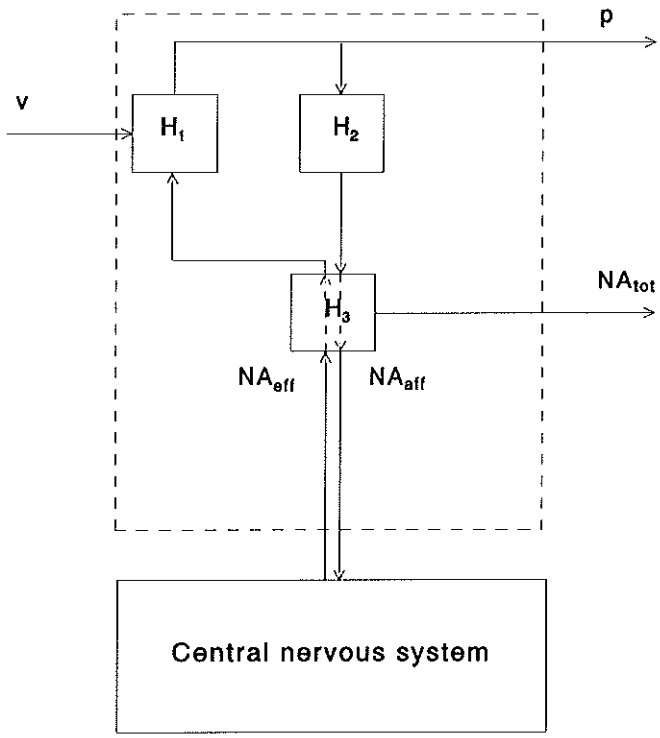

Figure 1. *Schematic drawing of the relations between pressure and both efferent (H_1) and afferent nerve activity (H_2). As it is practically impossible to separately measure efferent (Na_{eff}) and afferent nerve activity (NA_{aff}), in the experiments a combined signal is measured (NA_{tot}). H_3 is the transfer function that describes the relation between the combined signal NA_{tot} and NA_{eff} and NA_{aff}*

A method was developed to separate efferent and afferent nerve traffic, and the relations between bladder pressure and both efferent (H_1 in Fig. 1) and afferent nerve signals (H_2 in Fig.1) as well as the relation between total nerve activity and efferent and afferent nerve activity (H_3) were mathematically formulated. The assumed formulas were the simplest relations that could describe the results of previous studies (2,3,4,14). In a number of separate experiments the plausibility of the separate assumptions on which the model is based was investigated. Additionally the adequacy and reproducibility of the mathematical model were demonstrated.

MATERIALS AND METHODS

Surgery

Male Wistar rats were anaesthetized with urethan (1.2g/kg) intraperitoneally (8) and placed on a heated undercover. The trachea was dissected, tied and incised distally from the tie to avoid breathing difficulties caused by mucus.

An abdominal midline incision was made to access the nerves and the bladder. The left vas deferens and testicle were tied and laid outside the animal to put traction on the major pelvic ganglion and the underlying tissue. This traction facilitated the dissection of the pelvic nerve and one of the postganglionic bladder nerves (PBNs). These PBNs lead from the major pelvic ganglion (MPG) to the bladder. The pelvic nerve and a PBN on the left side were carefully freed from the underlying tissue and marked with sutures. The nerves on the right side were left intact. The abdomen was kept moist with warm saline. The animal was placed in a frame with a heated ground plate. The abdominal wall was then tied to the frame to create a basin which was filled with warm paraffin oil during the measurements.

Experimental setup

Two bipolar platinum-iridium electrodes mounted on the frame were lowered into the paraffin oil basin. The electrodes consisted of two metal hooks at a distance of approximately 0.5 mm. A postganglionic bladder nerve and the pelvic nerve were guided over the electrodes, which were slightly elevated. The pelvic nerve electrode was used for stimulation only, the bladder nerve electrode was used for recording. The stimulation signal was generated by a Hameg HM8130 programmable function generator. The recorded nerve signal was amplified by a DISA 15C01 EMG amplifier (amplification range: 5,000-50,000) and band-pass filtered (Bessel, 4^{th} order; Krohn-Hite model 3944). Cut off frequencies were manually set in a way that maximized the difference between action potentials and background noise. For this reason the signals were displayed on an oscilloscope. The pass band was usually set from approximately 200 Hz to approximately 2 kHz, thus rejecting 50 Hz power supply interference and allowing measurement of spikes with pulsewidths down to 0.5 msec (15). The recording electrode was moved along the PBN until the difference between background noise and action potentials on the oscilloscope was maximal. The combination of the symmetrical electrodes and an EMG amplifier with a very high common mode rejection ratio (CMRR >100 dB) ensured a minimal 50 Hz power supply interference and radio frequency interference.

A 23G needle was inserted near the top of the bladder. Through this needle the bladder was filled with room-temperature saline while the intravesical pressure was measured using a disposable pressure transducer and a Statham SP1400 blood pressure monitor. The bladder was filled with a Hospal K10 pump at an infusion rate of either 0.05 or 0.1 ml/min. The pressure and bladder nerve signals were read into a personal computer at sample rates of 10 Hz and 25 kHz respectively, using especially developed software driving a PCL 818 A/D converter. To select an appropriate measurement interval the data was continuously read into a 30 seconds ringbuffer and the pressure was displayed on the PC screen. After voiding this buffer was saved as soon as the pressure had returned to baseline.

Modeling

A model was developed, relating bladder pressure and both efferent and afferent nerve activity during 30 seconds episodes consisting of an initial pressure rise ($t < t_1$), the actual voiding ($t_1 < t < t_2$) and a pressure decline after voiding ($t > t_2$) (see Fig. 2).

A measure for total nerve activity as a function of time (NA(t)) was calculated as the 100 msec time integral of the rectified nerve signal (1,6):

$$NA(t) = \frac{\int_{100msec} ENG(t)}{100msec} \tag{1}$$

Where ENG (electro neurogram) represents the nerve signal.

The value of the integral was divided by 100 msec so that NA(t) represents the average amplitude of the nerve signal (18). In our setup this signal consisted of 2,500 samples in each

$$NA(t) = \frac{\sum_{i=1}^{2500} |ENG[i]|}{2500} \tag{2}$$

integration interval, so that Eq. 1 was approximated by:

A number of assumptions was made. It was assumed that efferent nerve activity was negligible during the pressure decline immediately after voiding ($t > t_2$ in Fig. 2).

The simplest relations for H_1, H_2 and H_3 were assumed that could describe the results of measurements done by others (2,3,4,14): the calculated total nerve activity ($NA_{tot}(t)$) was assumed to be the linear sum of efferent ($NA_{eff}(t)$) and afferent ($NA_{aff}(t)$) nerve activity, it was assumed that the relation between bladder pressure and afferent nerve activity could be described by a linear equation and that the bladder pressure increase above a constant baseline pressure could be estimated by low-pass filtering of the (delayed) efferent nerve activity. This last assumption was based on the amplitude attenuation and phase-shift of the transfer function relating nerve activity and force development in the triceps surae muscle (14), which can adequately be described by a low-pass filter. The system of assumptions that constitutes the model can thus be formulated as follows:

$$NA(t)_{tot} = NA(t)_{eff} + NA(t)_{aff} \tag{3}$$

$$NA_{eff}(t) = 0 \;\forall\; t > t_2 \tag{4}$$

$$P_{est}(t) = K_1 \cdot \exp(-\alpha t) * NA_{eff}(t-\tau) + P_0 \;\forall\; t \notin [t_1, t_2] \tag{5}$$

$$NA_{aff}(t) = K_2 \cdot P_{meas}(t) + NA_0 \tag{6}$$

In these equations p_0 represents baseline pressure and NA_0 covers both background noise and background nerve activity, which may be found in some fibers (2,3). The four model parameters of interest are K_1, K_2, α and τ. In Eq. 5, which describes a low-pass filter in the time domain, the asterisk denotes a convolution, α is the cut off frequency of the low pass filter and K_1 is the gain. τ is the latency between nerve activity and pressure development.
The model equations were fitted to the experimental data by minimizing the mean squared error ($\Sigma|P_{meas} - p_{est}|^2$) using a linear regression algorithm. To estimate the relative quality of a fit the mean difference between measured (p_{meas}) and estimated pressure values (p_{est}) was calculated as a percentage of the mean pressure during the recorded episode.

$$Fiterror = \frac{\sum |p_{meas} - p_{est}|}{\sum p_{meas}} \cdot 100\% \tag{7}$$

The model was not fitted during voiding ($t_1 < t < t_2$ in Fig 2) since the effect of flow on the bladder pressure is not accounted for in the model yet.

Measurements
Four different types of measurement were done:
1) In each rat the bladder was filled with saline until a voiding contraction occurred. Bladder pressure and postganglionic bladder nerve signals were recorded during the voiding contraction. As a result of differences in electrical coupling between nerve and electrode, the signal to noise ratio (SNR) in the nerve signal varied. The SNR was estimated as the difference between the maximum and minimum value of the calculated nerve activity during the recorded episode divided by the minimum. It was assumed that this minimum value represented noise only.

$$SNR_{est} = \frac{\mathrm{Max}(NA_{tot}) - \mathrm{Min}(NA_{tot})}{\mathrm{Min}(NA_{tot})} \tag{8}$$

To derive a stable estimate for SNR_{est}, $Max(NA_{tot})$ and $Min(NA_{tot})$ were taken as the mean values of the 10 highest samples and the 10 lowest samples respectively. Model parameters were only calculated from measurements with $SNR_{est} \geq 1$. This threshold value was based on audio-visual judgement of the nerve signals. It selected measurements with action potentials that could clearly be seen on the oscilloscope. To verify to what extent the SNR_{est}-criterion biased the results, the average parameter values were also calculated for a (larger) set of measurements, selected using a lower threshold value of 0.9.

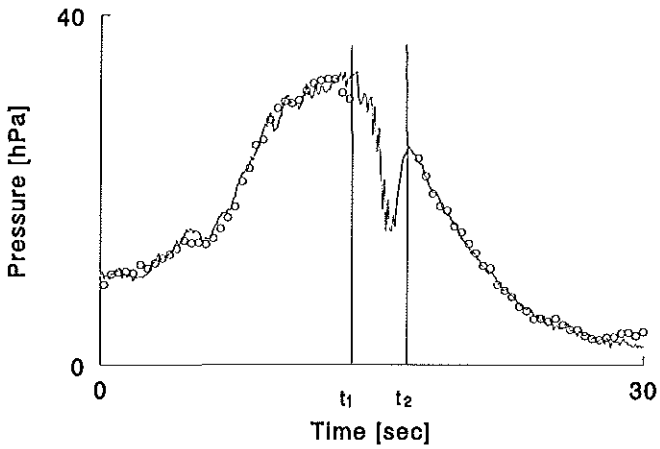

Figure 2. *Pressure development during a voiding contraction in the rat (line). In the first part of the graph ($t < t_1$) pressure developed isovolumetrically by bladder contraction. In the second part ($t_1 < t < t_2$) voiding occurred. In the last part ($t > t_2$) the pressure decreased.* o, *estimated pressure values calculated from the simultaneously measured nerve activity using the developed model.*

The calculation of the model parameters was done in four steps - K_2 was calculated from the episode immediately after voiding ($t > t_2$ in Fig 2) by fitting Eq. 6; in this period it was assumed that there was only afferent nerve traffic as indicated by Eq. 4. - Once K_2 was known, $NA_{aff}(t)$ was calculated from Eq. 6 for the periods $t < t_1$ and $t > t_2$. - Then $NA_{eff}(t)$ was calculated using Eq. 3 and finally - Eq. 5 was fitted yielding values for K_1, $\alpha$and τ. Thus there were two steps that involved parameter estimation: in the first step K_2 was estimated and in the last K_1, α and τ. A simultaneous estimation of all parameters is impossible because in that situation the error function ($\Sigma(P_{meas}-P_{est})^2$) has an absolute minimum at the non-physiological parameter values $K_1 \rightarrow 0$ and $K_2 \rightarrow \pm \infty$ (see appendix). The primary estimation of K_2 is possible because there is a period in the measurement ($t > t_2$) where NA_{eff} is assumed to be negligible, so that NA_{aff} is identical to the measured NA_{tot}. There is not such a period in the measurement during which NA_{aff} is negligible. Therefore efferent nerve activity was calculated in the second step by subtracting the calculated NA_{aff} from the measured NA_{tot}.
For the determination of t_1 and t_2, the pressure was displayed on a computer screen. Using a mouse cursor t_1 and t_2 were selected at the approximate pressure maxima immediately before and after voiding. To examine the sensitivity of the model parameters for changes in t_1 and t_2,

the relative changes in the parameter values were calculated after applying a small change of approximately ½ sec in t_1 and t_2.

2) After these baseline measurements in some animals the postganglionic bladder nerve in which the total nerve signal had been measured, was distally crushed. The nerve was firmly squeezed with a pair of tweezers between the electrode and the urinary bladder. In this way afferent nerve traffic was eliminated from the total nerve signal. Bladder contractions still occurred because the other left-side PBNs and all the PBNs on the right hand side were left intact. Since nerve activity in the crushed PBN was reduced to efferent activity, less total activity was measured. This resulted in lower values of SNR_{est}. For this reason type 2 measurements were accepted if $SNR_{est} \geq 0.5$. Again the model parameters were fitted to the measured data as described above. As it could not be avoided that the nerve moved with respect to the recording electrode when it was squeezed, possible changes in the model parameters could have been caused by two factors: the changed characteristics of the nerve and the change in electrical coupling. The effectiveness of the crush was therefore verified by calculating the dimensionless product $K_1 \cdot K_2$. This product is independent of changes in electrical coupling, which can be understood as follows:
A signal attenuation of a factor γ generally affects both efferent and afferent nerve activity. If the pressure remains unchanged, the value that is found for K_1 from Eq. 5 will increase by a factor γ, and the value of K_2 in Eq. 6 will decrease by this factor. Thus the product has a value which is independent of the electrical coupling. A crush however, should affect afferent nerve activity only and thus only the value of K_2 should change, resulting in a different value of K_1K_2. The only reason to calculate a value for K_2, was to verify wether afferent activity had adequately been eliminated. If so the value for K_2 should be (close to) 0. Measurements that had similar K_1K_2 values before and after an attempted crush were therefore not taken into account; it was assumed that the crush had not been effective.

3) In other rats nerve activity in the postganglionic bladder nerves was reduced to afferent activity by a proximal crush of these nerves. The effectiveness of this crush was tested by stimulation of the pelvic nerve before and after the crush. The applied stimulation had an amplitude and a frequency that generated action potentials that could clearly be detected in the recorded postganglionic bladder nerve signal before the crush. Measurements were accepted when stimulation at this frequency and amplitude did not evoke action potentials in the crushed nerve. Only Eq. 6 was fitted to these results.

4) In the last type of measurement the pelvic nerve was stimulated (duration: 15 sec, frequency: 10 Hz, amplitude: 3 V, pulsewidth: 400 μs) to evoke bladder contractions. During these contractions nerve activity in the postganglionic bladder nerve and bladder pressure were measured. The last 5 seconds of stimulation and a ten seconds period immediately after stimulation were recorded. In this way the effects of a sudden stop of efferent nerve activity on bladder pressure were studied. The stimulator caused artifacts in the recorded postganglionic bladder nerve signal that could easily be recognized. Since the calculated nerve activity was biased by these artifacts no reliable reading could be established for the nerve

stimulation. As a result no values for K_1 could be derived when Eq. 5 was fitted, but α and τ could adequately be estimated.

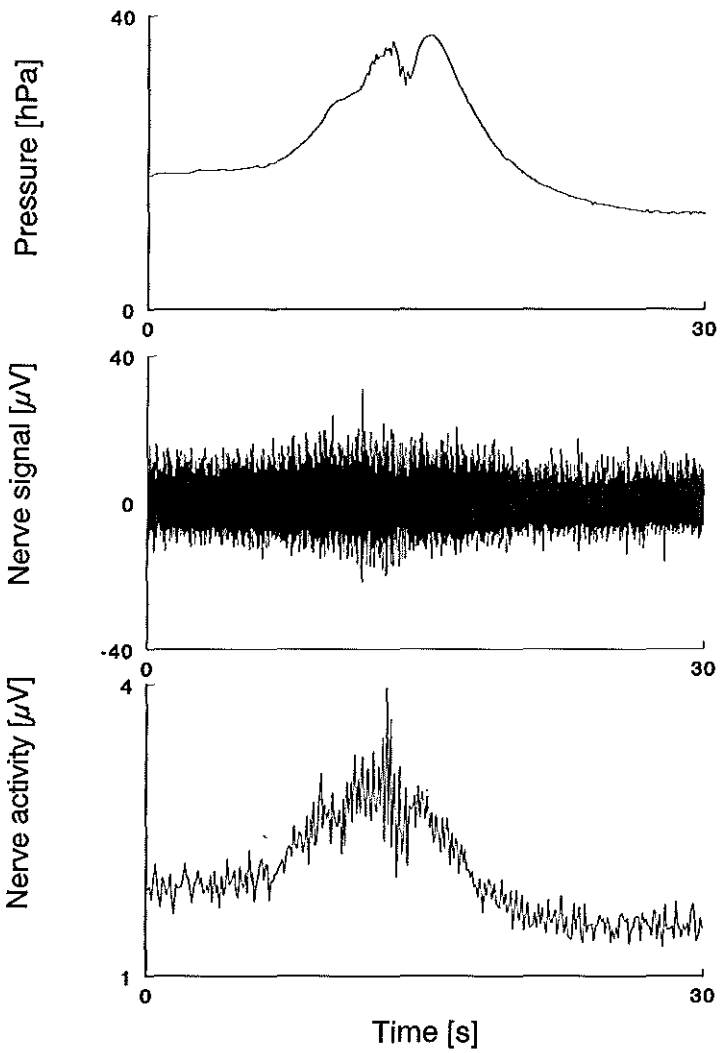

Figure 3. *Bladder pressure (upper trace) and postganglionic bladder nerve signal (middle trace) recorded during a voiding contraction. The lower trace shows the calculated amount of nerve activity (NA_{tot}).*

RESULTS

In the course of this study experiments were done in 45 rats. In the earliest animals attempts to record nerve activity were often unsuccessful but the experimental efficiency increased gradually, mainly as a result of increasing care in delicately handling the nerves. In the last ten rats, five experiments were successful.

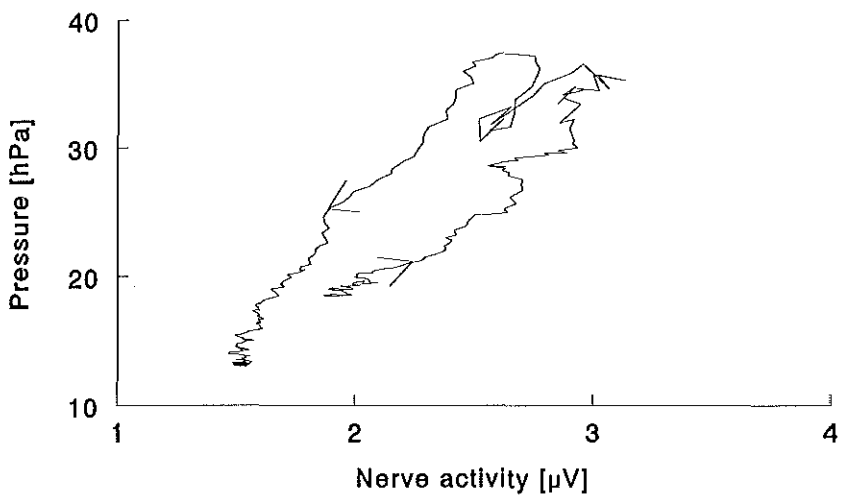

Figure 4. The bladder pressure and the postganglionic bladder nerve signal were recorded during a voiding contraction. The measured pressure was plotted against the nerve activity that was calculated from the nerve signal. The arrows indicate the time course. The "dip" at the top of the pressure signal is caused by the actual voiding.

In the type 1 measurements the bladder was filled with the infusion pump until a voiding contraction occurred (see Fig 2). From 12 rats 63 measurements were obtained with sufficiently high SNR_{est} (Eq. 8). In each rat at least three 30 seconds episodes of bladder pressure and postganglionic bladder nerve activity were recorded. Figure 3 shows an example of the recorded pressure and nerve signal, and the calculated nerve activity. From these recordings usually a relation between pressure and nerve activity was found as shown in Figure 4.

From the 63 measurements 2 were excluded that did not show passive relaxation of the bladder after voiding, indicated by a pressure that did not smoothly decline to baseline. The four model parameters K_1, K_2, α and τ and both baseline pressure (P_0) and background nerve

activity (NA_0) were fitted to the remaining 61 measurements. From these parameters an estimated pressure (P_{est}) was calculated using Eq. 5. An example of the measured pressure and the pressure estimated from the model is shown in Figure 2. In each rat the mean values of the parameters were calculated. The average values of these mean parameters for all rats (±SD) are shown in Table 1. Furthermore, in each rat the standard deviation of each of the four parameters was calculated as a percentage of the mean value of this parameter. This percentage (%SD) is a measure for the reproducibility of the parameters in one rat. Table 1 also shows the average values of this %SD for each parameter. Additionally the mean value and SD of the product K_1K_2 of all rats and the mean percentage of standard deviation are shown in Table 1 as well as the mean fit error as defined in Eq. 7. Figure 5 shows an example of the breakdown of the total nerve activity into efferent and afferent nerve activity using the model.

To test the dependence of the model parameters on the exact values of t_1 and t_2, two different values were used for t_1 and t_2 in 8 randomly chosen measurements from 8 rats. The difference between the values for t_1 was -0.64 ± 0.14 (mean ± SD) seconds and for t_2 +0.54 ± 0.16 seconds. There was no significant change in any of the parameters (changes were: K_1: -0.1 ± 3.6 %, K_2: +1.4 ± 10.1 %, α: +0.1 ± 8.7 % and τ: +2.3 ± 16.0%). To study the extent to which the SNR_{est}-criterion biased the results, the model parameters were recalculated for a set of measurements selected using the less restrictive threshold $SNR_{est} \geq 0.9$. This extended the original set with eight measurements from two rats (with mean SNR_{est} 0.90 and 0.97) and did not significantly change any of the average parameter values (2 tailed t-test: $p \geq 0.3$ for all parameters). When the parameters estimated in these two rats were considered separately, a higher value was found for K_1 and a lower value for K_2, both indicating that less nerve activity was measured. The value for K_2 was significantly different from that in the original population ($p<0.05$), K_1, α and τ were not.

In four cases the postganglionic bladder nerve was distally crushed after the above described baseline measurements. The crushes did not have any noticable effect on the micturition reflex. Again voiding contractions were recorded. An SNR_{est} was found of 1.4 ± 0.7. One of these measurements was excluded because the product K_1K_2 did not significantly change (2-tailed t-test: $p > 0.6$). In the other measurements (6 measurements from 3 rats) K_1K_2 changed from 0.029 ± 0.005 to 0.0059 ± 0.0039 (mean ± SD). An example of the pressure and the nerve activity that were recorded during a voiding contraction after the distal crush is shown in Figure 6. After the crush the model still fitted the data very well (mean fit error: 8.2 %). Figure 7 shows the averaged relative changes (± SD) in the four model parameters. The mean (± SD) of the ratio {mean value of a parameter in a rat before crush} / {mean value of this parameter after crush} is shown for each parameter.

Table 1. *Values and reproducibility of the model parameters, the product K_1K_2, and the fit error.*

parameter	*Mean ± SD*	*%SD*
K_1 [hPa/μV]	0.58 ± 0.55	21.2
K_2 [μV/hPa]	0.13 ± 0.15	27.2
α[Hz]	0.29 ± 0.13	24.2
τ[s]	0.78 ± 0.43	55.1
$K_1 \cdot K_2$	0.032 ± 0.019	24.8
error [%]	6.66 ± 1.94	

Mean and SD represent descriptives of the average parameter values in each rat. %SD is the average of the coefficients of variance in each rat. K_1,gain; K_2, slope of relation between bladder pressure and postganglionic afferent nerve activity; α, cut-off frequency; τ, latency between nerve activity and pressure development.

To study the relation between bladder pressure and afferent nerve activity, in three other rats 18 measurements were obtained after a proximal crush in the postganglionic bladder nerve. In all three rats the nerve was adequately crushed since after the crush only stimulus artifacts were measured (Figure 8). Again the bladder was filled with saline until a voiding contraction occurred. There was no noticeable effect on the micturition reflex. Nerve activity was plotted as a function of recorded pressure and Eq. 6 was fitted to the entire recording (Fig. 9). A fit error of 6.4 ± 1.2 % (mean ± SD) was found. The value that was found for K_2 (0.062 ± 0.024 μV/hPa) was significantly smaller (2-tailed t-test: $p < 0.05$) than its value before the crush, indicative of a smaller amount of afferent nerve activity.

To gain an impression of the error in the estimation of NA_{eff} in the type 1 measurements K_2 and NA_0 were also calculated from the decreasing part of the pressure only ($t > t_2$ in Fig. 2) using Eq. 6. It was found that in the type 3 measurements afferent nerve activity calculated from K_2 and NA_0 underestimated NA_{aff} during isovolumetrical pressure development ($t < t_1$ in Fig. 2) by 8.7 % on average. There was no difference in the values that were estimated for K_2 in these intervals. Figure 10 shows an example of the estimated and measured afferent nerve activity in this period. As can be seen from Figure 5 the values of NA_{eff} and NA_{aff} are comparable in this interval so that the error in NA_{eff} was approximately 9% as well.

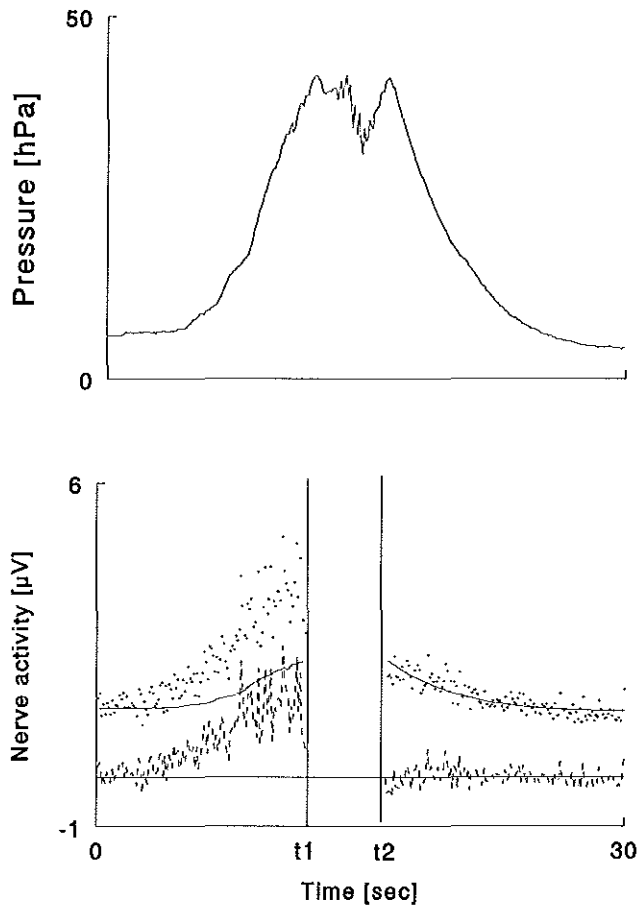

Figure 5. Bladder pressure (upper trace) during a voiding contraction. The lower trace shows the total post ganglionic nerve activity (NA_{tot}, dots) and the breakdown of NA_{tot} into efferent (NA_{eff}, dashed line), and afferent (NA_{aff}, solid line) nerve activity.

An example of the last type of measurement, the induction of bladder contractions by electrical stimulation of the pelvic nerve, is shown in Figure 11. Eight measurements were done in 6 different rats and again the model was fitted to the data. Because of the stimulus

artefact the absolute value of the nerve activity could not be measured. As this activity obviously changed stepwise, it was nevertheless possible to estimate the parameter α of the model, which does not depend on the amplitude of the signal.
A value was found that was slightly higher (0.37 ± 0.09) than the one found in the voiding contraction measurements (0.29 ± 0.13) but this difference was not significant (2-tailed t-test: $p > 0.2$).

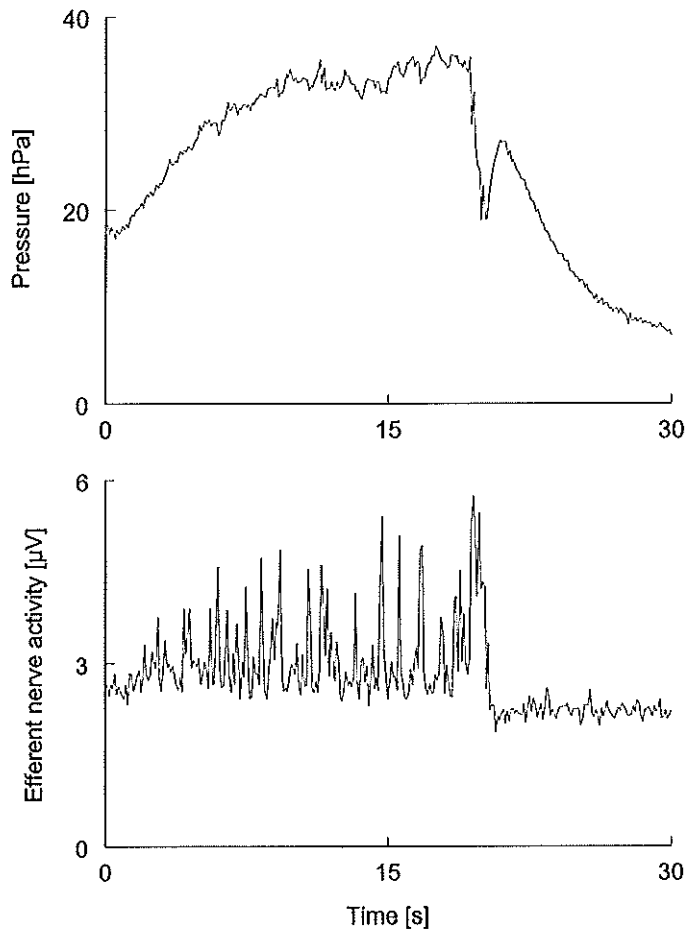

Figure 6. *Pressure and efferent nerve activity in a post ganglionic bladder nerve during a voiding contraction. Before the measurement the bladder nerve was distally crushed, resulting in a nerve signal that consisted of efferent nerve traffic only.*

DISCUSSION

As a first step towards a model of both the mechanical and the neurophysiological properties of the lower urinary tract, quantitative relations between the urinary bladder pressure and both efferent and afferent bladder nerve activity were established. These relations were based on measurements of pressure and postganglionic bladder nerve activity in the rat. In the model a linear relation between bladder pressure and post ganglionic afferent nerve activity was assumed (with a slope K_2) and bladder pressure was related to efferent nerve activity by the transfer function of a first order low pass filter with a certain gain (K_1), cut-off frequency (α) and latency (τ). Efferent and afferent nerve activity were assumed to add linearly.

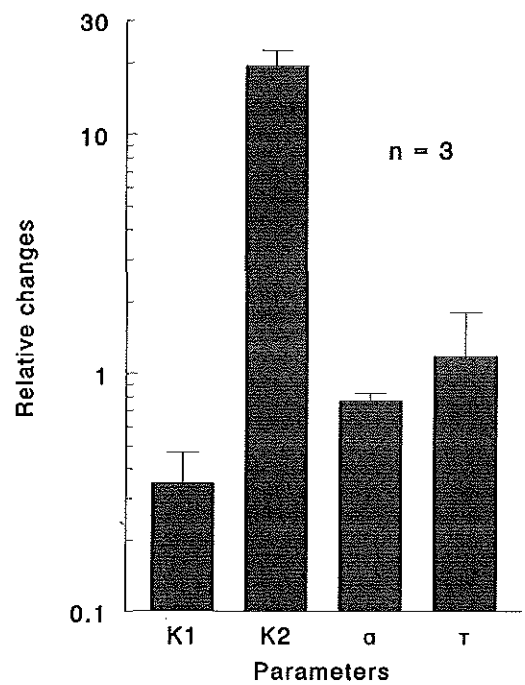

Figure 7. *Relative changes in model parameters after the postganglionic bladder nerve was distally crushed, measured in 3 different rats. For each parameter the ratio (mean value before crush) / (mean value after crush) is shown . K_1, gain;K_2, slope of relation between bladder pressure and postganglionic NA_{aff};α, cut-off frequency; τ, latency between NA_{eff} and pressure development.*

To verify the applicability of the model, experiments were performed in which the bladder was filled with saline until a voiding contraction occurred. The pressure and the nerve activity were recorded and the model was fitted. From the results it was concluded that the model described the measured data very well. A mean fit error of 6.7 (± 1.9)% was found. Furthermore the values of the model parameters showed a good reproducibility in each rat. On average the standard deviation of each parameter was less than 25% of the mean except for the latency τ.

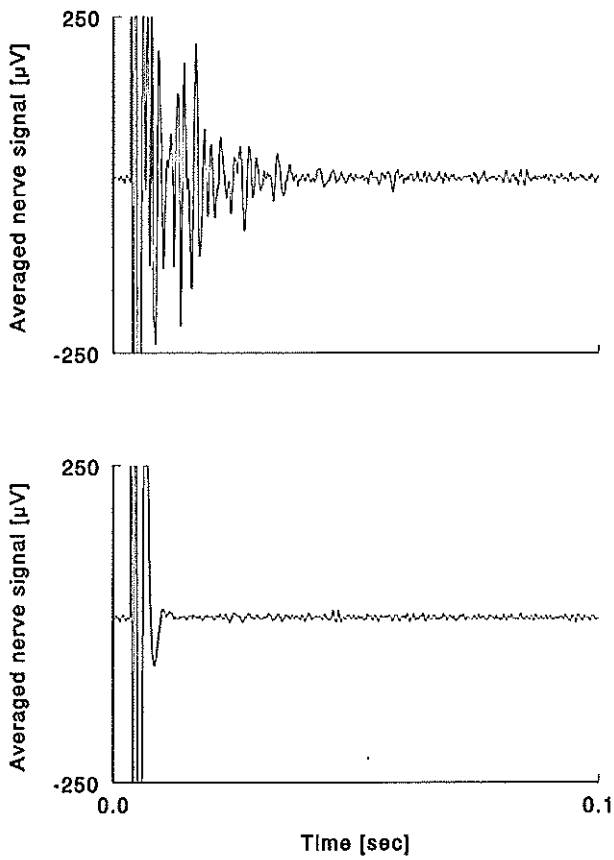

Figure 8. *The upper trace shows the action potentials that were evoked in a postganglionic bladder nerve by electrical stimulation of the pelvic nerve. The lower trace shows a similar recording when the bladder nerve was proximally crushed. Both traces show averages of 20 stimulations.*

The standard deviation of τ was relatively large because this parameter was determined with 0.1 s resolution which is very low considering its mean value of 0.78 s. This mean latency however, is in very good agreement with the value found by Noto et al, who found bladder contractions between 0.7 and 0.8 seconds after stimulation at the pontine tegmentum (13). These measurements were comparable with ours since the supraspinal latency is only a minor fraction of this period (16). The maximum frequency that is passed by the low pass filter in the model (Eq. 5, α) is the reciprocal of the time constant of pressure development in the urinary bladder. Thus a time constant of 3.4 seconds was found in our study. This value is in very good agreement with the time constant of force development in pig urinary bladder strips measured by Van Koeveringe and Van Mastrigt: 3.1 ± 1.1 sec (mean ± SD) (7).
The approximate 25% standard deviations in K_1 and K_2 could at least partially be explained by variations in electrical coupling between the nerves and the pick-up electrodes. In a number of rats two clusters of values for K_1 an K_2 were found, separated in time by a reconnection of the electrode to the nerve.

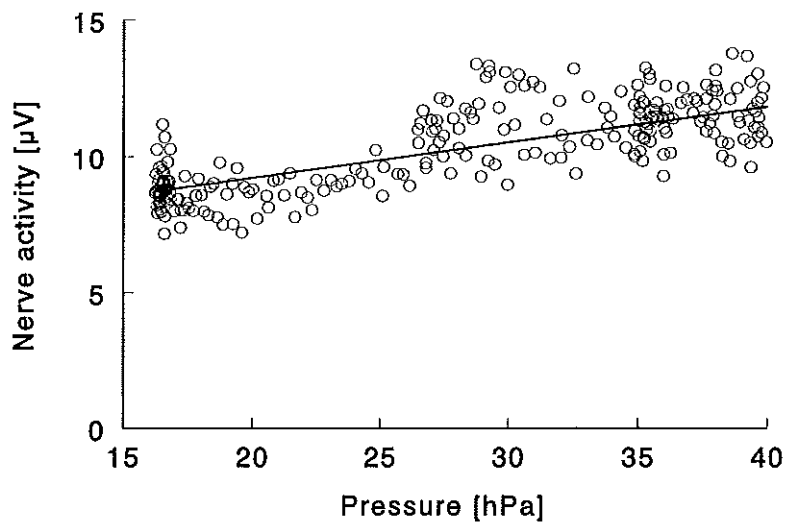

Figure 9. *The bladder pressure and the postganglionic nerve activity were measured after the bladder nerve was proximally crushed. Nerve activity was plotted against pressure (O). The fitted line represents the model equation:* $NA_{aff}(t) = K_2 \cdot P(t) + NA_0$.

The model was based on a number of assumptions. The plausibility of the separate assumptions was tested in type 2 (measurements in a distally crushed post ganglionic bladder nerve), type 3 (measurements in a proximally crushed bladder nerve) and type 4 (generation of bladder contractions by electrical stimulation of the pelvic nerve) experiments.
The assumption that total nerve activity is the linear sum of efferent and afferent nerve activity (Eq. 2) was based on the findings of Biró et al who showed that spikes add linearly (4). In our study it was made plausible that nerve activity adds linearly in bidirectional fibers as well, by the results of type 2 and type 3 measurements. Both efferent (Fig. 6) and afferent nerve activity (Fig. 9) were measured separately during voiding contractions in these experiments. These two signals were very similar to those estimated in the intact situation (Fig. 5).
In the type 2 experiments, in which the postganglionic bladder nerve was distally crushed, a

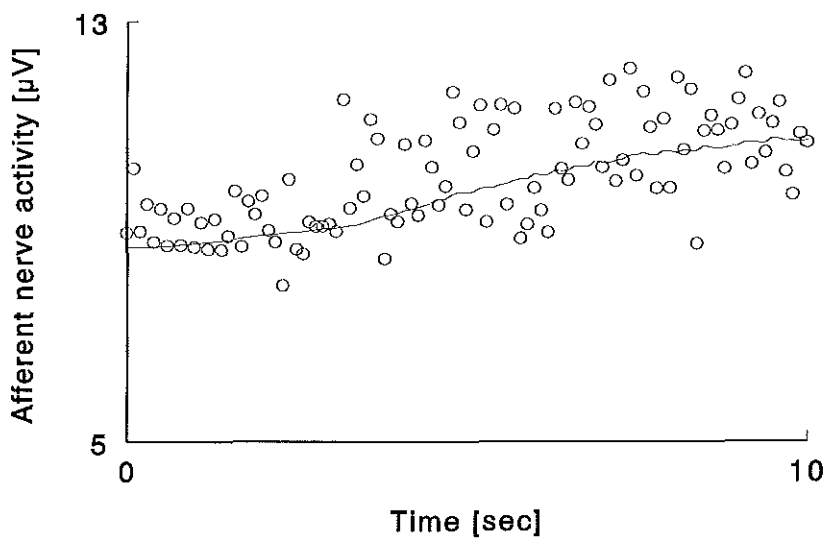

Figure 10. *Measured afferent nerve activity (NA_{aff}) in a proximally crushed postganglionic bladder nerve during isovolumetric pressure development (O) and estimated NA_{aff} (line). For this estimation K_2 and NA_0 were calculated from the measured pressure and nerve activity during pressure decrease at $t > t_2$ (Fig 1) by Eq. 6. From K_2 and NA_0, NA_{aff} was estimated during the pressure increase at $t < t_1$ (Fig 1). Afferent nerve activity was always slightly underestimated during this period.*

comparison was made between the values of K_1 before and after the crush. Ideally these values should be identical. After the crush however, the value of K_1 was always higher than before, indicating that less efferent nerve activity was measured. Theoretically a higher amount of efferent nerve activity might have been expected. As the chances of two action potentials coinciding at the two poles of the electrode increase at higher firing rates, an increasing number of action potentials go undetected. Therefore efferent nerve activity is always underestimated in the presence of afferent nerve activity if these do not add linearly. Probably the decrease of NA_{eff} is caused by a deterioration of the electrical coupling, overruling a slight increase in efferent nerve activity. A distal crush strongly reduced the value of K_1K_2, indicating that the characteristics of the bladder nerve were affected by these crushes and that the changes in the model parameter K_1 and K_2 were not caused by differences in electrical coupling only.

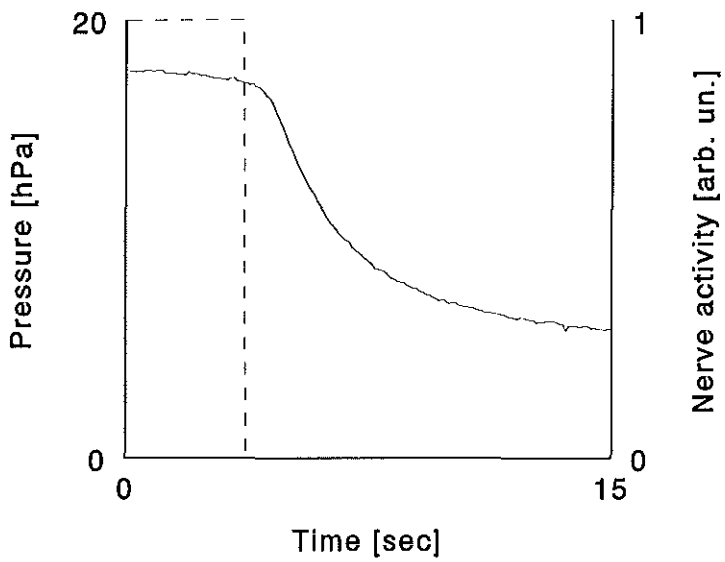

Figure 11. *The pelvic nerve was electrically stimulated to impose a stepwise change in efferent nerve activity in a post ganglionic bladder nerve. This caused stimulus artifacts that were very high compared to the evoked action potentials. Therefore an estimated nerve activity (arbitrary units, dashed line) is shown in the figure instead of the calculated nerve activity. The figure also shows the pressure response of the bladder (solid line) to such a stepwise decrease of efferent nerve activity.*

In type 3 experiments the bladder nerve was proximally crushed. After this crush the amount of measured afferent nerve activity was always less than before (K_2 always had a lower value after the crush). An improvement in electrical coupling after manipulation of the nerve was never found, probably due to the optimalisation of the coupling before the crushing procedure. Again, in case of non-linear addition, K_2 would be expected to have a higher value after the crush.
It can be concluded that although the results may be biased by a change in electrical coupling they do not indicate a non-linear addition of efferent and afferent nerve activity.

In the model it was assumed that efferent nerve activity was negligible during the pressure decrease immediately after voiding. This is a very important assumption as it allows the breakdown into efferent and afferent nerve traffic. It is known that electrical stimulation can also have an inhibitory effect on bladder contractions. Noto et al (13) found that electrical stimulation of the dorsal pontine tegmentum could have both excitatory and inhibitory effects on bladder pressure. Inhibitory effects however, were only observed in the absence of efferent nerve activity in postganglionic bladder nerves. Furthermore the assumption (Eq. 4) is supported by the results of the type 4 measurements. When electrical stimulation of the pelvic nerve was suddenly aborted, resulting in a sudden stop of efferent nerve activity, a decrease in bladder pressure was found that was very similar to the decrease that occurred during voiding contractions. No significant difference was found between the values of α that were obtained in both types of measurements. From these measurements it follows that the 50% reduction of the value for K_2 after proximal crushing of the nerve should probably be attributed to a deterioration in electrical coupling rather than to some residual efferent activity during relaxation.

The relation between efferent nerve activity and pressure (Eq. 5) in the model was based on the measurements done by Partridge (14) who showed that skeletal muscles responded to changes in nerve activity with an amplitude attenuation and phase shift that were frequency dependent. This dependence can very well be described by a low pass filter. Partridge found "an amplitude ratio that was essentially independent of frequency up to a frequency between 0.6 and 1.2 Hz" so if he would have used a low-pass filter to describe his results, he would have found a cut-off frequency between 0.6 and 1.2 Hz. This is approximately 3 times higher than the cut-off frequency that was found in our study (α = 0.29 Hz). This difference may be explained by the fact that his measurements were done in striated muscle, which generally shows a faster response than smooth muscle.

The relation between bladder pressure and afferent nerve activity was investigated qualitatively in 18 measurements obtained from 3 rats. In all animals the assumed linear relation (Eq. 6) fitted the measurements very well (fit error: 6.4 ± 1.2 %, mean ± SD), although there was always a slightly higher amount of afferent nerve activity during pressure development at the onset of the voiding contraction than during the pressure decrease at the end. This difference might have been caused by adaptation in the receptors in the bladder wall, where the afferent action potentials are evoked. It could also result from the yet unknown mechanism that evokes these potentials which might be dependent on both stretch

and movement of the receptor fields. The difference however, was so small (8.7%) that it was not taken into account in the model. A linear relation was accepted for the entire period. This finding was supported by the linear part at the period of pressure decrease in Figure 4 and the measurements of Bahns et al, who found that the temporal shape of the intravesical pressure was well encoded by the discharge rates of the afferent units (3). In this respect it is relevant to note that as reported by other researchers a good measure for nerve activity in whole nerve recordings is hard to define (1,4,10,18). In this study integration of the rectified electro neurogram was chosen as the best method available (1,6). It was shown that this measure for nerve activity is not a linear one since it underestimates nerve activity at high firing rates (1,4,18). As a consequence of this non-linear relationship, it appears that the linear relationship which was found between pressure and the derived afferent nerve activity in our study should not be interpreted as evidence for a linear relationship between pressure and firing rates. It might result from similarities between the non-linearity in the used measure for nerve activity and that in the afferent excitation. Furthermore the measured nerve activity depends on the electrical coupling between nerve and electrode. To exclude measurements with insufficient coupling the SNR_{est}-criterion was introduced. Application of this procedure causes measurements that have a very high basal activity (more than half of the maximum activity) to be excluded as well. Bahns et al (3), however, found that there was some basal activity only in 1 out of 26 afferent units.

In summary it can be concluded that a sudden stop of efferent nerve activity as was imposed in the type 4 experiments resulted in a pressure decrease that could not be distinguished from the one seen in voiding contractions (α was not significantly different). This supported the assumption that efferent activity was absent during the pressure decrease immediately after voiding (Eq. 4). Possibly due to alterations in electrical coupling between nerve and electrode the assumption that efferent and afferent nerve activity linearly add (Eq. 3) could not quantitatively be justified by the results of the type 2 and type 3 experiments, but it was not contradicted by these results either. However the similarity in the shapes of separately measured NA_{eff} (Fig. 6) and NA_{aff} (Fig. 9), with those estimated in the intact situation (see Fig.5) did support this assumption. Furthermore the experiments showed that the relation between bladder pressure and the delayed efferent nerve activity can be described by the transfer function of a first order low pass filter (Eq. 5). A linear relation between pressure and afferent nerve activity as in Eq. 6 was tested and found to be adequate. Finally it was shown that the model described the data very well (an average fit error of less than 7% was found) and that the parameters that were found showed good reproducibility.
It can thus be concluded that the measurements done support the proposed model, that establishes quantitative relations between post ganglionic bladder nerve activity and the detrusor pressure in the rat.

APPENDIX

In our study the parameter estimation was performed in two steps; in the first step K_2 was estimated and in the second step K_1, α and τ were estimated. As an alternative approach, simultaneous estimation of all parameters was studied.
Such simultaneous estimation would require elimination of the terms NA_{eff} and NA_{aff} from the system of equations 3-6.
This can be achieved by substitution of Eq. 6 in Eq.3, which yields:

$$NA_{eff}(t) = NA_{tot}(t) - K_2 \cdot P_{meas}(t) - NA_0 \qquad (A1)$$

Substitution in Eq. 5 gives:

$$\begin{aligned} P_{est}(t) &= K_1 \exp(-\alpha t) * [NA_{tot}(t - \tau) - K_2 \cdot P_{meas}(t - \tau) - NA_0] + P_0 \\ &= K_1 \exp(-\alpha t) * NA_{tot}(t - \tau) - K_1 K_2 \exp(-\alpha t) * P_{meas}(t - \tau) + P_0' \end{aligned} \qquad (A2)$$

where

$$P_0' = P_0 - K_1 \cdot \exp(-\alpha t) * NA_0 \qquad (A3)$$

From Eq. A2 it can be seen that the error function $\Sigma(P_{meas}-P_{est})^2$ would reach a minimum at the non-physiological values $0 < |K_1| \ll 1$ and $K_1 \cdot K_2 = -1$. With $\alpha \rightarrow -\infty$ and $\tau = 0$ this would yield $P_{est}(t) = P_{meas}(t)$ and thus the error function would reach an absolute minimum of 0. Therefore either K_1 or K_2 has to be determined separately.

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3. Neurophysiological modeling of voiding in rats: urethral nerve response to urethral pressure and flow.

Joost le Feber, Els van Asselt and Ron van Mastrigt
Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43) R1473-R1481, 1998.

ABSTRACT

In male urethane anaesthetized rats, activity was measured in nerves that run over the proximal urethra. The urethral nerve response to stepwise urethral perfusion could be described by a four-parameter model (fit error <6%). At the onset of perfusion, the urethra was closed and the pressure increased with the infused volume. The nerve activity (NA) increased linearly with this inserted volume to a maximum (NA_{max}), which was proportional to the instantaneous pressure. The duration of this first episode (δt) was inversely proportional to the perfusion rate. After infusion of a fixed volume, the urethra opened and the nerve activity decreased with a time constant φ^{-1} (~1.8 s) to an elevated level (NA_{level}). NA_{level} was linearly related to the steady state pressure. Accordingly, sensors in the urethra are sensitive to pressure rather than to the perfusion rate. The parameters NA_{max}, NA_{level}, and δt showed very good reproducibility (SD ~19% of mean). The measured activity was most likely afferent, and conducted to the major pelvic ganglion.

Key words: Urethral perfusion; quantitative model; nerve activity; rat

INTRODUCTION

In recent years, the mechanical properties of bladder and urethra have been extensively investigated and described (5). Based on these properties, clinical methods have been developed that allow discrimination of different causes for voiding complaints, such as low bladder contractility or high urethral resistance (25). In a similar way, a model, combining mechanical and neurophysiological properties of the lower urinary tract, would be very useful for differentiation between myogenic and neurogenic causes for dysfunction.
The neuroanatomy of the lower urinary tract has been studied extensively, and many of the pathways that play a role in the voiding reflex are known (3, 4, 8 - 10, 15 - 19, 21). Neurophysiology, however, has received far less attention and most studies did not quantitatively describe the relations between neural activity and system dynamics (i.e. bladder pressure and urethral flow).
In a previous study, we quantitatively described the relations between bladder pressure and both efferent and afferent nerve activity in bladder nerves (12). The present study addresses the innervation of the proximal urethra. The proximal urethra has been described as the part of the urethra that lies between the bladder neck and the urogenital diaphragm. The upper half of the proximal urethra wall consists mainly of smooth muscle cells. In the lower half, a layer of striated muscle fibers, known as the external urethral sphincter, encircle the smooth muscle layers. Additionally, a layer of intermingled smooth and striated muscle fibers in this half of the proximal urethra has been described (26).
Efferent innervation of the striated muscle layers of the urethra is provided by the pudendal nerves (8, 9, 16, 23). Nerve branches originating from the major pelvic ganglion innervate the smooth muscle fibers. Afferent activity from the proximal urethra is probably

conducted the nerves leading through the major pelvic ganglion (2, 10, 13). Figure 1 shows a schematic drawing of this configuration.

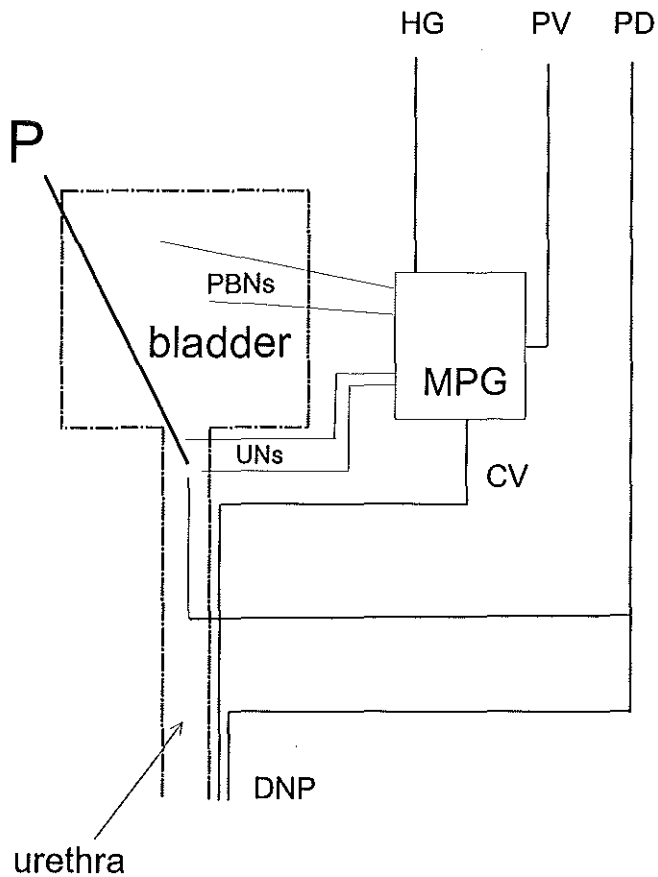

Figure 1. *Schematic drawing of the innervation of bladder and urethra. HG: hypogastric nerve, PV: pelvic nerve, PD: pudendal nerve, CV: cavernous nerve, DNP: distal nerve of the penis, MPG: major pelvic ganglion, PBNs: postganglionic bladder nerves, UNs: urethral nerves. P: needle, inserted into the urethra and connected to a pump and a pressure transducer.*

In the present study, the relations between urethral flow, transurethral pressure and neural activity in nerves running over the proximal urethra are quantitatively described.

MATERIALS AND METHODS

Surgery

Male Wistar rats (458 ± 26 g) were anesthetized with urethane (1.2 g/kg) intraperitoneally (14) and placed on a heated pad. An incision was made at the left side of the spine, just below S3, to access the pudendal nerve from the dorsal side. Two trimel coated silver wires were twisted around the compound nerve (containing efferent and afferent branches, 16) at a distance of ~2 mm. Then the wound was closed and the animal was turned over on its back.

An abdominal midline incision was made to access the urethra. The left vas deferens and testicle were tied and placed outside the animal. Nerve branches running over the left side of the proximal urethra were freed from the underlying tissue and marked with sutures. The pelvic nerve, the hypogastric nerve and the cavernous nerve were dissected and similarly marked. The right side of the urethra was left intact. Warm saline kept the abdomen moist during surgery. The animal was placed in a frame with a heated groundplate and the abdominal wall was tied to the frame to create a basin. During the measurements, this basin was filled with warm paraffin oil.

Experimental setup

A 24-gauge (6 animals) or a 17-gauge angiocatheter (6 animals) was inserted into the urethra, just beyond the bladder neck. Through this catheter, the pressure at the entrance of the urethra was measured with the use of a disposable pressure transducer and a Statham SP1400 blood pressure monitor. Furthermore, the catheter was used to pump saline through the urethra with a Harvard Apparatus (Millis - Massachusetts 2202) infusion pump. The pressure loss in the tube and angiocatheter that connected the pressure transducer to the urethra was described by a second order polynomial This polynomial was used to calculate pressure losses at each flow rate applied (1.1, 2.2, 5.4, 10.8 or 21.4 ml/min). The calculated pressure loss was subtracted from the measured pressure, to yield the transurethral pressure (P_{ura}). The bladder was tied and emptied to prevent high bladder pressures and leakage from the urethra to the bladder.

A bipolar platinum-iridium electrode, that consisted of two metal hooks at a distance of ~0.5mm, was lowered into the paraffin oil basin. One of the urethral nerves was guided over the electrode, which was then slightly elevated. The recorded nerve signal was amplified by a DISA 15C01 electromyogram (EMG) amplifier (amplification range 10,000 - 200,000) and band-pass filtered using a Krohn-Hite model 3944 (Bessel 500-2500 Hz, 4th order). The combination of the symmetrical electrode and an amplifier with a very high common mode rejection ratio (>100dB) ensured a minimal radio frequency and power supply interference. The recording electrode was moved along the urethral nerve until action potentials were clearly detected on an oscilloscope at the onset of urethral flow. Only those nerves that showed increased nerve activity during urethral perfusion were used for further measurements.

The silver wires around the pudendal nerve provided a bipolar electrode, which was used for stimulation only. The pelvic nerve, hypogastric nerve or cavernous nerve were

stimulated with an electrode similar to the one used for recording urethral nerve activity. A Hameg programmable function generator, HM8130, generated the stimulation signal.
During the measurements, the abdominal wall was electrically grounded.
The pressure and the urethral nerve signal were read into a personal computer at sample rates of 10 Hz and 25 kHz, respectively, with the use of specially developed software driving a PCL 818 analog-to-digital converter. A measure for nerve activity (NA) was calculated as the mean value of the rectified nerve signal in 100 ms intervals:

$$NA(t) = \sum_{i=1}^{2500} |ENG(i)| / 2500 \quad (1)$$

Where ENG(i) (electroneurogram) represents the i^{th} sample of the nerve signal in each 100 ms interval containing 2500 points (1, 7, 27).

Measurements
Four types of measurement were done. The *major nerve stimulation* measurements were done to identify the origin of the urethral nerves. *Urethral perfusion* measurements were done to determine quantitative relations between urethral nerve activity and urethral pressure and flow and *penile clamp* measurements to determine the nature of the receptors (pressure or flow dependent firing). *Lateral transection* measurements were done to determine the nerve composition (efferent and/or afferent fibers).

1) *Major nerve stimulation.* Recordings were made from a urethral nerve that responded to urethral perfusion. Either the pelvic nerve, hypogastric nerve, cavernous nerve or the pudendal nerve was electrically stimulated with an increasing amplitude until a response was seen (max. 10V, stimulation frequency 10 or 100 Hz, pulse width 50 μs or 400 μs). Urethral flow at an arbitrary rate (0.5 or 1.1 ml/min, occasionally higher) was imposed during the stimulation, only to enable measurement of urethral pressure. 100 Hz stimulation was used to investigate urethral pressure changes in response to the stimulation. Since 100 Hz stimulation caused stimulus artifacts every 10 ms, and we wanted to investigate responses that might have longer latencies, additional 10 Hz stimulations were performed to examine urethral nerve activity in 100 ms intervals.

2) *Urethral perfusion.* We induced increased activity in the urethral nerve by pumping saline through the urethra. Urethral pressure and urethral nerve activity were recorded during 60 s episodes that consisted of three phases: (1) initially, there was no flow through the urethra, (2) the pump was switched on and there was a constant fluid current through the urethra, and (3) the pump was switched off.
The signal to noise ratio (SNR) of each measurement was estimated as:

$$SNR_{est} = [\max(NA) - \min(NA)] / \min(NA) \quad (2)$$

To derive a stable measure for SNR_{est}, averages of the ten lowest and the ten highest values were taken for min(NA) and max(NA). Measurements that had an SNR < 0.3 were excluded from further analysis. In measurements with an SNR above this threshold, action potentials could clearly be distinguished from background noise.
The nerve activity pattern during flow (phase 2) was described by a four parameter model (see results section, eqs. 5-6) with flow dependent parameters. This model was fitted to the data using a least squares algorithm. The quality of the fit was assessed by calculation of the relative error:

$$\text{Fit error} = [\Sigma \left| NA_{meas} - NA_{est} \right| / \Sigma NA_{meas}] \times 100\% \quad (3)$$

The flow dependence of the parameters was statistically verified by one-way analysis of variance.

3) Penile clamp. To determine whether the receptors in the urethral wall are pressure or flow sensitive, we alternated measurements in the presence (mode I) and absence (mode II) of flow. In mode II the penis was clamped and inserting or withdrawing small volumes of saline with a syringe slowly varied pressure in the urethra. The pressure variations were in a range similar to that in the urethral perfusion measurements.
The nerve activity that was generated in both modes was compared.
In *mode I*, the 5 flow rate settings were each applied once. In *mode II*, nerve activity was measured during 5 consecutive maneuvers in which the urethral pressure was manually varied. Mode I and II were alternated in the same animal (in the order I-II-I), to allow a direct comparison of the responses.
From the *mode I* measurements, the steady state nerve activity during urethral perfusion (NA_{level}, see eq. 6) was calculated as a function of the steady state pressure (mean pressure in the last 5 seconds of perfusion). This relation was described by a linear function. In the *mode II* measurements, the relation between pressure and nerve activity was described by a linear relation as well, and offset and slope of both relations were compared.
As we often found deterioration in the nerve activity over the course of an experiment, especially during the first 10-15 minutes after connection of the electrode to the nerve, a stabilization period of 15 minutes was introduced before the measurements were started.

4) In the *Lateral transection* measurements, we tried to determine whether the urethral nerve conducted efferent or afferent activity. After completion of the urethral perfusion measurements, in some animals the urethral nerve was cut at one side of the electrode to establish unidirectional nerve traffic. Since we were often unable to trace the urethral nerve back to one of the major nerves innervating the urethra, we could not determine whether the transection was proximal or distal. Saline was pumped through the urethra again, and the activity in the urethral nerve was recorded. The recorded nerve activity in this situation was compared with the measured activity in the intact nerve. Parameter values were compared by a t-test or analysis of variance (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". All data are presented as mean ± standard deviation.

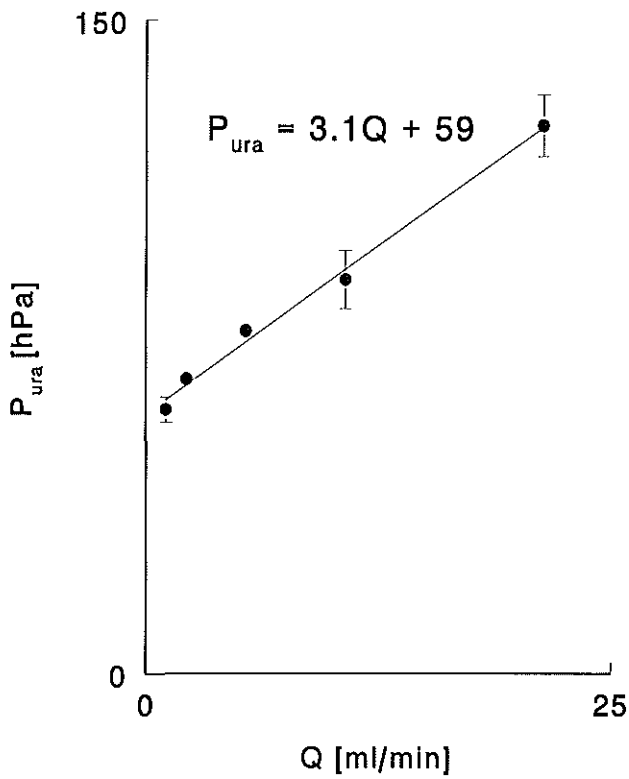

Figure 2. *Average transurethral pressure (P_{ura}) induced by urethral perfusion at various flow rates (Q). In 12 rats, all pressures were normalized to the value at Q = 5.4 ml/min. The curves were averaged and then multiplied by the mean P_{ura} at 5.4 ml/min. Line represents linear regression equation.*

RESULTS

Urethral nerve activity was successfully measured in 15 out of 36 rats. In 225 measurements obtained from 12 rats, saline was pumped through the urethra. Nerve activity and pressure at the proximal urethra, P_{ura}, (just beyond the bladder neck) were measured at 5 different flow rate settings (1.1, 2.2, 5.4, 10.8 and 21.4 ml/min).In each rat, dividing by P_{ura} at 5.4 ml/min normalized the measured pressures and the resulting fractions were averaged for all rats.

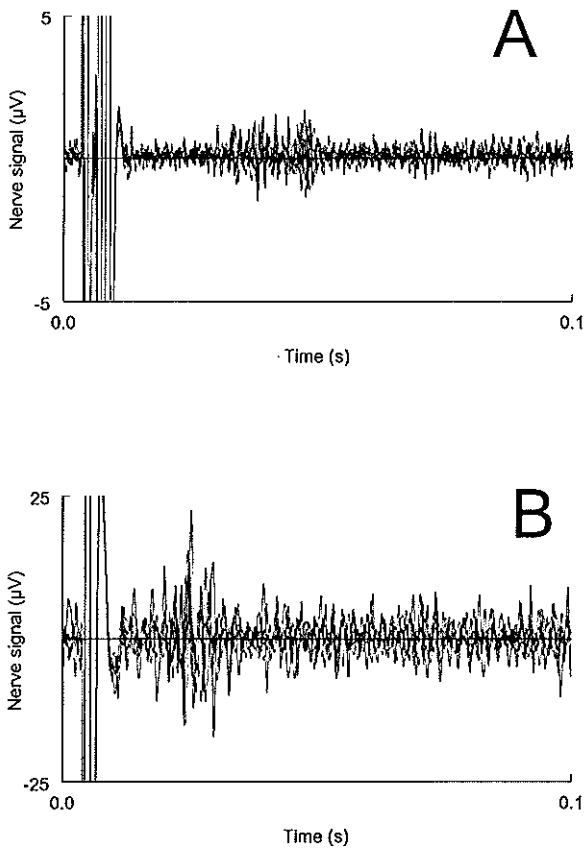

Figure 3. *Responses to electrical stimulation of the pudendal nerve (A) and the pelvic nerve (B). Both traces show 5 consecutive responses. The first points of the stimulus artifacts were synchronized. Computer averaging of the responses removed the action potentials from trace A and greatly reduced the response in trace B.*

In Figure 2 the relative transurethral pressures are multiplied by the mean P_{ura} at 5.4 ml/min (79 ± 24 hPa), which gives the average P-Q relation:

$$P_{ura} = 3.1 \cdot Q + 59 \quad (4)$$

Standard deviations in Figure 2 refer to differences between rats.

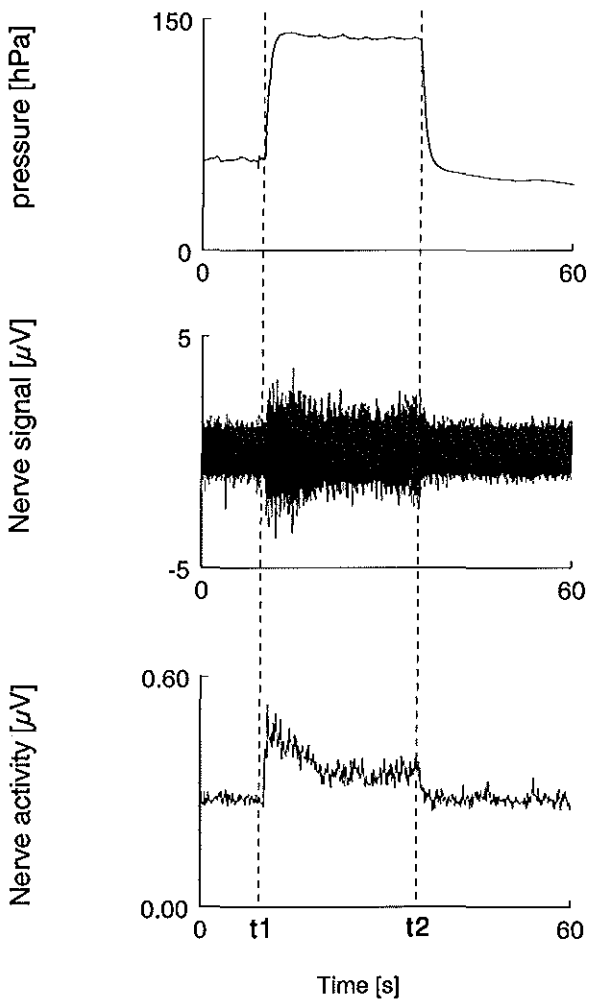

Figure 4. *Pressure at the proximal urethra (top trace), nerve signal in a nerve running over the proximal urethra (middle trace) and the calculated nerve activity (bottom trace), during urethral perfusion (Q = 10.8 ml/min). At t_1 the infusion pump was switched on and at t_2 it was turned off again.*

In the *major nerve stimulation* measurements, the nerves that innervate the lower urinary tract were electrically stimulated with increasing amplitude (max 10V), until a response was seen in the urethral nerve. Stimulation of the hypogastric nerve (2 animals) or cavernous nerve (5 animals) never induced action potentials in the urethral nerves. Stimulation of the pudendal nerve (8 rats) showed two responses: one with an ~8 ms latency in all animals and one with an ~35 ms latency (range 25-50 ms), in 63% of the animals. Pelvic nerve stimulation elicited action potentials with a ~25 ms delay in 4 out of 5 rats. Figure 3 shows 5 subsequent responses to pelvic nerve or pudendal nerve stimulation. Computer averaging removed the action potentials associated with pudendal nerve stimulation, and greatly reduced the response to pelvic nerve stimulation. Pudendal nerve stimulation always increased P_{ura} and thus, since a constant flow rate was applied, urethral resistance. Stimulation of pelvic, cavernous or hypogastric nerve generally did not change the urethral resistance.

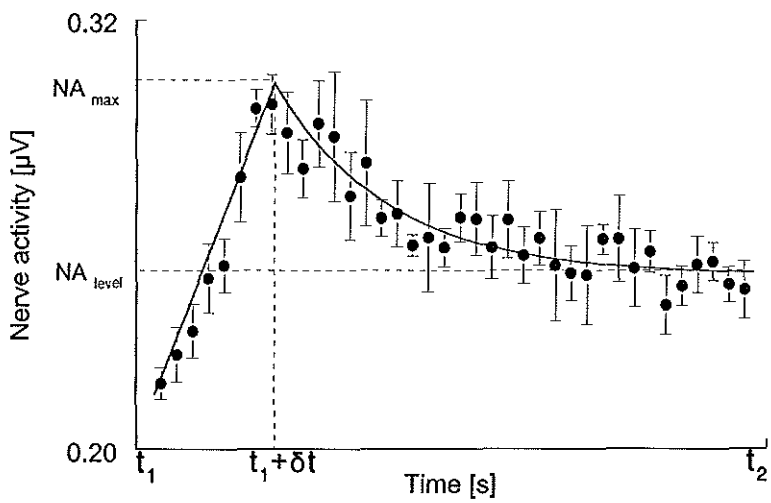

Figure 5. *Between t_1 and t_2 (Fig. 4), the nerve activity in a urethral nerve was described by a model (eqs. 5-6). This consisted of a linear increasing nerve activity during a period δt, until a maximum NA_{max} was reached. Subsequently, the nerve activity decreased with a certain time constant to an elevated level NA_{level}. Averages (±SD) of every 5 consecutive nerve activity values are displayed as filled circles. The drawn line represents the fitted model.*

Urethral perfusion measurements were done in 12 rats. In these measurements, nerve activity was evoked by a forced flow through the urethra. Three phases were distinguished. *1)* At the start of the measurement, there was no flow through the urethra, *2)* there was a stepwise change to the selected flow rate, which was maintained for ~30 s, *3)* the flow was suddenly set to zero again by switching off the pump. P_{ura} and urethral nerve activity usually showed a pattern as in Figure 4.

Table 1. *Statistical significance of the flow dependency of the four model parameters.*

Rat no.	NA_{max}	δt	NA_{level}	φ
1	p<0.003	p<0.001	n.s.	n.s.
2	p<0.003	p<0.001	n.s.	n.s
3	p<0.001	p<0.005	n.s.	n.s.
4	p<0.002	p<0.003	n.s	n.s
5	p<0.001	p<0.04	p<0.01	n.s.
6	p<0.02	p<0.004	n.s.	n.s.
7	p<0.05	n.s.	n.s.	n.s
8	p<0.02	p<0.001	p<0.005	n.s.
9	p<0.001	p<0.02	n.s.	n.s.
10	p<0.001	p<0.001	p<0.02	p<0.001
11	p<0.001	n.s.	p<0.001	n.s
12	p<0.001	n.s.	n.s.	p<0.001
% of animals significant:	100%	75%	33%	16%

During urethral perfusion, the induced nerve activity was described by a four parameter model. The four parameters depended on the applied perfusion rate. The statistical significance of these dependencies were assessed by one way analysis of variance. Dependencies were considered significant if p<0.05; n.s. = not significant. NA_{max} *maximum nerve activity;* φ*, time constant of decreasing nerve activity;* NA_{level}*, level to which the nerve activity decreased during perfusion;* δt*, period of increasing nerve activity.*

In phase 1 ($t < t_1$), there was only noise and possibly some baseline activity (NA_0). In phase 2 ($t_1 < t < t_2$), the neural activity was modeled by an increase during a period δt, until a maximum NA_{max} was reached. Then the nerve activity decreased with a certain time constant φ^{-1} to an elevated level NA_{level}, see Figure 5. This can be mathematically formulated as in eqs. 5-6.

$$NA(t) = NA_0 + [(NA_{max}-NA_0)/\delta t]\cdot(t-t_1) \qquad \forall\ t_1 < t \leq t_1+\delta t \qquad (5)$$

$$NA(t) = NA_{level} + (NA_{max}-NA_{level})\cdot\exp[-\varphi\cdot(t-(t_1+\delta t))] \qquad \forall\ t_1+\delta t < t \leq t_2 \qquad (6)$$

In these relations, NA is nerve activity, NA_0 is noise and/or background activity, and t_1 and t_2 are the moments when flow through the urethra was turned on and off (see Figure 4). During phase *3* ($t > t_2$), when there was no flow, the nerve activity returned to the baseline value (NA_0), and the pressure decreased to a level at which the urethra closed (56 ± 22 hPa). This pressure did not depend on the applied flow rate and showed excellent reproducibility in each rat (average coefficient of variation 2.9 ± 1.9%).

The model parameters δt, NA_{max}, φ and NA_{level} (eqs. 5-6) were estimated in each rat by minimizing the sum of squared deviations (average fit error (eq 3): 5.2 ± 1.4 %). 7.6 % of the measurements were excluded because SNR_{est} was lower than 0.3.

One way analysis of variance (see Table 1) showed that NA_{max} and δt were significantly dependent on the applied flow rate ($p<0.05$) in 100% and 75% of the animals respectively. The flow dependency of NA_{level} and φ was only significant in 33% and 16% of the animals. To adjust for differences between rats, all parameters were normalized to the value at 5.4 ml/min. These relative parameter values were averaged for the twelve rats, and then multiplied by the average parameter value at 5.4 ml/min. The model parameter NA_{max} was found to increase with the flow rate, and could be described by a two-parameter function (Figure 6).

$$NA_{max} = 0.32\cdot Q^{0.24} \qquad (7)$$

In many measurements, particularly at low flow rates, φ could not adequately be determined because of the poor signal to noise ratio. This resulted in a large coefficient of variation (SD as a percentage of mean ~59%). We found a slight increase with Q, but this was significant in only two out of twelve animals. We therefore modeled φ by a constant (φ=0.56, Table 2).
δt decreased with Q (Fig. 6) and was described by:

$$\delta t = 15.9/Q \qquad (8)$$

Thus $\delta t\cdot Q$ had a constant value (15.9). Divided by 60, this represents the average volume that was pumped into the urethra before the maximum nerve activity was reached (V = 0.27 ml). When this volume was separately calculated at each flow rate in each rat, and the mean volumes were averaged, a volume of 0.26 ± 0.19 ml was found. The average coefficient of variation of these volumes, however, was only 29% (Table 2). The relation between Q and NA_{level} could be described by a linear function (Fig. 6):

$$NA_{level} = 2.9\cdot 10^{-3}\cdot Q + 0.29 \qquad (9)$$

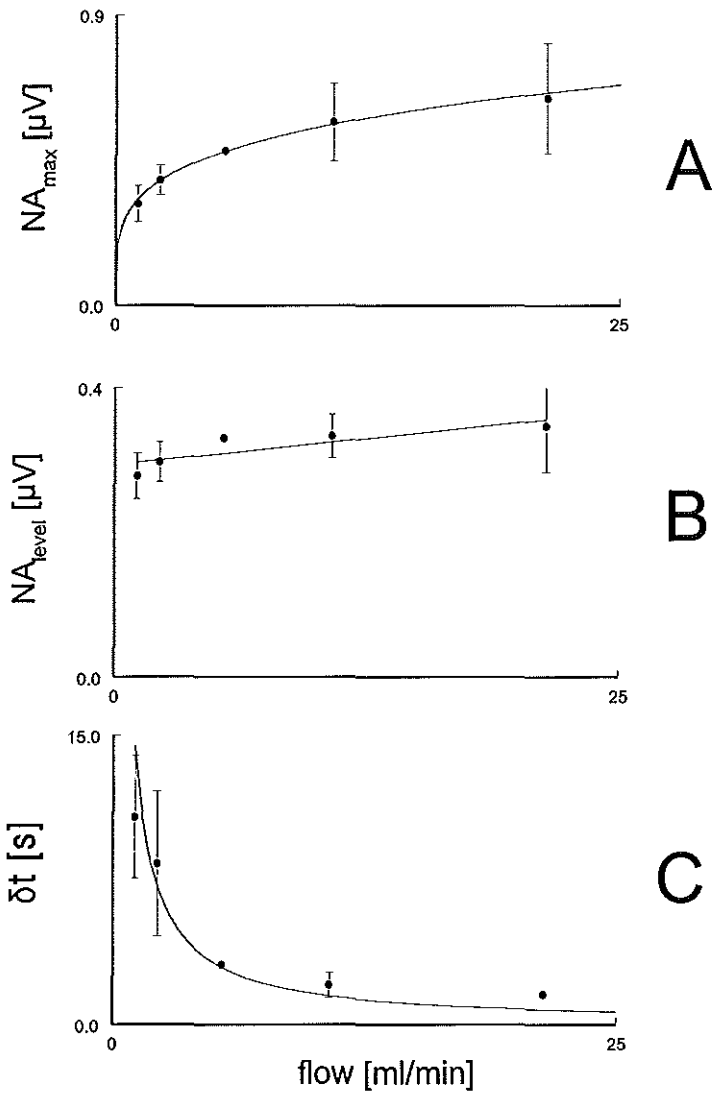

Figure 6. *The model presented in Fig.5 and eqs.5-6 was fitted to data obtained from measurements at 5 different perfusion rates (Q). The values of NA_{max} (graph A), NA_{level} (graph B), and δt (graph C), were normalized to the values at Q = 5.4 ml/min in all rats. These normalized values were averaged, and then multiplied by the mean values at Q = 5.4 ml/min (table 2). Lines represent least sum of squares fits: $NA_{max}=0.32 \cdot Q^{0.24}$, $NA_{level}=0.0029 \cdot Q+0.29$, $\delta t=15.9/Q$.*

To assess the reproducibility of the model parameters, coefficients of variation were calculated. On average the coefficient of variation of the model parameters was ~ 29% (Table 2).

Table 2. *Model parameters that describe nerve activity in a urethral nerve during urethral perfusion, and the infused volume at maximum nerve activity.*

parameter	Value (at 5.4 ml/min)	%SD
δt (s)	3.1 ± 2.9	29 ± 15
NA_{max} (μV)	0.48 ± 0.4	16 ± 11
φ (s^{-1})	0.56 ± 0.19	59 ± 31
NA_{level} (μV)	0.33 ± 0.19	11 ± 10
V (ml)	0.26 ± 0.19	29

Mean and SD are descriptives of the average parameter values at 5.4 ml/min in each rat. %SD is the average of the coefficients of variance in each rat.
δt: period of increasing nerve activity; NA_{max}: maximum nerve activity; φ: time constant of decreasing nerve activity; NA_{level}: level to which the nerve activity decreased during perfusion. V: inserted volume at maximum nerve activity.

The model parameter NA_{max} was also modeled as a function of the pressure at $t_1+\delta t$, $P_{ura}(t_1+\delta t)$. There was an average difference of 5.8 ± 5.1 hPa between $P_{ura}(t_1+\delta t)$ and the steady state pressure (pressure at $t_1+\delta t << t < t_2$), which tended to increase at higher perfusion rates. In all rats, the mean values of NA_{max} and $P_{ura}(t_1+\delta t)$ were calculated at each flow rate. As we could not exclude possible differences in electrical coupling between nerve and electrode in different rats, the values of NA_{max} had to be normalized before averaging all rats. Since pressure readings were not exactly equal, this normalization could not be made to the value of NA_{max} at a certain pressure. We therefore fitted a linear function to the data of each rat and calculated the value of NA_{max} at 100 hPa, which was used as a calibration factor. All NA_{max} values were scaled by this calibration factor. Overall we thus found a linear relation between NA_{max} and $P_{ura}(t_1+\delta t)$ (Fig.7):

$$NA_{max} = 0.0039 \cdot P_{ura}(t_1+\delta t) - 0.02 \qquad (10)$$

Penile clamp measurements were done in 3 animals. In these experiments, nerve activity was measured in two modes. In *mode I*, nerve activity was measured during urethral perfusion. In *mode II* the urethra was closed at the distal end and the urethral pressure was manually varied. In mode I a linear relation was found between steady state pressure and NA_{level} ($NA_{level} = a_I \cdot P + b_I$, see Figure 8). Mode I was alternated with mode II measurements, in which urethral pressure and nerve activity were measured and a linear function was fitted ($NA = a_{II} \cdot Q + b_{II}$, Figure 9). After a 15 minutes stabilization period, the penile clamp experiments were performed in the order I-II-I. A complete series took ~30 minutes. Nerve

activity usually decreased with time. Therefore, we estimated the degeneration of the nerve activity by plotting the relative values of NA_{max}, NA_{level} (mode I) and NA(140hPa) (mode II) versus time. On average, the measured nerve activity decreased 5.7 ± 9.9 % with time in these measurements (range -20% to +10%). The mean ratios of parameters a and b that described the relation between nerve activity and urethral pressure in mode I (a_I, b_I) and in mode II (a_{II}, b_{II}) were averaged and are shown in Figure 10. On average, there were no differences in the evoked nerve activity between the two modes.

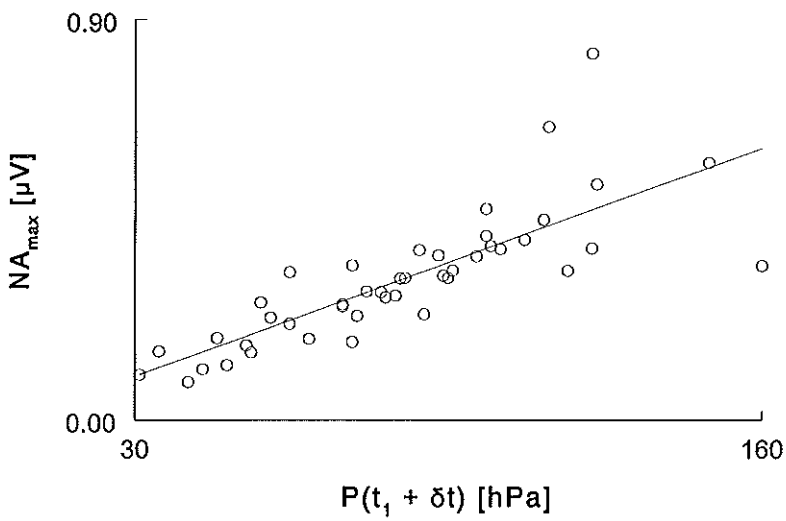

***Figure** 7. In the urethral perfusion measurements, pressure and nerve activity simultaneously reached a maximum ($P(t_1+\delta t)$ and NA_{max}). In all rats NA_{max} increased with $P(t_1+\delta t)$, and the nerve activity at 100 hPa was estimated from a fitted first order polynomial. This value was used as a normalization factor, to account for differences in electrical coupling between nerve and electrode. After normalization, the data from all 12 rats were pooled (○), and the relation between both variables was described by a linear equation ($NA_{max} = 0.0039 \cdot P - 0.02$).*

Lateral transection measurements were done in 4 rats, after the urethral perfusion measurements. The urethral nerve was cut to establish unidirectional nerve traffic. In one animal no nerve activity was measured after the transection. In the other three rats, nerve activity could still be measured and was compared to the activity in the intact nerve. In one animal, nerve activity was only measured at one flow rate setting after the transection. The differences in the four model parameters were statistically verified by 4 t-tests, and none of them was significant ($p > 0.14$, Table 3). In two rats, nerve activity was measured at various flow rates. In these animals, the effect of the transection was analyzed by an ANOVA procedure. In both animals, NA_{max} and δt depended significantly on the perfusion rate Q ($p < 0.01$) but not on *cut* (intact vs. transected, $p > 0.3$). NA_{level} and φ did not significantly depend on either Q or *cut* ($p > 0.15$, see Table 3).

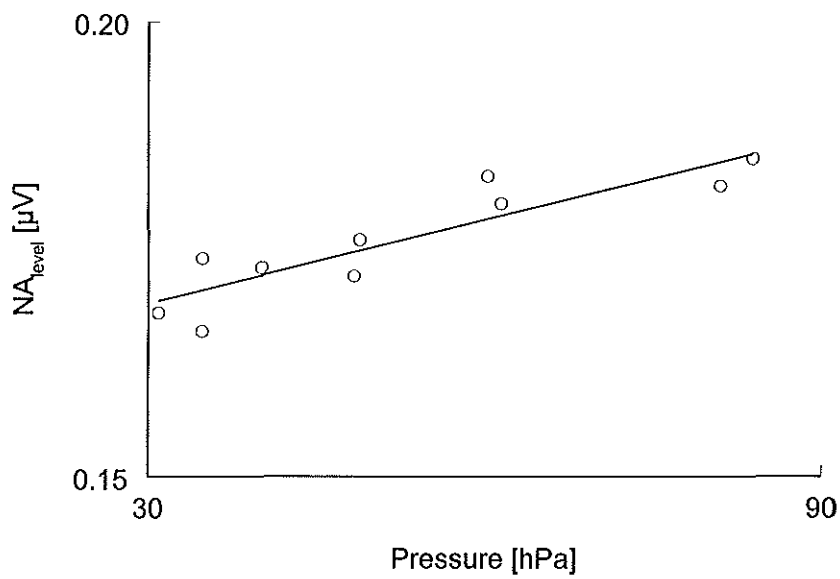

Figure 8. *Example of the relation between urethral pressure and NA_{level}, during perfusion. NA_{level} represents steady state urethral nerve activity at the end of perfusion; pressure is the average pressure during the last 5 seconds of perfusion. Line represents linear equation that minimized the sum of squares.*

DISCUSSION

In this study, we measured neural activity in nerves running over the proximal urethra in rats (urethral nerves). The measured activity was related to urethral pressure and flow rate. A four-parameter model adequately described the pattern of nerve activity measured in response to a stepwise change in urethral flow. This model consisted of a linearly increasing nerve activity during a period δt, until a maximum NA_{max} was reached, followed by a decrease with a time constant φ^{-1} down to an elevated level NA_{level}. NA_{level} depended linearly on the amplitude of the step change in flow rate (Q) (eq.10) and δt decreased inversely proportional to it (eq.9). The maximum activity, NA_{max}, increased at a decreasing rate with Q (eq.8).

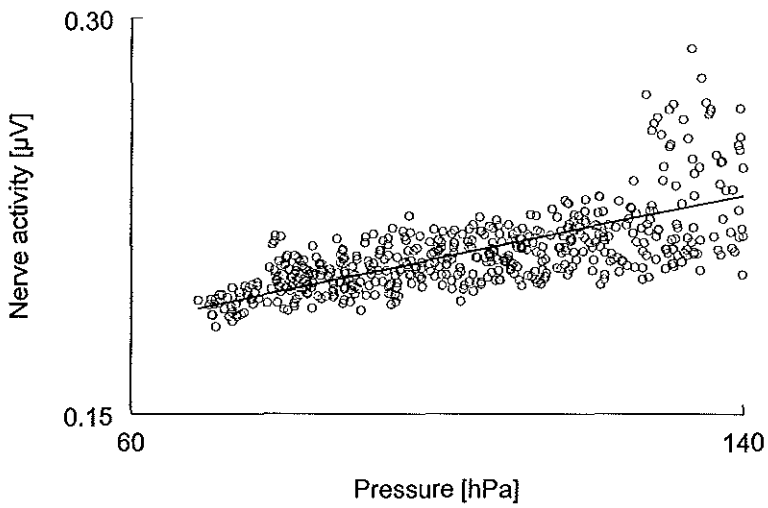

Figure 9. *Example of the relation between urethral pressure and urethral nerve activity. The urethra was clamped at the distal end and the urethral pressure was manually varied by insertion or withdrawal of small volumes of saline. Line represents linear equation that minimized the sum of squares.*

The values of the parameters NA_{max}, δt, and NA_{level} showed good reproducibility (mean coefficient of variation ~18%, table 2). This means that, as in postganglionic bladder nerves (12), a quantitative model of nerve activity and urethral dynamics is possible. One way analysis of variance showed that the flow rate dependency of the parameters was

significant ($p<0.05$) in 100%, 75%, and 33% of the animals (NA_{max}, δt, and NA_{level}). Since φ depended significantly on the flow rate in only 2 out of 12 animals, it was modeled by a constant value ($\varphi = 0.56\ s^{-1}$). The model described the data very well (fit error ~5.3%).

Figure 2 shows that in our measurements, urethral flow and transurethral pressure (P_{ura}) were linearly related. Others obtained similar results during perfusion of guinea pig urethra (6). Therefore, the nerve activity, which was modeled as a function of the flow rate in our analysis, could also result from pressure sensitive sensors, or from tension or extension receptors, which would also show a pressure dependent behavior.

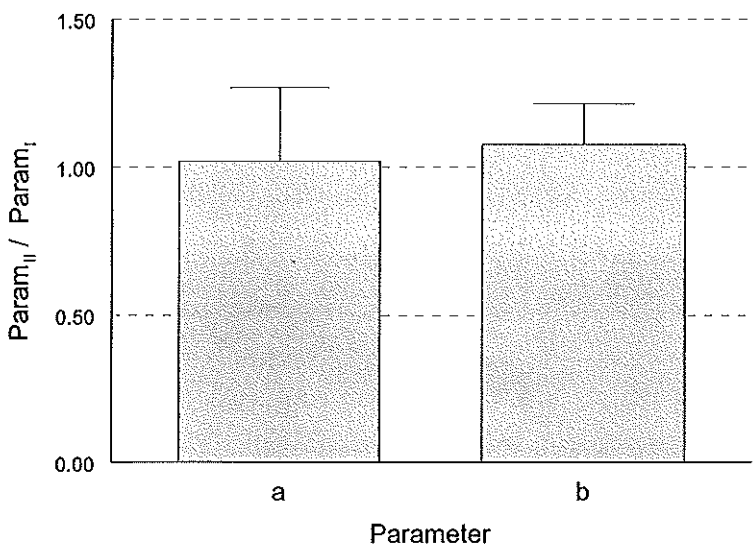

Figure 10. The relation between urethral pressure and nerve activity was described in two modes: I) during urethral perfusion and II) during manual variation of the pressure in the distally clamped urethra. In both modes a linear relation was found, with slopes a_I and a_{II}, and offsets b_I and b_{II}. In all rats, the ratios a_{II} / a_I and b_{II} / b_I were calculated. The average ratios are shown.

In the *penile clamp* measurements, in which the urethral resistance was increased to infinity by clamping of the urethra, the nerve activity at a certain urethral pressure was compared to that at a similar pressure during urethral perfusion. It was found that nerve activity always decreased during these measurements. This finding is supported by the results of others, who found nerve deterioration in the course of their experiments as well (18). The deterioration may be caused by the dissection from the underlying tissue and/or traction on the nerve. However, the decrease became less significant after ~15 minutes and evoked

nerve activity in both modes could be compared. As the nerve response in the urethra at a certain pressure, caused by perfusion, was identical to the response to a similar isometric pressure, we concluded that the activity in the urethral nerve is related to pressure, rather than flow. As the flow increases, P_{ura} increases as well, and so does the measured nerve activity. Thus the flow rate dependency of the model parameters NA_{max} and NA_{level} should be interpreted as a pressure dependency. In the steady state ($NA(t) = NA_{level}$), this can be established by inserting eq.4 into eq.8:

$$NA_{level} = 9.4 \cdot 10^{-4} \cdot P + 0.23 \qquad (11)$$

Due to transient effects, eq.4 is probably not valid at $t=t_1+\delta t$ (Fig.5). We therefore directly compared NA_{max} to the instantaneous pressure at $t_1+\delta t$. Eq. 10 shows that the transient nerve activity was also proportional to the pressure. A noticeable difference between eq.10 and eq.11 is the non-zero offset in eq.11. This is probably caused by the non-linear addition of noise and action potentials. At very low firing rates, the measured nerve signal consists mainly of noise, which results in a non-zero nerve activity after rectification and integration (eq.1). At high firing rates, this contribution of noise vanishes (11). Apparently, firing rates were high enough at $t=t_1+\delta t$ to conceal the noise contribution to the nerve activity. In the steady state, however, there was far less nerve traffic. This probably resulted in a significant contribution of noise, which would explain the offset in eq.11.

Table 3. *Statistical significance of the effect of nerve transection ("cut") and applied flow rate ("Q") on the model parameters.*

parameter	significance					
	rat 2		rat 5		rat 6	
	cut	Q	cut	Q	cut	Q
NA_{max}	n.s	-	n.s	$p<0.005$	n.s.	$p<0.005$
NA_{level}	n.s	-	n.s	n.s.	n.s.	n.s.
δt	n.s	-	n.s	$p<0.005$	n.s.	$p<0.005$
φ	n.s	-	n.s.	n.s.	n.s.	n.s

In three rats the urethral nerve was transected and model parameters before and after transection were compared. In rat 2 this comparison was done at a single flow rate setting. Parameter values were compared by t-tests. In the other rats, this comparison was done at various flow rates, by use of analysis of variance. Differences were considered significant if $p<0.05$; n.s. = not significant.

Between t_1 and $t_1+\delta t$, urethral nerve activity increased linearly with time and thus with the volume in the urethra ($V_{ura}(t)=(t-t_1)\cdot Q/60$). The inverse proportional relation between Q and δt suggests that maximum nerve activity was always found at a constant injected volume

(V = 0.26 ± 0.19 ml). This volume showed very good reproducibility in each rat (average coefficient of variation ~29%). Since nerve activity was linearly related to pressure a maximum pressure was also found at this inserted volume. This maximum pressure probably reflects the pressure necessary to open the urethra (and start the actual flow).
After this maximum, the nerve activity always decreased. This decrease may be the result of the pressure decrease that we measured (~6 hPa), which is probably caused by the vanishing of the so-called unsticking pressure. This is an extra pressure needed to unstick the walls of the collapsed urethra. After opening of the urethra, an extra pressure drop results (6). Because the tip of the needle connected to the pressure transducer was at the proximal end of the urethra, and the receptors were probably located further distally, the pressure gradient along the urethra during flow may result in a pressure at the location of the receptors which is lower than that measured by the pressure transducer. Thus, the pressure at the location of the receptors may decrease more than the measured ~6 hPa. However, the pressure decrease is probably not large enough to explain the decreasing nerve activity after $t_1+\delta t$. According to eq.10, a 10 hPa pressure drop would result in a decrease in nerve activity of ~0.04 μV. Yet the actual decrease is much larger.
A phenomenon that may play a more important role in this decreased activity, is adaptation of the receptors. Perfusion lasted much longer in our experiments than normal voiding in rats (~30s vs. <3s) (24). Since NA_{level} represents nerve activity in the adapted situation, and NA_{max} represents the immediate response to a pressure change, this would also explain the difference between the slopes of eq. 10 and eq. 11.

It has been shown that the urethral opening pressure is higher than the closure pressure (5), which as explained above, is not a sufficient reason for the decrease in the nerve activity from NA_{max} to NA_{level}. In the present study, the closure pressure could be determined accurately. In the last part of the urethral perfusion measurements, the flow through the urethra was turned off, which resulted in a pressure decrease down to a level at which the urethra closed. This pressure showed excellent reproducibility (coefficient of variation = 2.9%) and was independent of the applied flow rate ($P_{ura.clos}$ = 56 ± 22 hPa). This is in very good agreement with the data in Fig.2, where extrapolation of the pressure flow curve leads to $P_{ura.clos}$ = 59 hPa. In guinea pigs, $P_{ura.clos}$ was reportedly independent of the state of urethral relaxation (6). In our measurements, however, this can hardly be the case since rats normally void at bladder pressures ~41 hPa (24).

In the *lateral transection* measurements, the urethral nerve was cut on one side of the electrode to establish unidirectional nerve traffic. After transection of the nerve there was either no nerve activity left, or there was no significant change in activity. We therefore concluded that the intact nerve conducted unidirectional activity.
This was either efferent or afferent activity, but not both. Due to the difficulty in tracing the urethral nerve back to one of the major nerves, we could not determine whether the it was transected centrally or peripherally. However, the urethral nerves responded both to urethral perfusion and urethral pressure variations. Therefore it is probable that these nerves were conducting afferent activity. This finding should not be interpreted as evidence for a total absence of efferent fibers in the urethral nerves. Efferent activity to the urethra is

probably triggered by bladder afferents (3, 8, 17). Since we always maintained low bladder pressures to avoid urethral oscillations, afferent activity in postganglionic bladder nerves (Fig.1) was very low (2, 12) and efferent urethral fibers were probably not activated, even though they may exist (10).

According to the literature, nerves to and from the major pelvic ganglion, and branches of the pudendal nerve innervate the proximal urethra (2, 10, 20). These pudendal nerve branches conduct efferent activity to the striated urethral musculature (8, 13, 16). Urethral afferents are most likely conducted by the nerves that lead to the major pelvic ganglion, since they project through the pelvic nerve and, to a lesser extent, the hypogastric nerve (13).

In the *major nerve stimulation* experiments, we found that electrical stimulation of the pelvic nerve resulted in activity in 80% of the urethral nerves tested, after a 25 ms delay. Pudendal stimulation elicited similar activity after 35 ms in 63% of the tested urethral nerves and, moreover, resulted in a prolonged stimulus artifact. This prolongation was probably due to movement of the hind leg and/or the pelvic floor muscles. These results are very similar to those obtained by Steers et al (22). They found induced nerve activity in the cavernous nerve after stimulation of either the pelvic nerve (after 25 ms) or the distal nerve of the penis, which is a branch of the pudendal nerve (18 and 35 ms latencies). The activity that was evoked after pelvic nerve stimulation was considered efferent, and the responses to stimulation of the distal nerve of the penis were probably movement artifacts (18 ms) and afferent activity (35 ms response). In our measurements, responses to pudendal nerve stimulation coincided with a urethral pressure increase. The urethral nerve responded to pelvic nerve stimulation without an increase in urethral pressure. The evoked signals may thus very well be afferent (after pudendal nerve stimulation) and efferent (after pelvic nerve stimulation). The different latencies between the movement artifacts and stimulation of the distal nerve of the penis, or the pudendal nerve (8 vs. 18 ms), may be explained by the longer pathway through the distal nerve of the penis. The evoked activity in the cavernous nerve (which originates from the major pelvic ganglion), as measured by Steers et al (22), was very similar to our responses in a urethral nerve. Nevertheless, it is very unlikely that the urethral nerves are branches of the cavernous nerve, since stimulation of the cavernous nerve did not result in any activity in the urethral nerves. Other researchers described nerves running between the major pelvic ganglion and the urethra (10, 20). In 2 animals we were able to trace the urethral nerves back to the major pelvic ganglion. Thus it is most likely that the urethral nerves we have been recording from were leading to the major pelvic ganglion.

Computer averaging of consecutive responses to pudendal nerve stimulation removed the response, indicating that the individual responses had a different latency. Therefore the fibers were probably indirectly stimulated. The action potentials may have been induced by an increase in urethral pressure, which always occurred with pudendal nerve stimulation. Similar averaging of potentials evoked by pelvic nerve stimulation, did not remove all

activity, indicating a direct pathway between pelvic nerve and urethral nerve. In cats, the conduction velocity of urethral afferents is ~30 m/s (2). In our experiments, the distance between stimulation and recording electrode was ~10mm, which would result in a 0.3 ms latency. In our measurements, such a response would coincide with the stimulus artifact, which lasted ~2 ms. The 25 ms response found, may have been induced by efferent fibers, which are known to synapse in the major pelvic ganglion (15, 22). This would suggest the existence of efferent fibers in the urethral nerves.

In summary, it is concluded that the activity in urethral nerves of the rat is most likely afferent, and conducted through the major pelvic ganglion. During urethral perfusion, a nerve activity pattern was measured that could adequately be described by a four parameter model (mean fit error ~5.3%). This model consisted of a linearly increasing nerve activity during δt s, until a maximum NA_{max} was reached. Then the activity decreased with a time constant φ^{-1} to a steady state level NA_{level}. NA_{max}, δt and NA_{level} depended significantly on the perfusion rate in a large number of animals (70 ± 36 %). Evidence has been provided though, that the nerve activity was generated by pressure sensors in the urethra, rather than flow rate sensors. NA_{level} increased linearly with the steady state pressure and the NA_{max} was proportional to the instantaneous pressure after δt s (at $t=t_1+\delta t$). δt was the time needed to insert a fixed volume to open the urethra. After opening of the urethra, the receptors probably adapted to the higher pressure with a time constant φ^{-1} (~1.8 s).

ACKNOWLEDGEMENT

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4. Threshold for efferent bladder nerve firing in the rat.

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Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45) R1819-R1824, 1999.

ABSTRACT

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 using a theoretical model. The beginning of an active bladder contraction was defined by the onset of bladder efferent firing at a certain time t_0. From t_0 onwards, 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 the 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 it 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.

key words: nerve activity, micturition threshold, rat

INTRODUCTION

Many of the mechanical properties of the lower urinary tract have been described (6), as well as the anatomy of the major innervating nerves (9,13,21). Our aim is to develop a model which describes the quantitative relationships between mechanical properties of the bladder and the 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, 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 (12). 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 be able 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 (4). In the present study, the beginning of a contraction was defined as the onset of firing in efferent bladder nerves, and nerve signals that might be involved in the initiation of bladder contractions (bladder and urethral afferent) were investigated.

MATERIALS AND METHODS

Male Wistar rats (10, mean weight 434 ± 37 g) were anesthetized with urethane (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 23G 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 using 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 EMG amplifier (DISA 15C01, amplification range 5000-50000x, common mode rejection >100dB, pass band 20 - 2000 Hz). Pressure and nerve signals were recorded on tape (Racal tape recorder, frequency band 0 - 5000 Hz) or read into a personal computer directly at sample rates of respectively 10 and 25000 Hz, using a specially developed program. The tape recorded nerve signals were read into the computer as well. All nerve signals were filtered using 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 (7). All settings were adopted from previous work in which optimal conditions for recording, amplification and filtering were determined (4). Overall, the nerve signal that was used for analysis was filtered with a pass band 100 - 2000 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 which 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 due to movement artifacts. The quality of the nerve recording was assessed by the signal-to-noise-ratio (SNR). Assuming that the minimum activity represented noise only and that noise and nerve activity add linearly, the SNR was estimated as the mean of the ten highest nerve activity values minus the mean of the ten lowest values divided by the mean of the ten 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 (3). The real SNR values are thus a little higher than the ones calculated. Animals which 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.

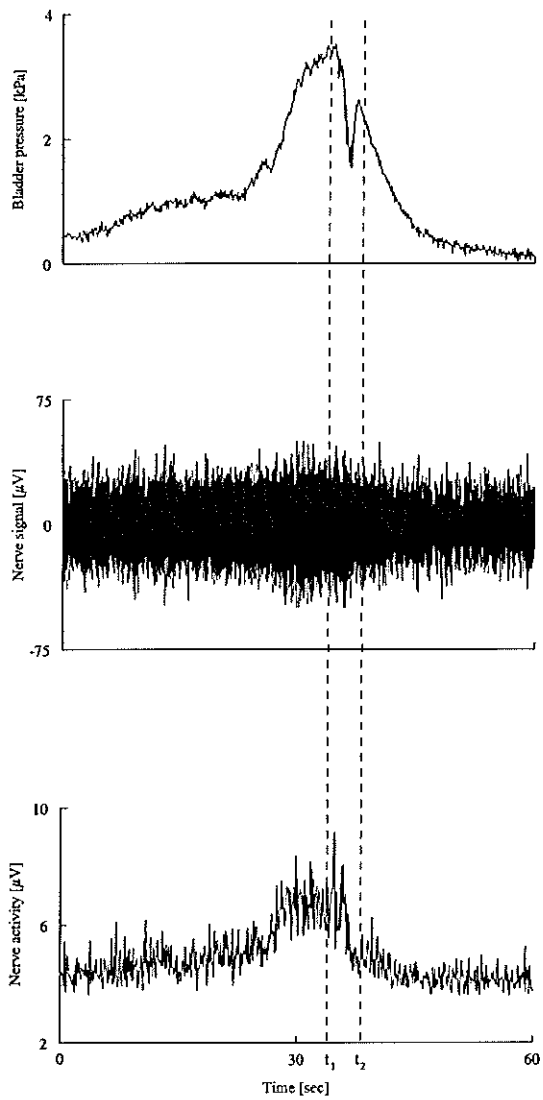

Figure 1. *Bladder pressure (top trace) and the postganglionic bladder nerve signal (middle trace) recorded during a detrusor contraction. The bottom trace shows the calculated total bladder nerve activity. Between t_1 and t_2 voiding took place, pressure oscillations disturbed adequate measurement of nerve activity, therefore this time period was omitted from analysis.*

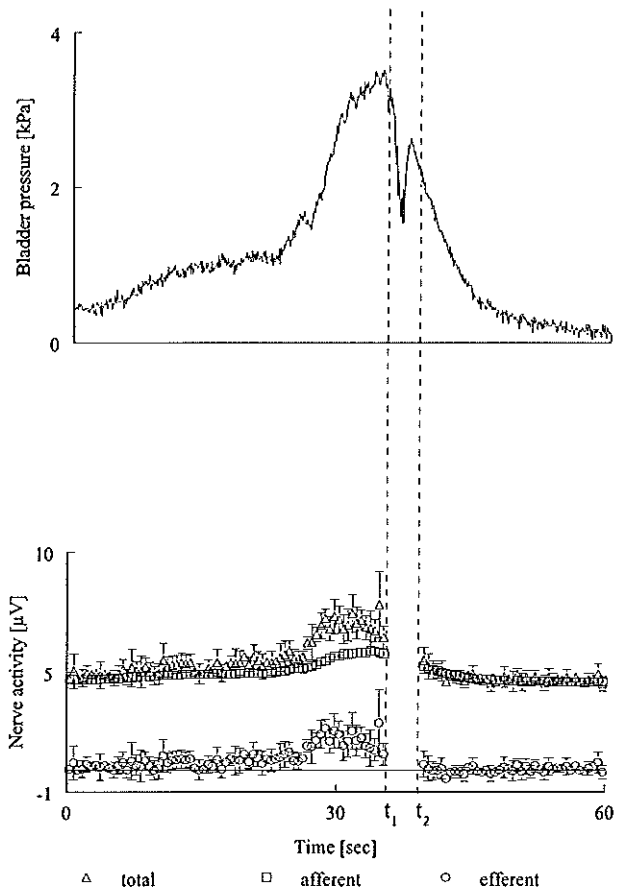

Figure 2. *Bladder pressure and bladder nerve activity during a contraction. Total nerve activity (Δ) was resolved into afferent (□) and efferent (○) activity (mean ± SD in 0.5 s intervals).*

In a previous study, a bladder afferent/efferent model has been developed which allows the distinction of afferent and efferent bladder nerve activity in intact postganglionic bladder nerves (Fig. 2) (4). This model is based on the following assumptions:

- efferent bladder nerve activity is negligible during the pressure decline immediately after voiding
- total bladder nerve activity is the linear sum of afferent and efferent activity
- the relation between bladder pressure and afferent bladder nerve activity can be described by a linear equation.

As 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 assumptions:

- the onset of a voiding contraction was defined at $t = t_0$
- efferent activity is 0 until t_0
- after t_0, efferent activity increases linearly during δt seconds, to a maximum value

The pressure at t_0, the afferent nerve activity at t_0, and the time during which efferent activity increased (rise time) δt, were determined in 30 contractions measured in 6 animals. The model was fitted to measured data by minimizing the mean squared error using 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 which 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 minutes of recording including 37 contractions from 6 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 contractions). This urethral afferent model is based on the following assumptions:

- up to a certain bladder pressure, afferent urethral activity is constant
- 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 to the bladder pressure at t_0, to determine whether urethral afferents are involved in the initiation mechanism.

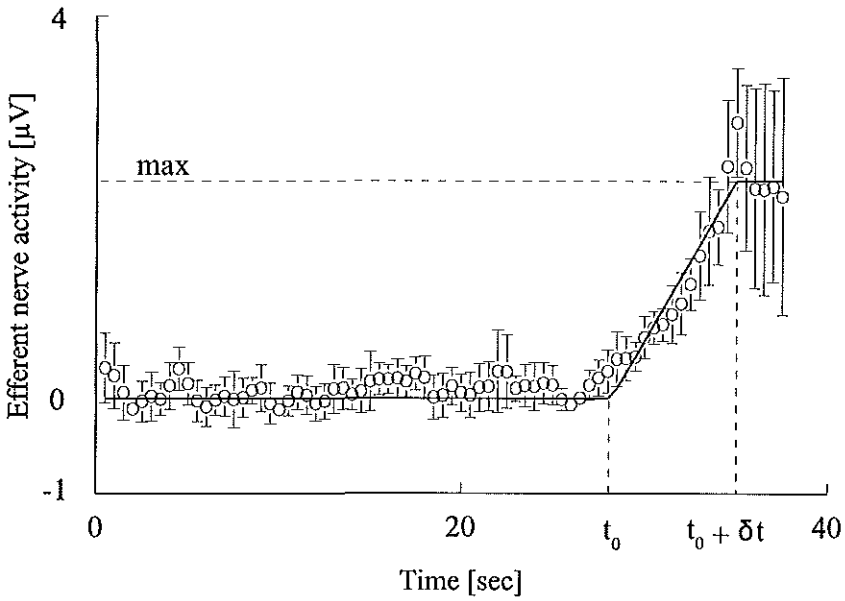

***Figure 3** Calculated (open circles) and fitted (line) efferent bladder nerve activity during the pressure rise of a bladder contraction (mean ± SD in 0.5 s intervals). Efferent activity was assumed to be 0 until t_0, then increased linearly to a maximum value in δt s. The fit error in this example was 5.8 %.*

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 mean ± SD. Parameter values were compared using the Mann-Whitney U test, Student 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 (4).
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) (4). Furthermore, building up pressure takes time, the smooth muscle of the bladder wall itself is slow (the time constant of the pressure development was 3.4 s) (4).

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 the maximum total nerve activity. t_0, Onset of bladder efferent activity; rise time, time during which efferent activity increased.

Bladder nerve activity was measured during 30 contractions in 6 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 the

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 7 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).

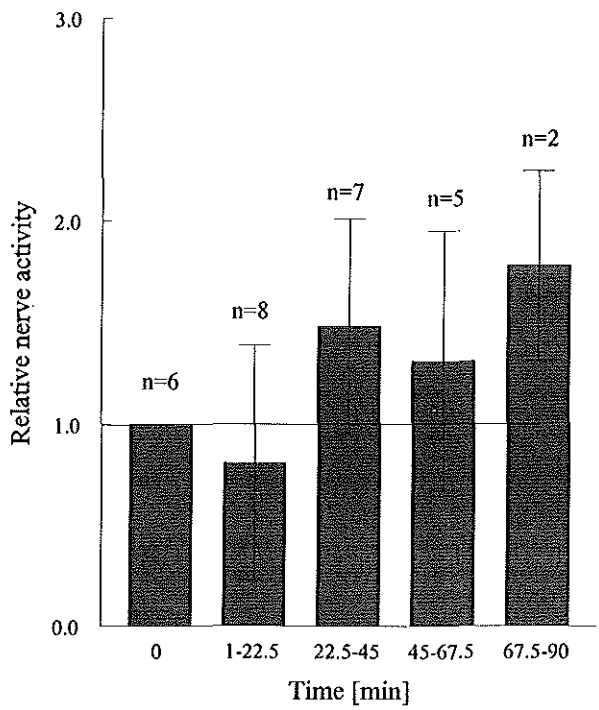

Figure 4. *Relative nerve activity as a function of time. For each of the 6 animals, the recorded nerve activity during the experiment was expressed as a percentage of the activity at the beginning (t=0, nerve activity =1). Bars represent the mean ± SD of all animals.*

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).

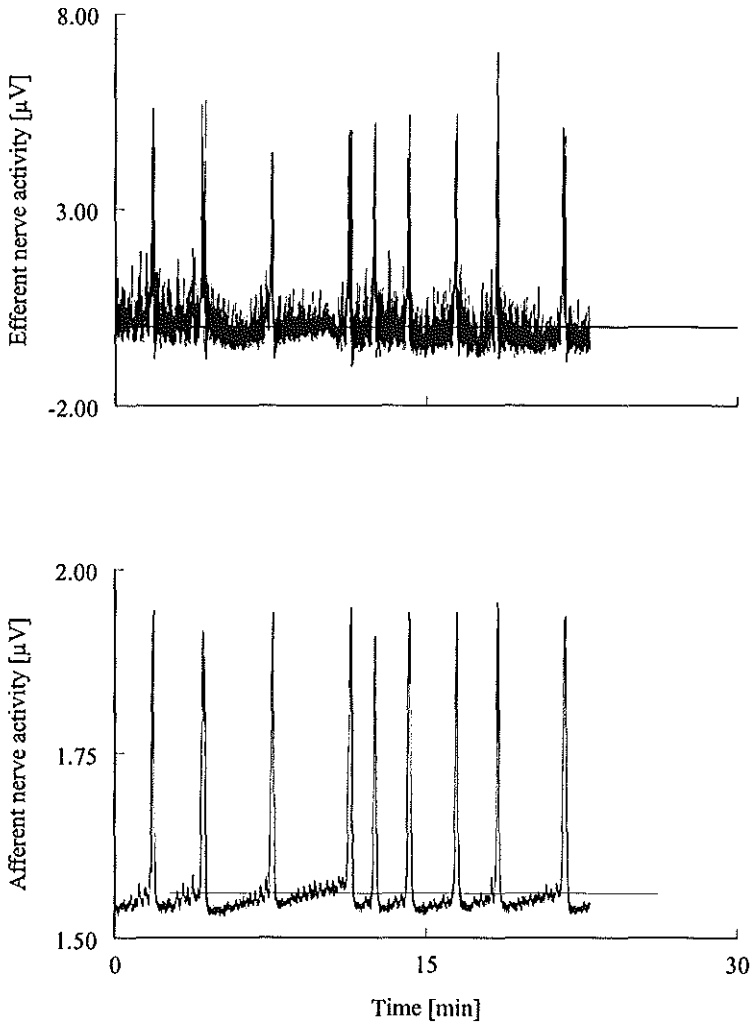

Figure 5. *Example of efferent bladder nerve activity (top trace) and afferent bladder nerve activity (bottom trace) during a continuous recording (30 minutes period includes 9 spontaneous contractions). Line shows the mean afferent bladder nerve activity ± SD at t_0 (1.56 µV) for all contractions included.*

It seemed to increase slightly with time, but the change was not significant (One-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.

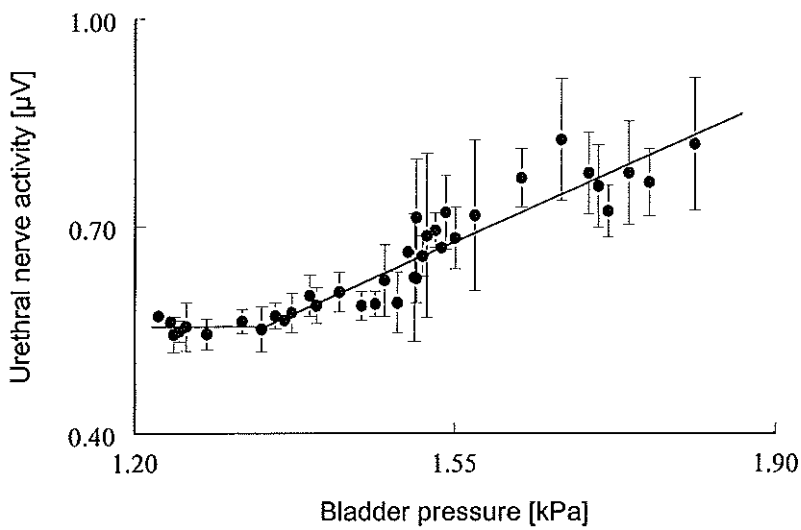

Figure 6. *Example of urethral afferent nerve activity as a function of bladder pressure (mean ± SD in 0.3 s intervals). A straight line was fitted to the increase. The fit error in this example was 5.3%.*

In one animal, contractions occurred without filling the bladder, these 10 spontaneous contractions were 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 (One-way ANOVA, $p< 0.005$).
The analysis of the long-term recordings, in total 107 minutes 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 non-voiding contraction (not shown).
Urethral nerve activity was recorded simultaneously with bladder pressure in 10 contractions from 4 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, $p<0.01$).

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 (10) (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: 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 (10) 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. 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. 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 (1). 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 (11). 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 stated in their simulation model that bladder afferents initiate and urethral afferents sustain voiding contractions (8). 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 6 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, exceeding the afferent bladder nerve activity at t_0 led to a small non-voiding contraction which 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 which 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 (10).

When afferent urethral nerve activity was recorded simultaneously with bladder pressure (5), 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 contraction and that there is a definite, reproducible threshold in afferent bladder nerve activity which has to be exceeded in order 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.

ACKNOWLEDGMENT

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5. Modeling of afferent bladder nerve activity in the rat.

ABSTRACT

In neurophysiological models of the lower urinary tract several mechanical variables have been proposed as the primary stimulus for afferent bladder nerve activity. In this study we used rats to measure bladder pressure and volume, and we calculated strain, surface, tension and stress. These were correlated with the measured afferent nerve activity. The highest correlation was found between stress and afferent nerve activity. In our measurements stress was proportional to bladder pressure × volume (P×V). Afferent nerve activity depended linearly on P×V within 6% and both slope and offset were independent of the bladder filling rate.

We also calculated the levels of afferent bladder nerve activity at the onset and cessation of efferent firing to the bladder. Both were highly reproducible with coefficients of variation ≤ 15%. We propose a bladder model in which afferent activity is proportional to P×V, bladder contraction is initiated when an afferent threshold is exceededed due to an increasing pressure and volume, and continues until afferent activity drops below a threshold again because of a decreasing volume. Then the contraction stops and afferent activity decreases further with the pressure. Thus in both directions a minor excess of the threshold results in a decisive threshold crossing. On average the second threshold was slightly higher than the first.

INTRODUCTION

In the micturition cycle two phases can be distinguished. During the storage phase, when urine is collected in the bladder, the bladder pressure is low while urethral pressure must be high enough to prevent leakage. In the voiding phase urine is expelled from the bladder, which involves bladder contraction and urethral relaxation (6). Both processes are regulated by the central nervous system. To enable neural control over the bladder and the urethra, afferent nerve fibers supply the central nervous system with information on the physical state of bladder and urethra.

Detailed knowledge of the nervous control over the lower urinary tract may enable the development of new diagnostic methods or treatment of neurogenic dysfunction. In paraplegics, for example, the control of the lower urinary tract could perhaps be taken over by a computer that uses afferent nerve activity as input, if all the nerve signals involved were known in sufficient detail. Several models of the innervation of the lower urinary tract that combine physiological and neuronal properties have been proposed (7, 11, 27). The neuronal properties, however, have obtained far less attention in past research than the physiological properties and crude assumptions were made in the developed computer models.

Recently more data have become available that describe the relations between activity in the nerves that innervate the lower urinary tract and physiological variables, such as

bladder pressure or urethral flow. Due to the complexity of the urethra very few papers have been published that relate urethral afferents to urethral flow or pressure (14, 24). Bladder afferent nerve activity, however, has been described more extensively.
Generally, a division is made in two types of afferent fibres: C and Aδ fibers. The C fibers are also referred to as silent fibers and are generally assumed to be active only in situations of inflammation (8). During normal micturition the state of the bladder is monitored by Aδ fibers which are generally conducted through the pelvic nerve (3, 8). The activity in these fibers has been related to bladder pressure (2, 13, 19, 23), wall tension (5, 11, 20) or stress (21). Occasionally receptor area related activity (5) or volume receptors (18) have been reported. However, the definitions of the mechanical variables that may control nerve activity are often very unclear. For example, this confusion is reflected in the frequently quoted fundamental study by Iggo (12), entitled 'Tension receptors in ... the urinary bladder', where the afferent nerve activity is actually related to bladder pressure rather than to tension.
It is possible that the bladder wall contains several types of receptors. However, Häbler et al concluded that the myelinated sacral afferents of the urinary bladder form a homogeneous population which encodes all information necessary for the normal regulation of this organ (8). Moreover the assumption of several receptor types would lead to highly complex neurophysiological models of the lower urinary tract which are undesirable and may not be nessecary. Therefore, generally, black box models are used that relate the average nerve activity to either volume, pressure, wall tension or stress.

In an earlier study, we showed that afferent bladder nerve activity triggers efferent firing to the bladder (26) and thus plays an important role in any neurophysiological model of the lower urinary tract. In that study afferent activity was related to bladder pressure. It is possible that the relation between afferent nerve activity and pressure was found adequate only, because the bladder volume hardly changed in that study. It is yet unknown what causes the cessation of efferent bladder nerve activity. As this may involve the bladder volume, it is important to accurately determine the stimulus to relate afferent bladder nerve activity to.

This study was done to determine the primary stimulus for afferent bladder nerve activity, and to extend the existing neurophysiological models of the lower urinary tract with quantitative relations between afferent activity and mechanical bladder variables. Several variables were calculated from the bladder volume and pressure and were compared to determine which one correlated best to afferent bladder nerve activity. In the literature the concepts of wall tension and stress are sometimes confused. According to the definition of these quantities, we calculated tension as being proportional to $P \times r$ (r = radius) and stress proportional to $P \times V$ (see appendix). The best variable to relate afferent nerve activity to was used to investigate the levels of afferent nerve activity at the onset and cessation of efferent firing to the bladder.

MATERIALS AND METHODS

Surgery

Male Wistar rats were anaesthetised with urethane (1.2g/kg) intraperitoneally (16) and placed on a heated undercover. An abdominal midline incision was made to access the nerves and the bladder. A few (3 or 4) left side postganglionic bladder nerves (PBNs) were dissected at two distinct sites and marked with sutures. These two sites are referred to as central and peripheral (see Fig. 1). PBNs lead from the major pelvic ganglion (MPG) to the bladder (17). The nerves on the right side were left intact. Only one PBN was used for recording nerve activity, but several were dissected to increase the chance of successful recording from an undamaged PBN. The abdomen was kept moist with warm saline. The animal was placed in a frame with a heated ground plate. The abdominal wall was then tied to the frame to create a basin, which was filled with warm paraffin oil during the measurements.

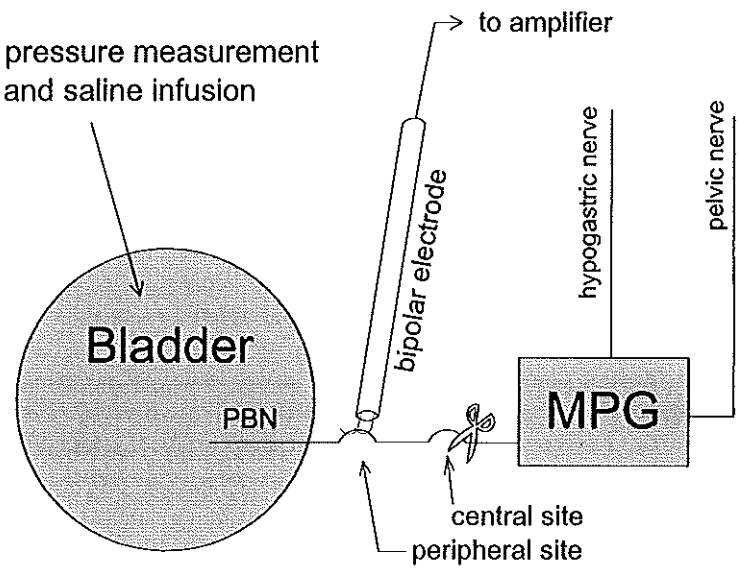

Figure 1. *Schematic drawing of the measurement setup. The bladder was filled with saline through a needle inserted in the bladder, until a voiding contraction occurred. Bladder pressure was also measured through this needle. Postganglionic bladder nerves were dissected between the bladder and the major pelvic ganglion (MPG) at two sites. The peripheral site was used for recording nerve activity with a bipolar platinum iridium electrode. For afferent nerve recordings, the bladder nerve was transected at the central site.*

Experimental set-up

A bipolar platinum-iridium electrode mounted on the frame was lowered into the paraffin oil basin. The electrode consisted of two metal hooks at a distance of ~ 0.5 mm. A postganglionic bladder nerve (at the peripheral site, see Fig. 1) was guided over the electrode, which was slightly elevated for recording nerve activity. The central site was used to enable transection of the nerve without changing the electrical coupling between electrode and nerve at the peripheral site. Transection excluded all efferent nerve activity from the signal. If there was no detectable nerve signal, another PBN was guided over the electrode.

The recorded nerve signal was amplified by a DISA 15C01 EMG amplifier (amplification range: 50,000 – 200,000) and band-pass filtered (Bessel, 4th order; Krohn-Hite model 3944). The pass band was set from 100 Hz to 2 kHz, allowing measurement of spikes with pulse widths down to 0.5 msec (22). The recording electrode was moved along the PBN until the difference between background noise and action potentials on the oscilloscope was maximal. The combination of the symmetrical electrodes and an EMG amplifier with a very high common mode rejection ratio (CMRR >100 dB) ensured a minimal radio frequency and 50 Hz power supply interference.

A 24 Ga angiocatheter was inserted near the top of the bladder. Through this catheter the bladder was filled with room-temperature saline while the intravesical pressure was measured using a disposable pressure transducer and a Statham SP1400 blood pressure monitor. The bladder was filled with a Harvard Apparatus (Millis-Massachusetts) infusion pump at infusion rates of 0.2, 0.5, 1, or 2 ml/min. The pressure loss in the connecting tubing was measured and corrected for.

The pressure and bladder nerve signals were read into a personal computer at sample rates of 10 Hz and 25 kHz respectively, using especially developed software driving a PCL 818 analog to digital converter.

After the experiments the bladder was removed from the animal and put into a measuring glass filled with water, to determine its tissue volume (V_t). The bladder weight was determined with a balance (Sartorius 2004 MP).

Modeling

NERVE ACTIVITY. The mean value of the rectified nerve signal in 100 ms intervals was calculated as a measure for total nerve activity (NA) (1, 9, 13, 30). As a result of differences in electrical coupling between nerve and electrode, the signal to noise ratio (SNR) in the nerve signal varied. We calculated a measure for the signal to noise ratio, SNR_{est} as described before (13). Measurements were analysed only if $SNR_{est} \geq 1$ in the intact nerve (combined signal of efferent and afferent nerve activity), or $SNR_{est} \geq 0.5$ in the centrally transected nerve when the measured nerve activity was purely afferent ($NA=NA_{aff}$). These threshold values were based on audio-visual judgement of the nerve signals. Measurements with action potentials that could clearly be seen on the oscilloscope were thus selected.

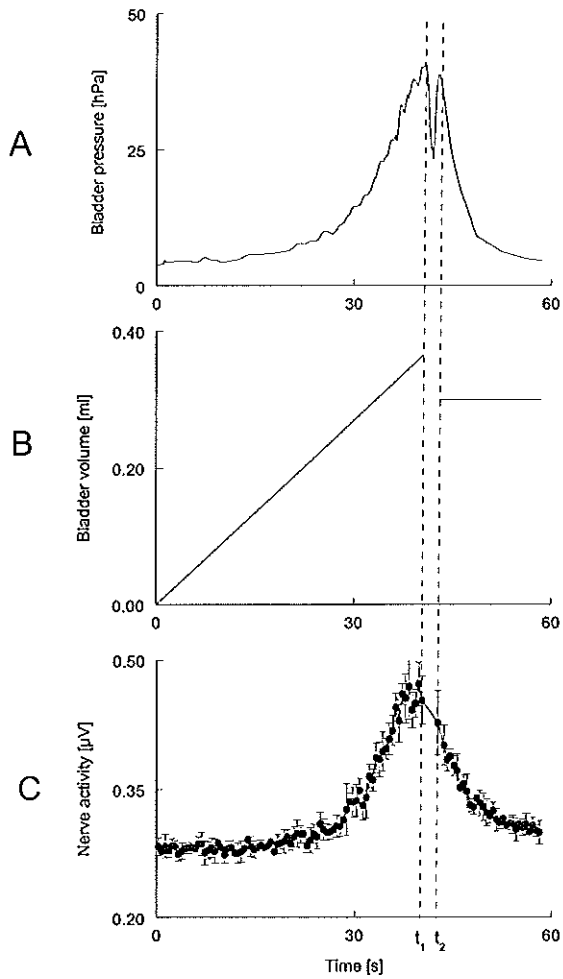

Figure 2. *Examples of the measured bladder pressure (A), volume (B) and afferent bladder nerve activity (C). The bladder was manually emptied before the measurement. At $t = 0$ the infusion pump was started and the bladder was filled until a voiding contraction occurred. Then the pump was switched off. After the measurement the residue was determined. The period with flow through the urethra ($t_1 < t < t_2$) was excluded from the analysis because there were movement artifacts in the measured nerve signal in this episode and the bladder volume was not exactly known.*

Nerve activity could not adequately be determined during the actual bladder voiding ($t_1 < t < t_2$, see Fig. 2) due to movement artefacts caused by rhythmic urethral sphincter contractions (13). Moreover, the bladder volume V was not exactly known during voiding. Therefore the period $t_1 < t < t_2$ was excluded from analysis. For the determination of t_1 and t_2, the pressure signal was displayed on a computer screen. Using a mouse cursor t_1 and t_2 were selected at the approximate pressure maxima immediately before and after voiding.

BLADDER MECHANICS. The afferent nerve activity (NA_{aff}) was related to the bladder pressure (P), the bladder volume (V) and the derived variables: strain (ε), bladder surface (A_{det}), bladder wall tension (T) and stress (σ). For the calculation of these variables, the bladder was approximated by a sphere with volume V and radius r (29).

$$\varepsilon = (l - l_0)/l_0 = \sqrt[3]{V/V_0} - 1 \quad (1)$$

$$A_{det} = 4\pi r^2 = 4\pi \cdot (3V/4\pi)^{2/3} \quad (2)$$

$$T : \text{proportional to } P \cdot r \text{ (see appendix)} \quad (3)$$

$$\sigma = 3PV/2V_t + P/4 - P_{out} \text{ (see appendix)} \quad (4)$$

$$\sigma_{est} : \text{proportional to } P \times V \text{ (see appendix)} \quad (5)$$

Here l is the length of a certain bladder segment with initial length l_0; V_0 is the initial bladder volume. V_t is the bladder tissue volume and P_{out} is the atmospheric pressure, which is considered a constant. We calculated the correlation coefficients of NA_{aff} with each of these variables. We also fitted linear relations between afferent nerve activity and the 7 variables ($NA_{aff} \sim$ slope·*variable* + offset), and calculated the relative fit error.

$$\text{relative error} = \frac{\Sigma \left| NA_{aff} - (\text{slope} \cdot \textit{variable} + \text{offset}) \right|}{\Sigma NA_{aff}} \times 100\% \quad (6)$$

BIDIRECTIONAL NERVE TRAFFIC IN INTACT NERVES. Nerve activity was measured either in intact nerves, or in centrally dissected nerves. The measurements with centrally dissected nerves were used to determine the mechanical variable that correlated best with afferent bladder nerve activity (see below). In intact nerves a combined signal of efferent and afferent activity was measured. The intact nerve measurements were analysed with a previously described model (13), which enabled estimation of afferent and efferent contributions to the nerve signal. However, this model was slightly modified:

- It was assumed that all nerve activity after t_2 (Fig.2) was afferent, which enabled determination of the relation between NA_{aff} and the selected variable (not necessarily the bladder pressure as assumed in the original model).
- It was assumed that NA_{aff} depended linearly on this variable. Slope and offset were determined from the episode $t>t_2$ (Fig. 2). Thus NA_{aff} could be calculated for $t < t_1$ (Fig. 2)
- Linear addition of efferent and afferent nerve activity was assumed, which enabled estimation of NA_{eff} by subtracting NA_{aff} from the measured total nerve activity.

Measurements

In each rat the bladder was filled with saline until a voiding contraction occurred. Bladder pressure and postganglionic bladder nerve signals were recorded from the start of the bladder filling until the pressure returned to baseline after the voiding contraction (maximum 300 seconds). After each measurement the bladder was manually emptied with a syringe to determine the residual volume. Thus, except from the actual voiding phase ($t_1 < t < t_2$, Fig. 2), the bladder pressure and volume were known throughout the recording. An example is shown in Figure 2.

The measurements were done under two conditions: intact or centrally transected nerves.

1) CENTRALLY TRANSECTED NERVE. In these measurements the recorded nerve signal was purely afferent. From each measurement, seven correlation coefficients were calculated for NA_{aff} with P, V, A_{del}, ε, T, σ, and σ_{est} (eqs. 1-5). Linear models (NA_{aff}= slope·*variable* + offset) were fitted between NA_{aff} and the best correlating variables. The applicability of the assumed linear relations was assessed by the relative error (eq. 6). In each rat both the differences between the 7 correlation coefficients and the differences between the fit errors were statistically verified using a paired t-test.

We also investigated the effect of the filling rate on the evoked NA_{aff}. To this end we fitted linear relations to data obtained at 4 different filling rates (0.2, 0.5, 1 or 2 ml/min). In each rat the slopes and offsets of the 3 best correlating variables were normalised to their values at 1.1 ml/min, and averaged. Thus, at each filling rate 3 mean normalised slopes and offsets were calculated. Filling rate dependence of slopes and offsets were statistically tested by one-way analysis of variance.

Furthermore, slope and offset of the relation between NA_{aff} and each variable during $t > t_2$ were determined, and the nerve activity during $t < t_1$ was calculated based on these parameters, to assess the error made in the calculation of afferent nerve activity at $t < t_1$ in the intact nerve measurements.

Finally we estimated the level of afferent nerve activity at the start of bladder contraction. The pressure signal was displayed on a computer screen and the point where it first started to increase progressively ($t_{0,est}$) was selected with a mouse cursor. Afferent activity at this moment was both determined from the nerve signal and calculated from the appropriate linear relation. It was compared with the afferent activity at $t = t_2$. In some measurements this

moment could not be detected clearly. These measurements were excluded from further analysis. In these measurements, the value of $t_{0,est}$ could not be determined more accurately than this rough estimation. For a better determined value, we did measurements in intact nerves.

2) INTACT NERVE. In these measurements a combined signal of efferent and afferent nerve activity was measured. The measurements were done to determine the level of afferent nerve activity at the beginning and end of efferent firing. The analysis of these measurements was done after, and depending on the results of the centrally transected nerve measurements, although the measurements were obviously done before the transection. From the centrally transected nerve measurements we found that stress or pressure×volume (P×V) correlated best to afferent nerve activity. Afferent and efferent contributions to the measured combined nerve signal were calculated with the model explained above, and afferent activity was assumed to be proportional to P×V. A model was fitted that assumed no efferent activity until t_0, and then a linear increase until the voiding started (26). The start of bladder contraction was defined as the moment that efferent firing to the bladder was initiated (t_0 in Fig 7), thus avoiding problems with measurements that lacked a clearly recognisable contraction start in the pressure signal.

The exact ending of efferent firing could not be determined because of the movement artefacts that were recorded during flow. To estimate the level of afferent activity at the end of efferent firing, we calculated afferent nerve activity immediately after the last oscillation and at t_2 (see Fig.2). The reproducibility of these levels of afferent nerve activity was assessed by their coefficients of variation (SD/mean × 100%). In a previous study, it was shown that efferent nerve activity to the bladder starts when the afferent activity (related to bladder pressure) exceeds a certain threshold (26). In this study we recalculated this threshold, with afferent activity now calculated from P×V. We compared this threshold with the estimated level of afferent nerve activity at the end of efferent firing using a paired t-test.

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. All data are presented as mean ± standard deviation. Differences were considered statistically significant if $p<0.05$.

RESULTS

In this study 16 rats (451 ± 47 g) were used to measure bladder pressure, bladder volume and bladder nerve activity during the micturition cycle. Bladder weight (m) and tissue volume (V_t) were determined in 10 rats: Vt = 0.23 ± 0.06 cm^3; m = 0.21 ± 0.06 g. The density of bladder tissue averaged 0.95 ± 0.23 g/cm^3.
In all measurements the bladder was filled with saline until a voiding contraction occurred (mean filled volume: 0.82 ± 0.19 ml). After bladder voiding the residue was determined. It averaged 0.58 ± 0.22 ml. To investigate whether the residue depended on the filling rate, we normalised the residues in each rat to their value at Q = 1 ml/min and averaged the normalised values. The residue tended to increase with the bladder-filling rate. However this effect was not significant (one way ANOVA: p = 0.12).
Nerve activity was recorded from intact bladder nerves in 5 rats with an $SNR_{est} > 1$, and in 6 rats we adequately measured afferent bladder nerve activity in centrally transected nerves with an $SNR_{est} > 0.5$.

1) CENTRALLY TRANSECTED NERVE MEASUREMENTS: In 93 measurements from 6 rats afferent postganglionic bladder nerve activity was recorded during $t < t_1$ and $t > t_2$ (see Fig. 2), and correlated to bladder pressure (P), volume (V), strain (ε), surface (A_{det}), tension (T) and stress (σ). An example is shown in Figure 3.
For the calculation of stress, the bladder tissue volume V_t is needed (see appendix). Because this parameter was not determined very accurately, we also approximated stress by σ_{est} = pressure × volume. The calculated correlation coefficients for stress and P×V differed less than 0.1%. The correlation coefficients are shown in Table 1. The best correlation was found between nerve activity and stress (or P×V). Nerve activity always correlated significantly better with stress than with strain, surface and volume (paired t- test: $p<0.05$). In 83% of the rats, nerve activity correlated significantly better to stress than to pressure, and in 67% also better than to tension.

Next, we fitted linear relations between afferent nerve activity and the 4 best correlating variables (NA = slope·*variable* + offset). The slopes, offsets and relative fit errors were calculated, and are also shown in Table 1. Stress (and σ_{est}) showed the smallest fit error, significantly smaller than for pressure in 83% of the animals, and in 33% also significantly smaller than for tension. An example is shown in Figure 3.
In each measurement, fit errors using stress or P×V were equal, offsets differed less than 0.25% and slopes differed a fixed factor, equal to $3/2V_t$ within 5%. We decided to calculate stress simply as being proportional to P×V at any bladder volume, thus avoiding the inaccurately measured tissue volume.

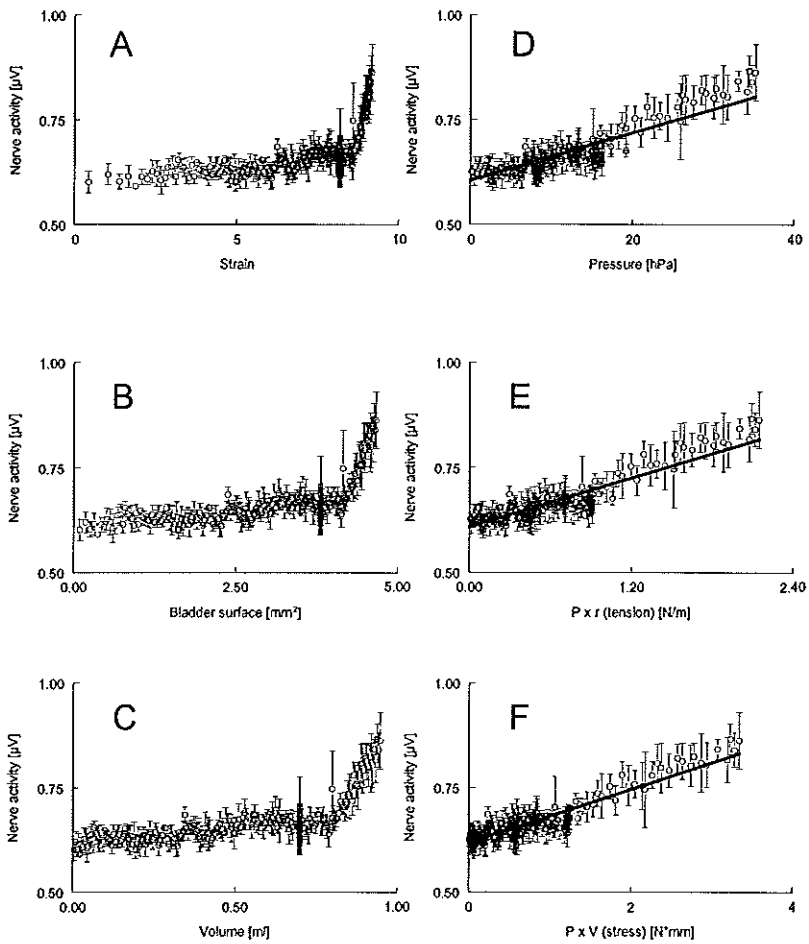

Figure 3. *An example of bladder nerve afferent activity measured during one filling/voiding cycle, related to strain (A), bladder surface (B), volume (C), pressure (D), p×r (tension, E) and P×V (stress, F), shown in order of increasing correlation coefficient (on average r^2 = 0.55, 0.60, 0.64, 0.78, 0.80, and 0.81 respectively). Linear equations were fitted to the relations between nerve activity and the 3 best correlating mechanical variables: pressure, tension and stress.*

In 5 rats the bladder was filled at 4 different filling rates: 0.2, 0.5, 1 and 2 ml/min (86 measurements). The filling rate had no significant effect on the average correlation coefficients or fit errors (one way ANOVA: $p>0.17$). In each rat slopes and offsets of the relations between nerve activity and pressure, tension, and stress were normalised to their values at 1 ml/min and averaged, as shown in Figure 4. One way ANOVA showed that none of the calculated slopes or offsets depended significantly on the applied filling rate. Therefore all measurements were pooled. The average coefficients of variation were 33% for the slopes and 8% for the offsets.

Table 1. *Correlation coefficients and linear fit parameters and errors.*

Variable	*Corr. Coeff.*	*Sign. < r^2(stress)*	Slope [$\cdot 10^{-2}$]	Offset	Relative error [%]	Sign. > error(stress)
Stress	0.81 ± 0.09	-	0.38±0.36	0.67 ± 0.56	6.2 ± 1.7	-
P×V	0.81 ± 0.09	-	2.2 ± 2.2	0.67 ± 0.56	6.2 ± 1.7	-
P×r	0.80 ± 0.10	67 %	2.5 ± 2.1	0.66 ± 0.55	6.5 ± 1.8	33 %
Pressure	0.78 ± 0.10	83 %	1.3 ± 0.9	0.65 ± 0.55	6.8 ± 1.8	83 %
Volume	0.64 ± 0.08	100 %			8.9 ± 2.3	100 %
Surface	0.60 ± 0.07	100 %			9.4 ± 2.4	100 %
Strain	0.55 ± 0.07	100 %			9.9 ± 2.6	100 %

Afferent bladder nerve activity was related to several mechanical variables. Mean ± SD of the correlation coefficients and linear fit parameters and errors of the variables are shown. The only difference between stress and P×V was the value of the slopes, that differed by a factor $3/2V_f$ in each rat, with V_f the individual bladder tissue volume. Also shown is the percentage of animals in which a lower correlation coefficient or a higher fit error (both compared to stress) were significant. Standard deviations refer to differences between rats.

We also calculated slopes and offsets for pressure, tension and stress from the data collected at $t > t_2$ (see Fig. 2) and estimated the afferent nerve activity during $t < t_1$ using the obtained relations. The thus calculated nerve activity was compared to the measured nerve activity in this period. On average the errors were 4.9% for pressure, 4.2% for tension and 3.4% for stress. These differences, however, were not significant. An example is shown in Figure 5.
Finally, we investigated the levels of afferent nerve activity from the bladder at the onset and cessation of efferent bladder nerve firing. Because efferent activity was not measured in these experiments, both time points were estimated as the moment when the bladder pressure started to increase progressively ($t_{0,est}$) and at t_2 (see Fig. 6), respectively. In all rats, both $NA_{aff}(t_{0,est})$ and $NA_{aff}(t_2)$ showed excellent reproducibility, with coefficients of variation averaging 9 ± 3 % and 15 ± 6 %. On average $NA_{aff}(t_{0,est})$ was 0.80 ± 0.67 μV and $NA_{aff}(t_2)$ was 0.86 ± 0.59 μV. In 4 rats afferent activity at $t_{0,est}$ and t_2 did not differ significantly, in 2 rats NA_{aff} was higher at t_2 than at $t_{0,est}$ ($p < 0.001$).

As the values of $t_{0.est}$ and thus of $NA_{aff}(t_{0.est})$ were rather rough estimations, we also did measurements in intact bladder nerves.

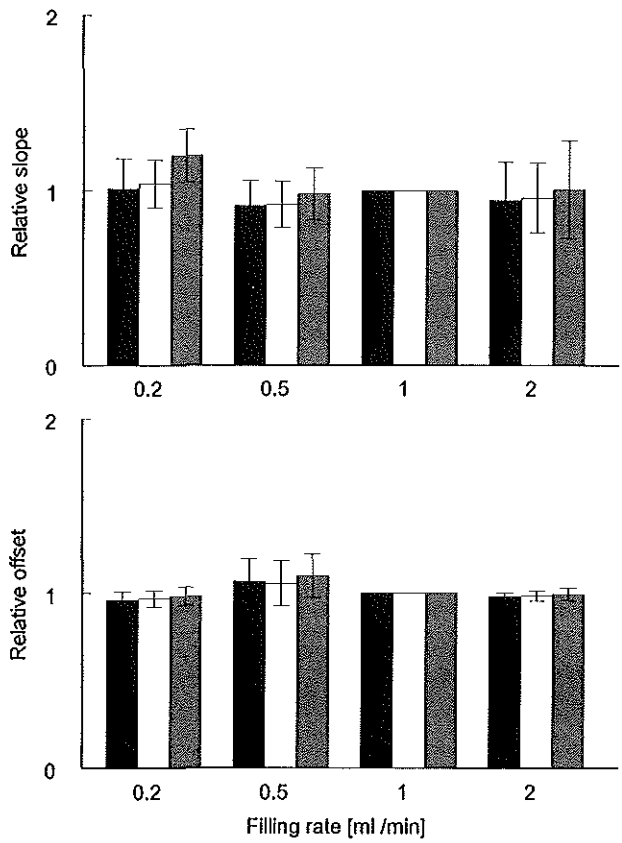

Figure 4. *To investigate the influence of the bladder filling rate on the slopes and offsets of the linear relations between afferent bladder nerve activity and bladder pressure (black), tension (white) and stress (hatched bars), the bladder was filled at 4 rates. In each rat all slopes and offsets were normalized to their value at 1 ml/min. Averages and standard deviations of all rats are shown. Neither slope nor offset depended significantly on the applied filling rate (1-way ANOVA: $p > 0.1$).*

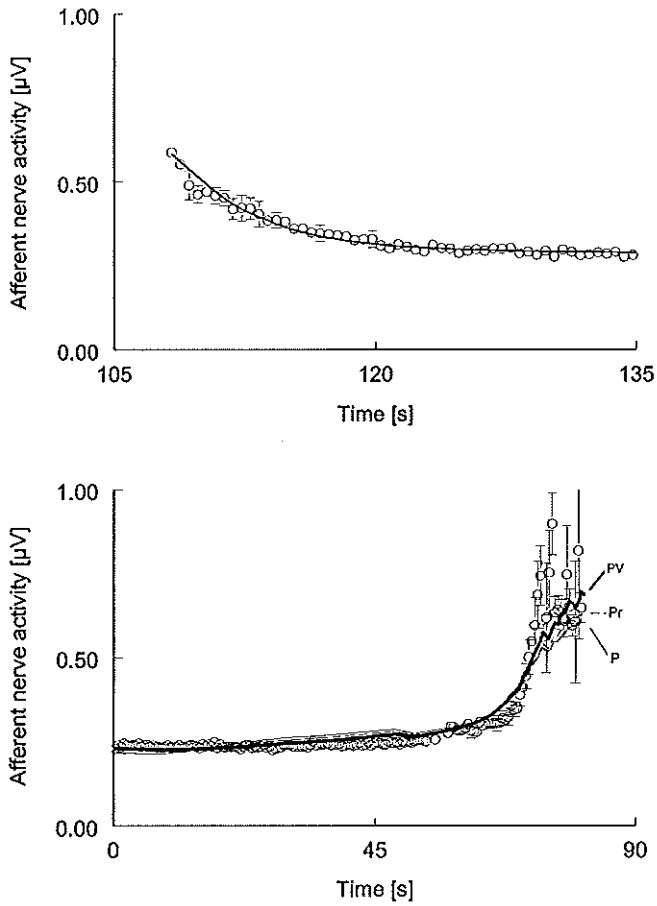

Figure 5. *Upper panel: Taking pressure (P, thin solid line), tension (Pr, dashed line) or stress (PV, thick solid line) as the primary stimulus for afferent bladder nerve activity, linear equations were fitted to data collected during the pressure decline immediately after voiding ($t > t_2$ in Fig. 2). Because the volume did not change in this period, the fitted curves are equal. The fitted parameters are different though.*
Lower panel: Using the obtained parameters, nerve activity was calculated from the bladder pressure and volume during the filling phase ($t < t_1$). Calculated afferent nerve activity was compared to measured activity and both differed less than 5% using either stimulus.

2) INTACT NERVE MEASUREMENTS: In intact nerve measurements, the bladder was again filled with saline until a voiding contraction occurred, but now a combined signal of efferent and afferent bladder nerve activity was recorded (see Fig. 7).
At $t > t_2$, the bladder volume was constant and measured (residue). In a previous paper it was shown that there is no efferent activity to the bladder during the pressure decline after t_2 so that the measured activity is purely afferent (13). From this episode, we calculated the relation (slope and offset) between afferent nerve activity and P×V. Using these parameters we calculated afferent nerve activity during $t < t_1$. Subtraction from the total nerve activity yielded an estimation of the efferent contribution. The exact ending of efferent firing could not be determined due to the movement artefacts during $t_1 < t < t_2$ (Fig. 7). However, after t_2 efferent activity was absent. We calculated afferent activity at $t = t_2$ and immediately after the last oscillation, just before t_2. In all rats, both showed high reproducibility, with coefficients of variation averaging 14 ± 11% and 15 ± 12%.
We also calculated the level of afferent activity at the onset of efferent firing (t_0 in Figure 7) using a model described before (26). This value had a very low coefficient of variation as well (17 ± 16 %). The level of afferent activity at the end of efferent firing (either at t_2, or immediately after the last oscillation) was higher than that at the onset of efferent nerve activity (paired t-test: $p<0.001$).

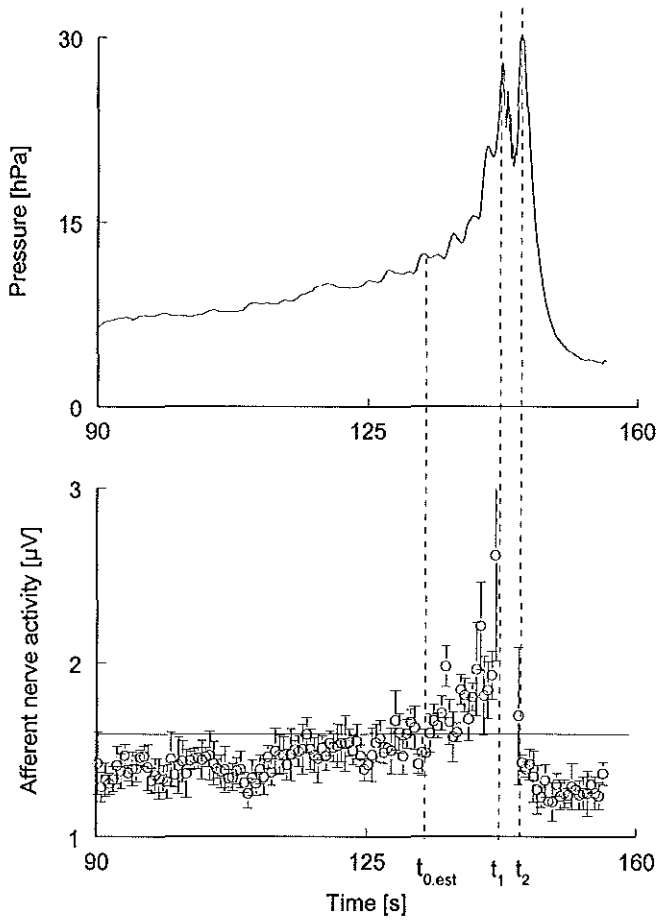

Figure 6. In the centrally transected nerve measurements the onset of the active bladder conraction was roughly estimated at the moment when the pressure started to increase progressively ($t_{0.est}$). The contraction was assumed to end at the pressure maximum immediately after voiding (t_2). The afferent nerve activity at $t_{0.est}$ was compared to that at t_2.

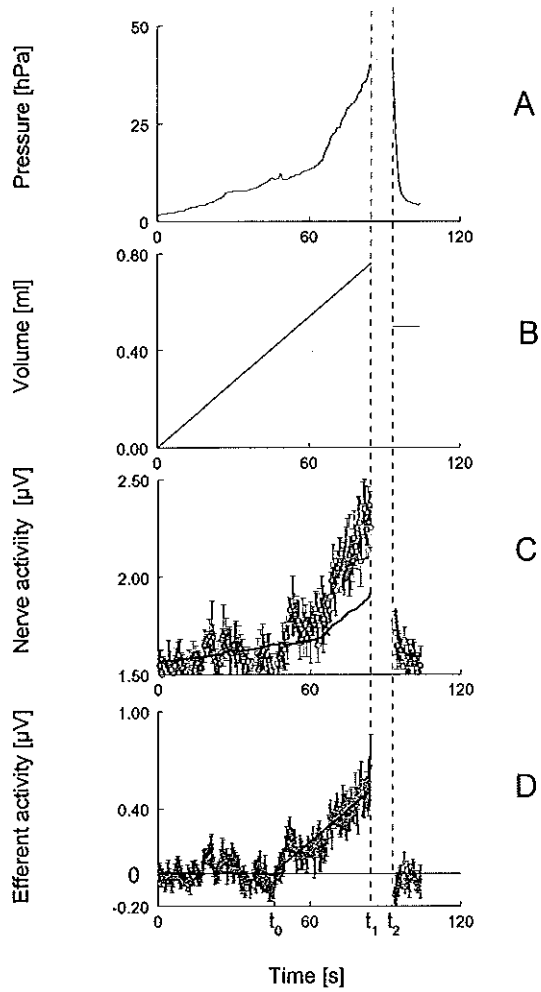

Figure 7. *An example of nerve activity in an intact bladder nerve during the micturition cycle. A,B: During $t > t_2$, the relation between afferent nerve activity and pressure × volume was determined (slope and offset). C: Then, afferent activity was calculated for the entire measurement using the obtained equation (line), and subtracted from the measured total activity (○). D. Thus efferent activity (NA_{eff}) to bladder was calculated (○). A model was fitted to NA_{eff}, assuming NA_{eff} is zero until t_0, and then increases linearly with time (line). The onset of efferent firing to the bladder was defined as t_0, and the levels of afferent bladder nerve activity at t_0 and t_2 were compared. In one rat $NA_{aff}(t_0)$equalled $NA_{aff}(t_2)$, in 4 rats $NA_{aff}(t_2)$ was significantly higher than $NA_{aff}(t_0)$.*

DISCUSSION

In this study the bladder pressure (P) was measured during the filling phase, during bladder contraction before voiding and bladder relaxation afterwards. The bladder volume (V) at any moment before bladder voiding was calculated from the filling rate and the residual volume after voiding was measured. The bladder volume averaged 0.82 ± 0.19 ml when voiding started, which is similar to the ~0.6 ml (17, 26) to 0.8 ml (25) found in other studies. The residue of 0.58 ± 0.22 ml agrees with the 0.5 ± 0.5 ml found by Mallory et al (17).
Because the bladder was empty at the start of the recording, and the renal excretion [~0.01 (10) to 0.02 ml/min (15)] was negligible compared to the imposed filling rate, the bladder volume was known throughout the filling phase and transection of the ureters was not needed which avoided unnecessary damage to the bladder.
In contrast to an earlier study (13), the bladder volume varied over a wide range, which enabled us to better investigate the influence of the bladder volume on the afferent bladder nerve activity.

Several variables were calculated from P and V: strain, bladder surface, wall tension, and stress. Stress showed the highest correlation with afferent bladder nerve activity, always higher than strain, bladder surface, or volume. Although the differences were small, stress correlated significantly better to nerve activity than pressure in 83% of the animals, and in 67% also significantly better than tension. It was expected to find only very small differences between the correlation coefficients because pressure and volume are closely related. In fact, if only the period before voiding was analysed ($t < t_1$), the correlation coefficients did not differ significantly. However, in the period immediately after voiding, the pressure changed independently of the (constant) bladder volume. This episode is therefore necessary in the analysis to discriminate between the influences of bladder volume and pressure on afferent nerve activity.

If two variables are linearly related, their correlation coefficient equals 1. Correlation coefficients lower than 1 are caused by either noise or by non-linearity. Figure 3 (A-C) shows that the low correlation coefficients between NA_{aff} and strain, bladder surface or volume are at least partially explained by non-linearities. The relations between NA_{aff} and P, P×r or P×V are approximately linear. It appeared that the relation between stress and nerve activity was described by a linear function best, with a fit error of only 6.2 ± 1.7 %.
It was concluded that stress was the stimulus that correlated best to afferent nerve activity, and that both these variables are linearly related.

In a previous study it was shown that afferent bladder nerve activity could linearly be related to the bladder pressure (13). However, in these experiments only minor changes in the bladder volume were recorded. At a constant bladder volume, pressure, wall tension, and stress are proportional, which makes a proper determination of the primary stimulus impossible.

Satchell and Vaughan showed that during bladder contraction ($t < t_1$) afferent activity is linearly related to bladder pressure, but that the slope depended on the filling rate (21). Due to the visco-elasticity of the bladder wall, at higher filling rates the pressure at a given volume will usually be higher (29), which changes the pressure-volume relation and would explain the different slopes. Therefore they suggested that afferents should be related to stress rather than pressure.
In our experiments the slope of the relation between pressure and nerve activity did not depend on the filling rate (ANOVA: p=0.71). This can probably be explained by the inclusion of the episode of pressure decrease immediately after voiding ($t > t_2$) in our analysis, when the bladder pressure changed while the volume remained constant.
Another finding suggesting that afferent activity is influenced by the bladder volume, was published by Moss et al who showed that the pressure threshold for afferent firing depended on the filling rate, whereas the volume threshold did not (20). In an earlier study, when afferent activity was linearly related to bladder pressure, we also found higher afferent activity during pressure development at the onset of the voiding contraction ($t < t_1$) than at comparable pressures during the pressure decrease at the end ($t > t_2$), when the bladder volume is lower (13).
It thus seems an oversimplification to relate afferent nerve activity to bladder pressure, which is only warranted if the volume does not show major changes. Otherwise, afferent bladder nerve activity should be related to stress.

A disadvantage of using stress as the stimulus for afferent activity in neurophysiological models is that it depends on the tissue volume of the bladder, which is difficult to determine accurately. The coefficient of variation in the tissue density that we found (24%) probably mainly reflects inaccuracy in the measurement of the tissue volume. Therefore we approximated stress by pressure × volume, which had no influence on the correlation coefficient or fit error. This approximation can be made only if $3V/2V_t >> ¼$ (see appendix). Apparently, in our measurements this was always true. During the pressure decrease after voiding the bladder volume remained constant. Thus in this period afferent activity was proportional to the bladder pressure, with a slope $a \cdot (3V/2V_t + 0.25)$ (see appendix). With a small residual bladder volume the term 0.25 becomes dominant. Thus, with a very small residue the slope of the relation between P×V and NA_{aff} may be different for the periods $t < t_1$ and $t > t_2$. The approximation that stress is proportional to P×V may be valid only if there is a substantial residual volume after voiding. The very small bladder volume at the beginning of each measurement introduces no problem in the used approximation, because in this phase the pressure is also very low, and the afferent nerve activity rises hardly above background activity / noise.

A previous study, relating afferent nerve activity to bladder pressure, showed that the level of afferent bladder nerve activity triggers efferent firing to the bladder. If a certain threshold is exceeded, efferent firing is initiated (26). This causes bladder contraction and

the bladder pressure rises, resulting in even more afferent activity. Thus a minor excess of the threshold results in a major increase in afferent activity. This model, however, fails to explain how efferent firing is stopped. Once the threshold is exceeded and the contraction has started, afferent activity will always be above threshold.
This problem is solved if afferent nerve activity is related to stress, rather than to pressure. Again, a minor excess of the threshold results in a major increase in afferent activity, which remains far above threshold until the actual voiding is started. Then, due to a decreasing volume, afferent activity decreases until it drops below a certain, possibly the same, threshold. Efferent activity ceases and the contraction terminates. Pressure decreases and afferent activity ends up far below threshold. Thus, in both directions a minor excess of the thresholds leads to a decisive threshold crossing. This bi-directional snowball effect makes the system bi-stable. This is illustrated in Figure 8.

In the centrally transected nerve measurements we roughly estimated the start of efferent nerve activity as the moment when the bladder pressure started to increase rapidly ($t_{0,est}$). Efferent firing was assumed to cease at the pressure maximum immediately after voiding. Efferent firing may stop at an earlier moment, but not later than at the local pressure maximum at t_2 (13). Since after voiding the volume is constant, the calculated afferent activity at the pressure maximum is higher than that just before or after t_2. Thus the level of afferent activity at the cessation of efferent firing may be overestimated. Both $NA_{aff}(t_{0,est})$ and $NA_{aff}(t_2)$ were highly reproducible, the latter being slightly higher than the first. This difference was significant in 2 of 6 rats.

In the intact nerve measurements, t_0 could be determined more accurately. In these experiments we estimated the efferent contribution to the total activity by subtraction of afferent activity, which was calculated from P×V. The slope and offset of the relation between NA_{aff} and P×V were calculated from the episode $t > t_2$, when the measured nerve signal was entirely afferent. This introduced an error of ~5% in the estimated NA_{aff} during $t < t_1$. A comparable value (6%) was found in an earlier study (13). The assumption of linear addition of efferent and afferent activity in compound nerve signals was supported by Biró and Partridge, who demonstrated that nerve or muscle action potentials of independent units are linearly superimposed in compound records (4), and by the results of an earlier study (13). Thus efferent activity could be determined adequately. We fitted a model to the efferent activity, which resulted in a more reliable value for t_0 than in the centrally transected nerve measurements.
Again, examination of the levels of afferent activity revealed that afferent activity was higher at the cessation than at the onset of efferent firing. This may have been caused by the overestimation of $NA_{aff}(t_2)$. A higher threshold at the cessation may also be explained by adaptation of the neurones that induce efferent firing to a higher afferent level. Thus, more afferent activity may be needed to continue the efferent firing.

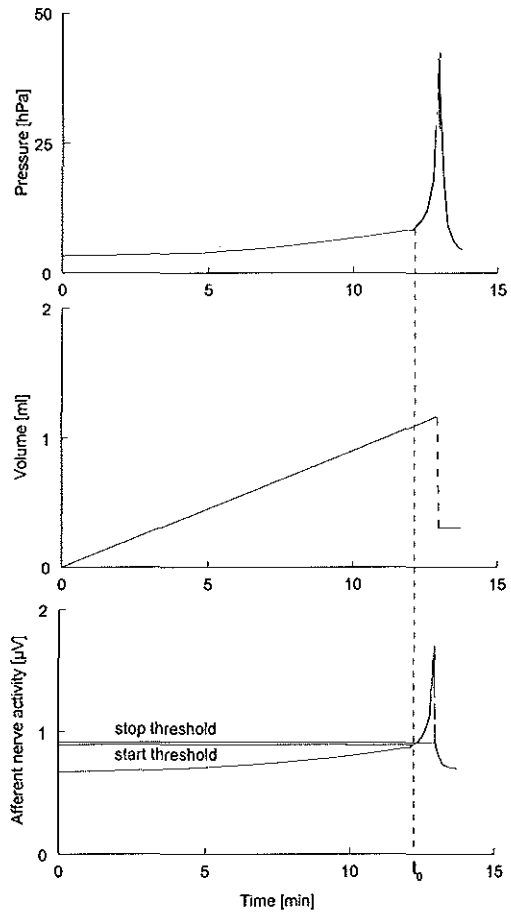

Figure 8. *Simulation of pressure and volume in a slowly filling bladder. At t_0 the active bladder contraction starts and the pressure rises. During the actual voiding ($t_1 < t < t_2$ in Fig.2) the volume decreases and rapid sphincter contractions cause an oscillatory pattern in the bladder pressure. These parts of the graphs are dotted as they can only be estimated in this period. Afferent nerve activity was calculated as $0.022 \cdot P \times V + 0.67$. The levels of afferent activity at the onset and cessation of efferent firing to the bladder are indicated as start threshold and stop threshold. Crossing the start threshold causes a large increase in afferent activity and crossing the stop threshold causes the afferent activity to further decrease, which makes the system bi-stable.*

We conclude that in the rat, afferent bladder nerve activity correlates best to bladder wall stress, which in our study was assumed to be proportional to bladder pressure × volume. The relation between P×V and afferent activity can be described by a linear equation, introducing an error of 6% on average. Afferent nerve activity triggers efferent firing to the bladder. Both start and cessation of efferent activity occur at afferent activity levels that are highly reproducible. The latter is slightly higher than the first. Crossing of either level is stable; a minor excess results in a decisive threshold crossing. We propose a bladder model with afferent activity proportional to P×V, bladder contraction initiated by exceeding of an afferent threshold, due to an increasing pressure and volume, and then continuing until afferent activity drops below a threshold as a result of the decreasing bladder volume.

APPENDIX

Calculation of bladder wall tension (T) and stress (σ).
The bladder is approximated by a sphere with radius R (Figure A1):

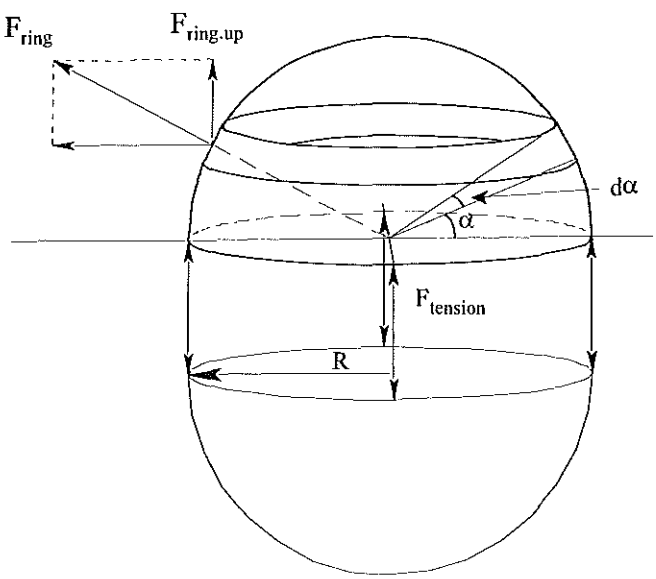

Figure A1. Calculation of bladder wall tension and stress. The bladder is approximated by a sphere with radius R. The force that drives the two semispheres apart has to be equal to the force keeping them together: $F_{tension}$. Tension is calculated as $F_{tension}$/ (circumference of cross section) and stress as $F_{tension}$ / (area of cross section).

Tension:
The ring area, A_{ring}, can be calculated as

$$A_{ring} = 2\pi \cdot R \cdot \cos\alpha \cdot R \cdot d\alpha = 2\pi \cdot R^2 \cdot \cos\alpha \cdot d\alpha \quad (A.1)$$

The force on this ring, F_{ring} equals $P \cdot A_{ring}$. P is bladder pressure. The vertical component, $F_{ring,up}$, equals $F_{ring} \cdot \sin\alpha$. Thus:

$$F_{ring,up} = 2\pi \cdot R^2 \cdot P \cdot \cos\alpha \cdot \sin\alpha \cdot d\alpha \quad (A.2)$$

Integration yields the force that drives the two semispheres apart, F_{up}:

$$F_{up} = \int_0^{\pi/2} \pi \cdot R^2 P \cdot 2 \sin\alpha \cos\alpha \cdot d\alpha$$

$$= \pi \cdot R^2 \cdot P \quad (A.3)$$

The force that keeps both semispheres together, $F_{tension}$, equals F_{up}. Wall tension is defined as $F_{tension}$ divided by the circumference of the cross section:

$$T = \frac{\pi \cdot R^2 P}{2\pi \cdot R} = P \cdot R/2 \quad (A.4)$$

Stress:
Stress (σ) is defined as $F_{tension}$ divided by the area of the cross section. If d is the wall thickness, this area equals $\pi R^2 - \pi(R-d)^2 = 2\pi Rd - \pi d^2$. Assuming that $d \ll R$ yields:

$$\sigma_{thinwall} = \frac{\pi \cdot R^2 P}{2\pi \cdot Rd} = \frac{PR}{2d} \quad (A.5)$$

However, when the bladder is almost empty this approximation is not valid.
With a non-negligible wall thickness, the tangential stress in the wall at radius r can be calculated (28):

$$\sigma(r) = \frac{P_{in} r_{in}^3 - P_{out} r_{out}^3}{r_{out}^3 - r_{in}^3} + \frac{r_{in}^3 r_{out}^3}{2r^3} \cdot \frac{P_{in} - P_{out}}{r_{out}^3 - r_{in}^3} \quad (A.6)$$

$$= \frac{P_{in} r_{in}^3}{2r^3} \cdot \frac{r_{out}^3 + 2r^3}{r_{out}^3 - r_{in}^3} - \frac{P_{out} r_{out}^3}{2r^3} \cdot \frac{r_{in}^3 + 2r^3}{r_{out}^3 - r_{in}^3} \quad (A.7)$$

With P_{in} = pressure inside sphere; P_{out} = pressure outside sphere; r_{in} = inner radius; $r_{out} = R$ = outer radius.

With V = bladder volume and V_t = tissue volume :

$$V_t = {}^4/_3\pi\,(r_{out}^3 - r_{in}^3) \quad \Leftrightarrow (r_{out}^3 - r_{in}^3) = \frac{3V_t}{4\pi} \tag{A.8}$$

$$V = {}^4/_3\pi\, r_{in}^3 \quad \Leftrightarrow r_{in}^3 = \frac{3V}{4\pi} \tag{A.9}$$

with (A.7) , (A.8) and (A.9):

$$\sigma(r_{out}) = \frac{3P_{in}}{2V_t}\cdot(V) \quad - \frac{3P_{out}}{2V_t}(V + {}^2/_3V_t) \tag{A.10}$$

$$\sigma(r_{in}) = \frac{3P_{in}}{2V_t}\cdot(V + \frac{V_t}{3}) - \frac{3P_{out}}{2V_t}(V + V_t) \tag{A.11}$$

$$\sigma(r_{out}) = \sigma(r_{in}) - \tfrac{1}{2}P_{in} + \tfrac{1}{2}P_{out} = \sigma(r_{in}) - \tfrac{1}{2}P \tag{A.12}$$

Mean stress in bladder wall:

$$\sigma = [\sigma(r_{out}) + \sigma(r_{in})] / 2 \tag{A.13}$$

from (A.12) and (A.13):

$$\sigma = \sigma(r_{in}) - \tfrac{1}{4}P \tag{A.14}$$

$$= \frac{3}{2V_t}(P_{in}-P_{out})V + \frac{1}{2}P_{in} - \frac{3}{2}P_{out} - \frac{1}{4}P$$

$$= \frac{3}{2V_t}\cdot PV + \frac{1}{4}P - P_{out} \tag{A.15}$$

Where $P = P_{in} - P_{out}$, and P_{out} is considered a constant. The term $^1/_4P$ becomes negligible if $(3PV/2V_t)/0.25P>10$, i.e. if $6V/V_t>10$.
In rats V_t averages 0.2 cc. Thus, the pressure term is negligible if $V > 0.33$ cc and should be accounted for only at low bladder volumes.

If nerve activity is proportional to stress then:

$$NA = a \cdot stress + b = a(3V/2V_t + {}^1/_4) \cdot P - [a \cdot P_{out} + b] = a(3V/2V_t - {}^1/_4) \cdot P + b'$$
$$\text{with } b' = -a \cdot P_{out} - b \qquad \text{(A.16)}$$

Or under the above assumption:

$$NA = a(3/2V_t) \cdot PV + b' = a' \cdot PV + b'$$
$$\text{with } a' = a(3/2V_t) \qquad \text{(A.17)}$$

ACKNOWLEDGEMENT

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6. Pudendal nerve stimulation induces urethral contraction and relaxation.

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Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46) R1368-R1375, 1999.

ABSTRACT

In this study we measured urethral pressure changes in response to efferent pudendal nerve stimulation in rats. All other neural pathways to the urethra were transected, and the urethra was continuously perfused. We found fast twitch-like contractions, superimposed on a slow relaxation. The amplitude of the twitches was independent of the stimulation frequency below 26 Hz, whereas the relaxation depended highly on this frequency. The twitches were caused by striated urethral muscles, and the relaxation was caused by smooth muscles. Both were mediated by acetylcholine. We calculated the effective urethral relaxation as the absolute relaxation multiplied by the time fraction between the twitches. Maximum effective relaxation occurred at 8-10 Hz, exactly the frequency of spontaneous oscillations during bladder voiding in rats. Although the oscillatory sphincter contractions in rats during voiding may be needed in other mechanisms for efficient voiding, our data suggest that they may be a side effect of the actual purpose: urethral relaxation.

Keywords: rat; urethral oscillations; nitric oxide; acetylcholine

INTRODUCTION

Dynamic properties of the lower urinary tract have been extensively studied over the years, and mechanical models have been developed (12, 42). More recently, attention has been paid to the innervation of the pelvic organs (1, 17, 26, 38), and the activity in the innervating nerves is being incorporated in these models (18, 19, 25, 29, 39). The extended models may enable differentiation between myogenic and neurogenic causes for dysfunction of the lower urinary tract or may improve the clinical use of neurostimulation as a treatment for lower urinary tract dysfunction in patients with spinal cord injury.
Most studies on lower urinary tract innervation have been done in animals, mostly rats. Like humans, rats have three major nerves that innervate the pelvic organs: the (predominantly sympathetic) hypogastric nerve, the (predominantly parasympathetic) pelvic nerve, and the (predominantly somatic) pudendal nerve. The sympathetic system is assumed to be active during urine storage, whereas the parasympathetic system controls bladder voiding. Despite the many similarities, there are some major differences between the human and the rat urinary system; one of the most striking is the oscillatory contractions of the external urethral sphincter (EUS) during voiding in rats (3, 6, 22, 24, 28, 40). The oscillations occur more often and are stronger in male rats than in female rats (22). The rat's normal pattern of bladder-EUS coordination has some superficial similarities to the dyssynergic pattern observed in humans with spinal cord injury. However, unlike the spinalized human, the rat is able to void quite successfully, and knowledge of the differences between the rat and human mechanism of bladder-EUS coordination has

therefore been suggested to be of use in evaluating micturition problems in patients with spinal cord injuries (16).
The reason for the sphincter contractions in the rat, which occur at a rate of 6-10 per second (24, 28, 40), has been a subject of discussion for years. A pumplike function (22), a mechanism to sustain a high level of bladder afferents and therefore bladder contraction (16), and a mechanism necessary for territory marking (40) have been suggested. However, none of these explanations seems very satisfactory. Furthermore, it remains unclear why the oscillations occur at the specific frequency range of 6-10 Hz.
In this study, we investigated the relationship between efferent pudendal nerve activity and urethral resistance, as well as some of the major neurotransmitters involved. Urethral oscillations normally occurring during voiding contractions (6-10 Hz) were simulated by pudendal nerve stimulation at 2-70 Hz. We distinguished smooth muscle and striated muscle responses to pudendal stimulation. We will introduce a third explanation for the urethral oscillations, taking into account the frequency of the oscillations during spontaneous bladder voiding.

MATERIALS AND METHODS

Surgery

Sixteen male Wistar rats (444 ± 47 g) were anesthetized with urethan(1.2 g/kg ip) (23) and placed on a heated pad. A dorsal incision wasmade on the left side of the spine, just below S3, to access the pudendal nerve. Two trimel-coated silver wires were twisted around the nerve (the undivided efferent and afferent branch) (26) at a distance of ~2 mm. The wound was then closed, and the animal was turned over on its back.
An abdominal midline incision was made to access the urethra. The right side pelvic and hypogastric nerves were transected. The left side pelvic and hypogastric nerves were freed from the underlying tissue and marked with sutures. Warm saline kept the abdomen moist during surgery. The animal was placed in a frame with a heated ground plate, and the abdominal wall was tied to the frame to create a basin. During the measurements, this basin was filled with warm paraffin oil.

Experimental Setup

A 20-gauge angiocatheter was inserted through the bladder into the urethra. Through this needle, the pressure at the entrance of the urethra (P_{ura}) was measured with the use of a disposable pressure transducer and a Statham SP-1400 blood pressure monitor. Furthermore, the needle was used to pump saline through the urethra with a Harvard Apparatus (Millis, MA) infusion pump at a flow rate (Q) of 0.5 ml/min. The bladder was

emptied and tied to prevent leakage from the urethra to the bladder and high bladder pressures.
A bipolar platinum-iridium electrode that consisted of two metal hooks at a distance of ~0.5 mm was lowered into the paraffin oil basin. Either the pelvic or the hypogastric nerve

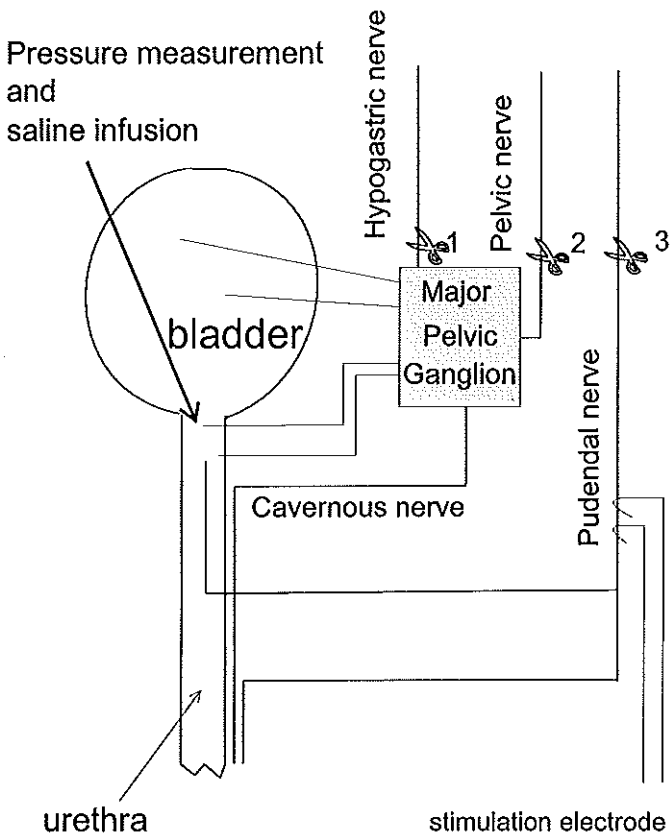

Figure 1. *Schematic drawing of left side innervation of rat lower urinary tract. The pudendal nerve was stimulated by an electrode that consisted of 2 silver wires twisted around the nerve. In our measurements a needle was inserted through the bladder into the urethra for saline infusion and pressure measurement. To determine the effect of only efferent pudendal stimulation, either the hypogastric and pelvic nerves were transected at* ✂1 *and* ✂2 *or the pudendal nerve was transected at* ✂3 *.*

was guided over the electrode, which was used for stimulation. The silver wires around the pudendal nerve provided another stimulation electrode. A Hameg programmable function generator, HM-8130, generated the stimulation signals. The pressure signal was read into a personal computer at sample rates of 200 or 1,000 Hz, with the use of specially developed software driving a PCL 818 analog-to-digital converter. The stimulation signal was also sampled, at a rate of 25 kHz.

Measurement conditions

Measurements were done under two conditions: 1) with intact left pelvic and hypogastric nerves and 2) with bilaterally transected pelvic and hypogastric nerves.

INTACT LEFT PELVIC AND HYPOGASTRIC NERVES. After transection of the right side pelvic and hypogastric nerves, in eight rats we electrically stimulated the left side pudendal nerve (frequency 2-70 Hz, amplitude 0.4-3.5 V, pulse width 50 μs) and measured the change in urethral pressure. Because the pudendal nerve electrode was very close to the sciatic nerve, pudendal stimulation also induced hindleg movement, which restricted the maximum stimulation amplitude. In four rats we measured the effect of hypogastric nerve stimulation on P_{ura}.

BILATERALLY TRANSECTED PELVIC AND HYPOGASTRIC NERVES. After exclusion of central reflexes by bilateral transection of the pelvic and hypogastric nerves, there were only pudendal efferent pathways to the urethra (see Fig. 1). The pudendal nerve was stimulated again (at maximum amplitude with only minor hindleg movement), and urethral pressure was measured. In seven rats, we determined the frequency dependence of the urethral pressure response to pudendal nerve stimulation by varying the stimulation frequency (f_{stim}) between 2 and 20 Hz (5 rats) or between 1 and 70 Hz (4 rats). In two of these rats both protocols were applied.

In the bilaterally transected rats, to investigate the neurotransmitters involved in urethral responses to the evoked pudendal nerve activity, we measured the effect of intravenous atropine [1 mg/ml, 0.2 ml (n = 1) or 0.4 ml (n = 3)] or N-nitro-L-arginine methyl ester [L-NAME; 50 mg/ml (n = 2) or 100 mg/ml (n = 3); 0.2 ml] administration. These series of measurements were done at fstim of 15 Hz. First, three control measurements were done, and then a drug was administered. If both drugs were given in the same animal, we allowed at least 1 h in between administrations.

Measurements

Under both conditions (bilaterally transected pelvic and hypogastric nerves), electrical stimulation of the pudendal nerve had a dual effect: fast twitch-like contractions were superimposed on a slow relaxation (see Fig. 2). The recorded urethral pressure was analyzed in blocks with a length equal to the stimulus period. The blocks were synchronized to the

stimulation signal, which was recorded for this purpose. In each block, the maximum (P_{max}) and baseline pressures (P'_0) during pudendal stimulation were determined. P'_0 was defined as the mean pressure in the block after exclusion of the first twitch and the subsequent pressure undershoot (see Fig. 2). Before stimulation and at the end of the measurements, P'_0 equaled the urethral pressure necessary to maintain a flow rate of 0.5 ml/min without nerve stimulation (P_0, see Fig. 2). P'_0 described the relaxant response. An exponential function was fitted to P'_0 to determine the time constant of relaxation, τ_{relax}.
The pressure amplitude of the fast contraction (P_{twitch}) was calculated as the mean value of P_{max} P'_0 during stimulation. The pressure amplitude of the slow relaxation (P_{relax}) was calculated as the difference between P_0 and the minimum value of P'_0.
In each rat P_{twitch} was expressed in decibels [= 20·log(amplitude)], and the responses were plotted on a logarithmic frequency axis (2). For each rat the horizontal part of the graph was shifted to 0 (normalization), and all graphs were averaged. A two-parameter model (cutoff frequency and slope) was fitted to the data. To evaluate the effect of the tested drugs, we had to distinguish between the decay with time that was always observed in the urethral pressure responses to pudendal nerve stimulation and the effect caused by the drug itself. We assumed that both drugs did not have any effect on P_{ura} after more than 30 min. The decay with time was estimated from the first three measurements (controls) and the measurements done more than 30 min after drug administration by fitting a straight line to these data. Urethral pressures measured within 30 min after drug administration were corrected using this line.

Materials

Drugs used were atropine (Pharmachemie BV) and L-NAME (Sigma), both dissolved in saline (0.9% NaCl), and urethan (Sigma), which was dissolved in water.
Experiments were carried out as outlined in the Erasmus University of Rotterdam Guidelines for the Care and Use of Laboratory Animals, which in general follow the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All data are presented as means ± SD.

RESULTS

In 16 rats, we measured urethral responses to electrical stimulation of the pudendal nerve. At a perfusion rate of 0.5 ml/min we measured a pressure loss of ~1 hPa in the connecting tubing. Pudendal nerve stimulation had a dual effect: fast twitch-like contractions on each stimulus pulse were superimposed on a slow relaxation (Fig. 2).

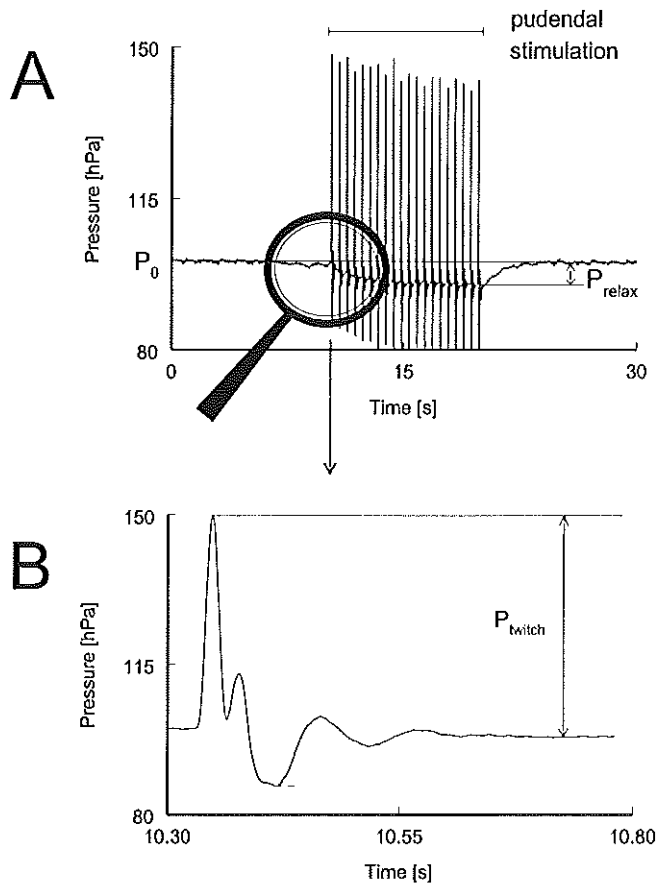

Figure 2. *A: electrical stimulation of the pudendal nerve (2 Hz in this example) had a dual effect: fast twitch-like contractions were superimposed on a slow relaxation.* P_0*, pressure necessary for urethral perfusion (after stimulation, urethral pressure slowly returned to baseline).* P_{relax}*, pressure amplitude of slow relaxation. B: magnification of portion of A. The oscillatory pressure waves after the first twitch are probably caused by the moving fluid that is suddenly stopped in a nonrigid chamber.* P_{max} *and* P_{min}*, maximum and minimum urethral pressure, respectively, during pudendal stimulation;* P_{twitch}*, pressure amplitude of fast contraction;* P'_0*, baseline urethral pressure during pudendal stimulation.*

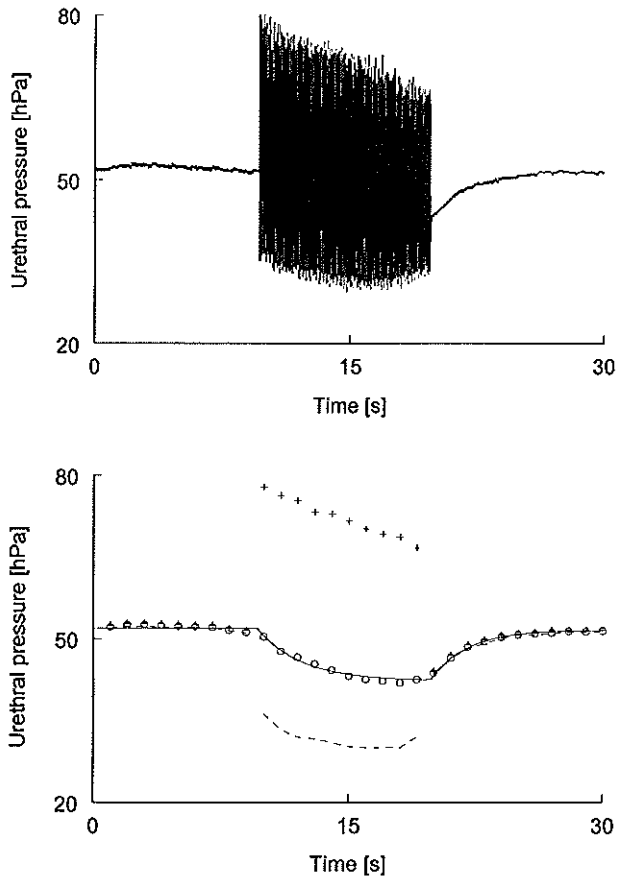

Figure 3. *Measured urethral pressure in response to pudendal nerve stimulation after bilateral transection of the hypogastric and pelvic nerves (A). The results were analyzed in blocks that had the same length as the stimulus period. An example of such a block is shown in Fig. 2B. B: in each block the maximum (+; P_{max}) and minimum pressures (—; P_{min}) were determined. Furthermore, the steady-state pressure in each block (P'_0) was calculated (O). Change over time of P_{max}, P_{min}, and P'_0 are shown in B [stimulation frequency (f_{stim}) = 15 Hz; for clarity, only one +, —, and are shown per second]. An exponential function was fitted to P'_0, and a time constant of 3 ± 1 s was found.*

The amplitude of the fast contractions (P_{twitch}), the amplitude of the relaxation (P_{relax}), and the pressure necessary for urethral perfusion (P_0) were measured. Stimulation of the hypogastric nerve (15-30 V) induced slow urethral contraction without twitches ($\Delta P = 6 \pm 3$ hPa) in three of four rats. In one rat we measured no effect.

To exclude the possible effects of afferent pudendal stimulation, either the stimulated pudendal nerve was transected centrally to the stimulation electrode (Fig. 1, ✂[3]; 1 rat), or the bilateral pelvic and hypogastric nerves were transected (Fig. 1, ✂[1] and ✂[2]; 12 rats). After transection, again twitches superimposed on slow relaxation were observed. Figure 3 shows an example of the development of P_{max}, P_{min}, and P'_0 before, during, and after pudendal nerve stimulation. P_0, P_{relax}, and P_{twitch} were determined. In all rats, P_{relax} and P_{twitch} increased with the stimulation amplitude. In two rats, pudendal nerve stimulation hardly had any effect, probably because of insufficient stimulation amplitude. In these rats, the amplitude could not be increased further because of violent hindleg movement; therefore, they were excluded from further analysis. P_0 did not change after pelvic nerve transection ($\Delta P_0 = 0 \pm 10\%$, 0.9 ± 5.4 hPa) and was slightly but significantly reduced after hypogastric nerve transection ($\Delta P_0 = 6 \pm 4\%$, 3.2 ± 2.1 hPa; paired t-test: $P = 0.02$). Transection of both nerves had no significant effect on P_0 ($\Delta P_0 = 4 \pm 11\%$, 1.7 ± 5.2 hPa; paired t-test: $P > 0.2$). Statistical tests were applied to absolute pressure changes. The average P_0 in all rats was 51 $\pm$ 18 hPa. Transection of the hypogastric and pelvic nerves had no influence on P_{twitch}.

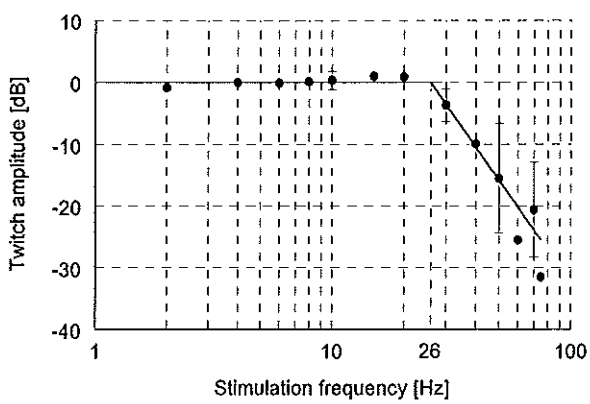

Figure 4. *Amplitude of twitches as a function of stimulation frequency, measured after bilateral transection of the hypogastric and pelvic nerves. As in a Bode diagram, Ptwitch is expressed in dB [= 20·log(amplitude)], and the frequency was plotted on a logarithmic scale. In each rat the horizontal part of the graph was shifted to 0 dB for normalization, and all graphs (n = 4) were averaged. , Mean Ptwitch (±SD). Fitted line had 2 degrees of freedom: cutoff frequency and slope.*

In four rats we determined the frequency dependence of P_{twitch}. We stimulated the pudendal nerve at frequencies between 1 and 70 Hz and plotted the frequency responses as in a Bode diagram. The amplitude of the twitches (P_{twitch}) was found to be independent of the stimulation frequency up to 26 Hz (Fig. 4). Beyond this frequency, the amplitude of P_{twitch} decreased because there was no longer complete relaxation between two consecutive twitches. In all rats, the relaxation (P_{relax}) depended highly on the stimulation frequency. In five rats we quantified this frequency dependence (Fig. 5). To avoid interference of the contractions with P_{relax}, these measurements were done up to 20 Hz only. For analysis each measurement was divided into blocks with a length equal to the stimulus period. P'_0 described the relaxant response during stimulation (see MATERIALS AND METHODS and Fig. 3). Fitting an exponential function to the relaxation showed a time constant of 3 ± 1 s (Fig. 3). The absolute values of P_{relax} were stimulation amplitude dependent, and the hindleg movement limited the maximum stimulation amplitude. Still, considerable urethral pressure decreases of ~30 hPa could be observed. On average, P_{relax} increased by 70% after bilateral transection of the pelvic and hypogastric nerves. However, this increase was not significant (P = 0.3, paired t-test).

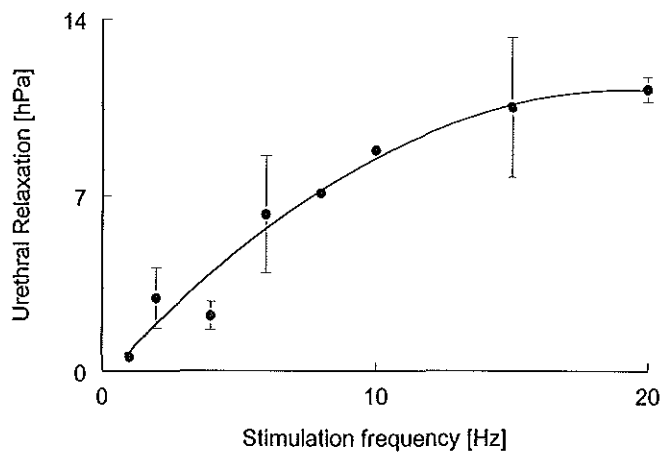

Figure 5. *To assess urethral relaxation as a function of the stimulation frequency, we normalized the relaxation at each frequency in all rats to the relaxation at 8 Hz in that rat. All normalized values were averaged and multiplied by the average relaxation at 8 Hz [P_{relax}(8 Hz) ~ 7.1 ± 5.6 hPa]. Measurements were done after bilateral transection of the pelvic and hypogastric nerves. ●, Measured P_{relax}; SDs refer to differences between rats. Fitted line: $P_{relax} = 0.42 + 1.1 \cdot f_{stim}\ 0.031 \cdot f^2_{stim}$*

On average a twitch-like contraction lasted 31 ± 4 ms and had an amplitude of 23 ± 15 hPa. The twitch amplitude (P_{twitch}) depended highly on the stimulation voltage. In contrast to P_{twitch}, the twitch width did not depend on the stimulation amplitude. In three rats we varied the stimulation amplitude and determined the ratio, P_{twitch}/P_{relax}. At very low stimulation intensities only slight twitches were observed; at higher voltages P_{relax} appeared to increase linearly with P_{twitch} (see Fig. 6). We found that $P_{twitch}/P_{relax} \sim 3$.

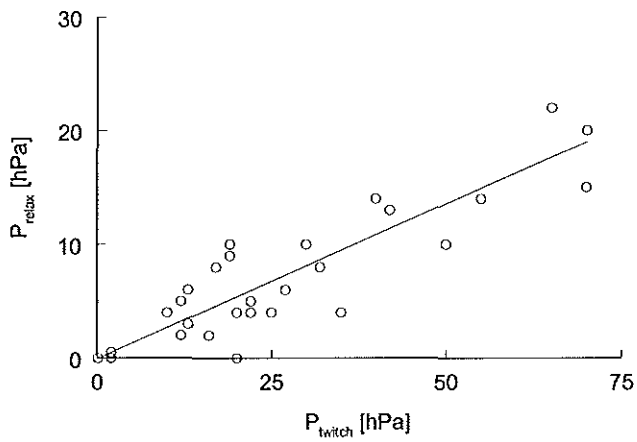

Figure 6. *In 3 rats, amplitude of pudendal nerve stimulation was varied. Amplitude of evoked twitches (P_{twitch}) and slow relaxation (P_{relax}) were measured. Measurements were done after bilateral transection of the pelvic and hypogastric nerves. Data of all rats were pooled (○). Fitted line: $P_{relax} = 0.3 \cdot P_{twitch} - 0.04$.*

We investigated the neurotransmitters involved in the pudendus-induced urethral relaxation by administration of the nitric oxide synthase (NOS) inhibitor L-NAME and the muscarinic receptor antagonist atropine. Over the course of the experiments (56 ± 9 min), the effects of pudendal nerve stimulation (P_{relax} and P_{twitch}) generally decreased. A straight line was fitted to the first three (control) values of P_{relax} and the values measured more than 30 min after drug administration. The slope of this line was used as an estimate for the decrease of P_{relax} with time and was used to correct for this time effect (mean slope, 0.14 ± 0.10 hPa/min).
Intravenous administration of L-NAME reduced P_{relax} in four of five rats by 30% on average and had no significant influence on P_{twitch} (ΔP_{twitch} was 0.7 ± 3.9 hPa; paired t-test: P = 0.74). Intravenous administration of atropine reduced P_{relax} in all rats by 40% (n = 4) and did not affect P_{twitch}.

An example of the effect of both drugs is shown in Fig. 7.

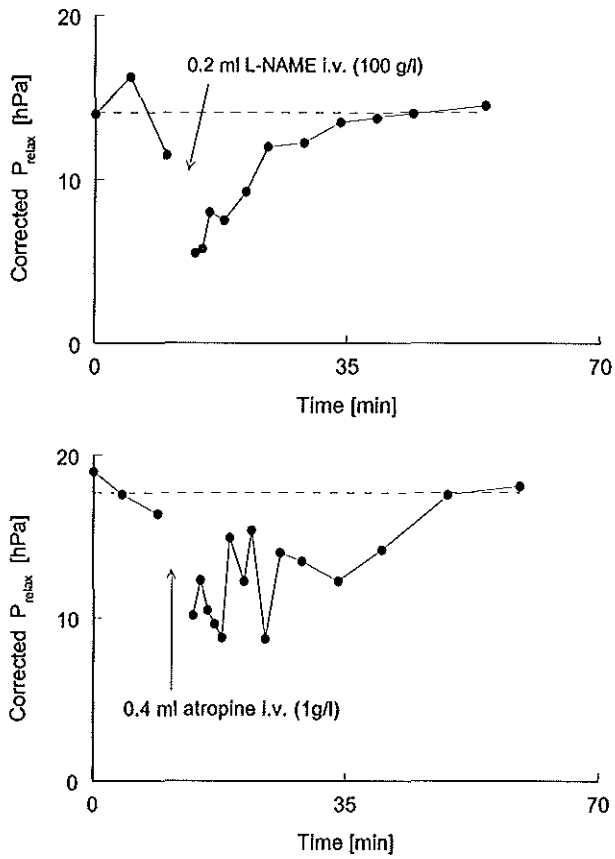

Figure 7. *Effect of N-nitro-L-arginine methyl ester (L-NAME; A) and atropine (B) on Prelax. After bilateral transection of the pelvic and hypogastric nerves, 3 control measurements were done in which the pudendal nerve was stimulated with an amplitude high enough to induce clearly detectable urethral relaxation (fstim = 15 Hz). Either drug was then administered, and pudendal nerve stimulation was repeated at the same frequency and amplitude. In both experiments a decrease of Prelax with time interfered with the effect of the drugs. It was assumed that the effect of both drugs had vanished after 30 min, which allowed estimation of the time effect from the first 3 (controls) and last 2 measurements. All responses were corrected for this estimated decrease with time (Prelax = 0.14 ± 0.10 hPa/min on average).*

DISCUSSION

In this study we measured urethral pressure (P_{ura}) in response to pudendal nerve stimulation. To allow urethral pressure measurement, the urethra was perfused at a constant flow rate (0.5 ml/min). At this flow rate the urethral pressure was 51 ± 18 hPa, which is in agreement with an earlier study (P_{ura} ~ 56 ± 22 hPa) (19).

The rat urinary sphincter has been shown to receive both autonomic and somatic input (43). The somatic input is conducted through the pudendal nerve (26), whereas the autonomic input is generally assumed to be provided via the major pelvic ganglion (7). The pudendal nerve anatomy allows a clear distinction between an efferent and an afferent trunk (26), the afferent trunk contributing ~84% to the total number of fibers (13). Although the pudendal nerve is generally considered somatic, the efferent trunk appears to contain ~25% sympathetic fibers. The other 75% are parasympathetic or somatic motor fibers. Only 12% of the efferent fibers are myelinated and are suggested to be somatic motor fibers (13).

After the initial transection of the right pelvic and hypogastric nerve, there were four possible neural pathways to control urethral pressure: left pelvicus, left hypogastricus, and left or right pudendus. As in the experiments done by Kihara and de Groat (15), electrical stimulation of the hypogastric nerve increased P_{ura}. In their experiments, the central pathway was transected. Therefore, the increased urethral pressure had to be caused by efferent hypogastric nerve stimulation. It is thus probable that the hypogastric nerve provides an excitatory (adrenergic) input to the smooth muscle of the proximal urethra. Fraser et al. (11) showed that electrical stimulation of parasympathetic pathways in the anesthetized rat produces urethral smooth muscle relaxation sensitive to NOS inhibition.

After subsequent transection of the left pelvic and hypogastric nerves only pudendal efferents controlled urethral resistance.

The first (rostral) half of the proximal urethra consists of three layers of smooth muscle, which receive mainly adrenergic innervation. In the innermost layer, cholinergic innervation has been described as well (43). In the second half, a single layer of smooth muscle cells is surrounded by a layer of mixed smooth and striated muscle cells and a layer of striated muscle. Both adrenergic and cholinergic nerves have been found in these layers. Ganglion cells have been described between the striated muscle layer and smooth muscular coat (43).

In our study, after bilateral transection of the pelvic and hypogastric nerves, electrical stimulation of the pudendus induced fast twitch-like contractions upon each stimulus pulse superimposed on a slow relaxation. At low stimulation frequencies (20 Hz) the pressure reached a stable level P'_0 before the next twitch contraction. The fast pressure undershoot (P_{min}, Fig. 2). was considered to be an artefact caused by the sudden stop of moving fluid in a flexible chamber. This point of view is supported by the fact that P'_0 at the end of stimulation (and not P_{min}) equals the urethral pressure immediately after stimulation (Fig. 2). Thus P'_0 described the relaxant response. We determined the time constant of this relaxation (relax = 3 ± 1 s). Other studies have shown that bladder smooth muscle contracts

with a time constant of ~3.1 ± 1.1 s (41) or ~3.4 s (18). We therefore concluded that the slow decrease in urethral pressure was caused by smooth muscle relaxation.

P_{twitch} was not frequency dependent up to 26 Hz, which corresponds to a time constant of ~40 ms, 80 times faster than the smooth muscle time constant. Skeletal muscles have been shown to follow frequencies up to 2 Hz (32). However, this latter study concerned load-moving skeletal muscle, which decreased the maximum frequency. Isometric twitch contraction in the soleus muscle of mice was shown to have a contraction time of 21 ms (21), even less than the 31 ms measured in our study. We concluded that the twitches in our study were caused by striated muscle contractions. Because all other neural pathways to the urethra had been transected, both the smooth muscle relaxation and the striated muscle contraction must have been induced by efferent pudendal nerve firing [caused either directly by efferent stimulation or indirectly via the pudendo-pudendal reflex (27)].

Transection of the left hypogastric and pelvic nerve had no influence on the tonic urethral pressure. Similar results were obtained by Kakizaki et al. (14) after ganglionic blockade. Tonic urethral resistance is probably not neurally regulated by the pelvic or hypogastric nerve.

In our study, the effect of hypogastric and pelvic nerve transection on P_{relax} was probably underestimated because there was always a spontaneous decay with time, which was not compensated for. Thus a possible increase is masked by a decrease with time. Yet, we still measured a P_{relax} that tended to increase after hypogastric and pelvic nerve transection, although not significantly.

If P_{relax} increased after bilateral transection of the hypogastric and pelvic nerves, what may have caused this increase?

Stimulation of pudendal afferent fibers (originating in sex organs) has been shown to have an inhibitory effect on the bladder (9, 20). Inhibition of the micturition reflex may include an excitatory reflex (sympathetic adrenergic or parasympathetic cholinergic) to the urethral smooth muscle. The most probable pathway would be the sympathetic system because this is generally accepted to be active during the urine storage phase (8). Furthermore, far more adrenergic nerves than cholinergic nerves were found in the proximal urethra (43). Stimulation of the hypogastric nerve induced increased urethral resistance (15). Thus, before transection of the pelvic and hypogastric nerves, the relaxant effect of pudendal nerve stimulation may have been masked by a central reflex via the hypogastric nerve that induced contraction of urethral smooth muscle.

In in vitro experiments done by others, electrical stimulation of urethral tissue also induced both relaxation and contraction of smooth muscle (14, 31, 34). The contraction was no longer observed after supramaximal α-adrenergic stimulation (31), which indicates that the smooth muscle contraction upon electrical stimulation was mediated by adrenergic pathways and therefore sympathetic.

Nonadrenergic, noncholinergic-mediated relaxation of urethral smooth muscle has been described (3, 31), whereas norepinephrine has been found to be the major excitatory

transmitter (5). Parlani et al. (31) reported atropine-sensitive relaxation of urethral muscle strips on acetylcholine stimulation.

In our study, atropine reduced the smooth muscle relaxation by ~40% and had no influence on the striated muscle contractions. Thus the smooth muscle relaxation was at least partially mediated by cholinergic activation of muscarinic receptors. Acetylcholine had an excitatory effect on the urethral striated musculature, but this effect was not blocked by atropine because the contraction is mediated by nicotinic receptors (14, 22).

The smooth muscle relaxation in our study was also reduced (by ~30 %) after L-NAME administration. We therefore concluded that the relaxation was also (partially) mediated by nitric oxide (NO). Persson et al. (33) described a dense innervation of NOS-containing nerves in the pig urethra and suggested a possible corelease of NO and acetylcholine because a considerable overlapping of NOS immunoreactivity and acetylcholinesterase (AChE) staining was found. In a more recent paper, they (34) suggested that the function of cholinergic nerves in the rat urethra is mainly to release inhibitory NO and to evoke urethral relaxation.

NO might also be released on cholinergic stimulation from endothelium cells (30). In the urethra, this may seem unlikely because cholinergic fibers were mainly found in the outer layers (43), whereas endothelium cells are located on the inner side. However, Persson et al. (35) found AChE-positive terminals that ran in close association with the urothelium, with branches arborizing into it. NOS-immunoreactive nerves were also observed in the suburothelial stroma (35).

NO may also be released from ganglion cells that are found in the urethra (43). In the major pelvic ganglion coexistence of cholinergic and nitrergic nerves has been demonstrated (34). This mechanism may play a role, but if so, it is not the single pathway because it does not explain the reduced relaxation after atropine administration that we found. Atropine has no effect on the nicotinic receptors in ganglion cells. Possibly, acetylcholine (also) has a relaxant effect on urethral smooth muscle, which is not mediated by NO. This is emphasized by our finding that a muscarinic receptor blocking agent induces stronger reduction of the urethral relaxation than an NO synthase inhibitor. Moreover, it was shown that L-NAME may block muscarinic receptors, and thus even the urethral relaxation that was reduced after L-NAME administration may have been mediated by acetylcholine instead of NO (4).

However, either of these mechanisms implies an important role of acetylcholine in pudendal nerve-controlled urethral relaxation. If acetylcholine has an inhibitory effect on urethral smooth muscle and an excitatory effect on urethral striated muscle, and these types of muscle cells intermingle rather freely in the rat urethra (43), the question arises: what is the predominant effect of acetylcholine release?

Our results show that the urethral relaxation is highly dependent on the frequency of pudendal nerve stimulation. The amplitude and the duration (~31 ms) of the striated muscle contractions, however, are independent of this stimulation frequency, up to 26 Hz.

On average the amplitude of the contractions was three times larger than the amplitude of the relaxation. We concluded that during contraction of the striated muscles, the urethra was always closed. The relaxation was therefore only effective in the fraction of time when the striated muscles did not contract.

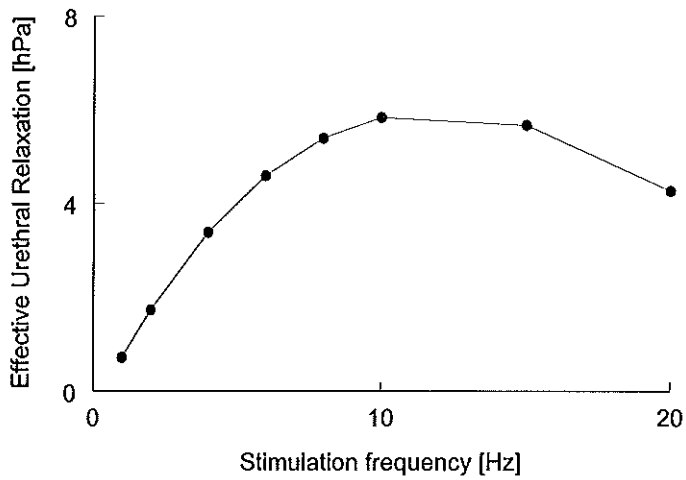

Figure 8. *Effective urethral relaxation was calculated by multiplying the urethral relaxation (Fig. 5, fitted line) with the time fraction in which the urethra was not closed because of a twitch contraction. Mean duration of twitches was 31 ms, and therefore this time fraction was calculated as 1 - (0.031·f_{stim}).*

We calculated the effective urethral relaxation (EUR) as the urethral smooth muscle relaxation multiplied by this fraction (1 - 0.031·f_{stim}). The result is shown in Fig. 8. From other studies it is known that rats normally void at bladder pressures of ~40 hPa (40). P_{ura} necessary for perfusion at a flow rate as low as 0.5 ml/min generally exceeded this pressure (~51 ± 18 hPa), and thus urethral relaxation is needed to enable bladder emptying. Figure 8 shows that the EUR is maximum at frequencies between ~7 and ~15 Hz, the frequency range of natural urethral oscillations during voiding. In our measurements the absolute value of the maximum EUR was ~7 hPa, but this depended highly on the stimulation amplitude and may be much larger if all nerves fire at maximum frequency.
Thus the dominant effect of acetylcholine on urethral resistance depends on the frequency of release. An oscillatory release at 7-15 Hz induces maximum EUR. The frequency of sphincter oscillations during evoked voiding contractions in anesthetized animals is in the lower half of

this frequency range with maximum EUR. This may be so because the lower frequencies require less metabolic activity, but it has also been described that the use of anesthetics lowers the oscillation frequency (28). Thus the oscillations during voiding occur at the frequency that maximizes urethral relaxation. Moreover, the oscillations are observed more frequently in male rats than in female rats. This may be explained by their higher need for urethral relaxation during voiding; male rat urethras are approximately four times longer than female rat urethras.

It is generally assumed that the striated urethral muscles are controlled by somatic motor fibers that are presumably myelinated (13) and that the smooth muscles receive unmyelinated parasympathetic innervation. Because myelinated nerve fibers show a lower stimulation threshold (37), it might be expected that only twitches at low stimulation amplitude would be found. From our measurements we could not determine such a threshold amplitude (Fig. 6). However, other studies showed that the stimulation threshold for unmyelinated fibers is one order of magnitude higher than the threshold for myelinated fibers (36). Thus we probably stimulated only myelinated fibers, and only the number of recruited myelinated fibers increased with increasing stimulation amplitude. Both the twitch amplitude and the relaxant amplitude increased. Inasmuch as it is unlikely that all muscle cells in the urethral wall receive direct innervation (10), there may be myelinated axons that have influence on both striated and smooth muscle cells.

The major argument for the theory that the oscillations are needed to pump the urine through the urethra is the observation that blocking the oscillations by either pudendal nerve transection (6) or administration of d-tubocurarine (22) increases the residual urine in the bladder or completely abolishes effective voiding. However, pudendal nerve transection obviously abolishes both the twitches and the slow relaxation, whereas d-tubocurarine also blocks ganglionic transmission, and ganglionic blockade has been shown to abolish the oscillatory reflex (22). In both situations there is no acetylcholine release and thus no effective urethral relaxation. Kakizaki et al. (14) showed that the striated muscle contractions could be blocked by α-bungarotoxin while there still was smooth muscle relaxation (although masked by adrenergic smooth muscle contraction).

In conclusion, urethral oscillation during bladder emptying in rats may have a function in territory marking, or it may pump urine through the urethra. In this study, we showed that the pudendal nerve induces urethral relaxation, which is, at least partially, mediated by acetylcholine. Acetylcholine also induces contraction of striated urethral muscle, and the overall effect on urethral resistance depended on the frequency of acetylcholine release. We therefore introduced the (frequency dependent) effective urethral relaxation that was maximum at the frequency of the spontaneous oscillations. Thus, although the oscillatory sphincter contractions in rats during voiding may be needed in other mechanisms for efficient voiding, they may very well be a side effect of the actual purpose of pudendal acetylcholine release: urethral relaxation.

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7. Summary.

SUMMARY

In this thesis we studied the innervation of the urinary bladder and urethra in the rat, which is constituted by the pelvic nerve, the hypogastric nerve and the pudendal nerve. Several models have been developed that describe the relations between the activity in these nerves and mechanical variables such as bladder pressure and urethral pressure and flow.

1. NERVE ACTIVITY. Nerve signals were measured in afferent fibers, which conduct sensory information to the central nervous system to monitor the physical state of the bladder and urethra, and in efferent fibers, which conduct organ regulating nerve signals from the central nervous system. Nerve signals consist of spike-shaped action potentials. The amount of nerve activity was quantified by calculating the mean value of the rectified nerve signal in 100 milisecond intervals. Thus 10 values per second were calculated, reflecting the number of action potentials in the appointed interval.
In some recordings a combined signal of efferent and afferent activity was measured. Our measurements supported the hypothesis that the measured total nerve activity is the linear sum of afferent and efferent nerve activity.

2. BLADDER. The urinary bladder is innervated by the pelvic and hypogastric nerves, which synapse in the major pelvic ganglion. Thin nerve fibers, called postganglionic bladder nerves, run between the ganglion and the bladder. We measured nerve activity between the major pelvic ganglion and the bladder, and related the measured activity to bladder mechanical variables, derived from pressure and volume. Bladder afferents appeared to be proportional to the stress in the bladder wall, which in our analysis was approximated by bladder pressure × volume.
We found that bladder afferents are essential for the initiation of bladder voiding. If the afferent activity exceeds a certain threshold level the bladder is neurally stimulated to contract. In our experiments, efferent activity in postganglionic bladder nerves always led to contraction, we never found postganglionic inhibitory signals. The increased bladder pressure as a result of efferent firing, can be calculated by low-pass filtering of the efferent signal with a cut-off frequency of $^1/_3$ Hz. During a contraction, the rising bladder pressure causes the afferent nerve activity to further increase above threshold.

While the bladder contracts, urine flows through the urethra and the bladder volume decreases. Due to the decreasing volume, the bladder wall stress decreases and so does the afferent bladder nerve activity. Efferent firing continues until the bladder afferent activity drops below a threshold again. After cessation of efferent firing to the bladder, the bladder pressure decreases, which causes the afferent activity to further decrease below threshold. Thus the system is bi-stable: in both directions a minor crossing of the threshold results in a decisive change.

Both the described efferent and afferent signals are conducted through the pelvic nerve. The afferent signals running through the hypogastric nerve were not investigated in this thesis. Other researchers, however, described afferent hypogastrical activity upon chemical stimulation of bladder receptors.

3. URETHRA. From the urethra, nerve signals may be conducted through the pelvic, hypogastric or pudendal nerve. Afferent nerve activity from the proximal urethra is proportional to the urethral pressure and thus increases with the flow through the urethra. In contrast to afferent bladder nerve activity, urethral afferents adapt rather rapidly to changes in urethral pressure, with a time constant of approximately 1.8 seconds. Afferent activity from the proximal urethra is probably conducted via the major pelvic ganglion and thus through the pelvic and / or hypogastric nerve.

Urethral afferents only start firing at pressures that are too high to play a role in the initialization of voiding contractions. Bladder contractions are already started when the bladder pressure, and therefore the urethral pressure, is too low to induce afferent nerve activity from the proximal urethra. Urethral afferents may be necessary to avoid stress incontinence. Afferent nerve activity that comes from the more distant parts of the urethra was not investigated.

The efferent innervation of the urethra is very complex as well, receiving impulses via three nerves, n. pelvicus, n. hypogastricus and n. pudendus. Furthermore, the urethra consists of smooth and striated muscle tissue with different responses to electrical stimulation of these nerves.

We found slight contraction of urethral smooth muscle upon hypogastric nerve stimulation. Pudendal nerve stimulation induced contraction of striated muscles and relaxation of smooth muscle tissue. Both responses were mediated by the neurotransmitter acetylcholine and the dominant effect of pudendal nerve stimulation depended on the stimulation frequency (the number of stimulus pulses per second). At high frequencies (more than 30 pulses per second) the urethra contracted continuously due to striated muscle contraction. At lower frequencies the striated muscles contracted upon each stimulus pulse, and the relaxation of the smooth muscles increased with the frequency. Effectively, the urethral relaxation was highest at approximately ten pulses per second. During natural voiding, rats show an oscillatory pattern of urethral sphincter contractions at this frequency. Thus, the rhythmic release of acetylcholine as seen in rats may be necessary for urethral relaxation.

Thus, although not complete yet, the developed model translates the physical state of the bladder and the proximal urethra into afferent nerve signals. It describes how these afferent nerve signals are processed in the central nervous system, and how they may effect efferent

firing to the bladder. It also gives quantitative relations between efferent nerve activity and the mechanical responses of the bladder and urethra.

8. Samenvatting.

SAMENVATTING

Alle levende wezens hebben voor hun voortbestaan een regelmatige aanvoer van brandstof en nieuwe bouwstenen nodig. Mede omdat het zichzelf hiervan voorzien meestal niet als onprettig wordt ervaren, levert dit ook een hoop afvalstoffen op, die onder andere via de nieren worden uitgescheiden. Dit is een proces dat in principe continu plaats vindt. In veel gevallen is een continue uitstoot van afvalstoffen echter onwenselijk. De natuur heeft hiervoor een voorziening getroffen, de continue urinestroom uit de nieren wordt opgevangen in de blaas, welke op gezette tijden kan worden geleegd via de urethra (plasbuis). Blaas en urethra vormen samen de lage urinewegen. Zowel de blaas als de urethtra bestaan uit spierweefsel dat wordt bestuurd door het centrale zenuwstelsel, dat bestaat uit de hersenen en het ruggenmerg. Het centrale zenuwstelsel zorgt voor de coördinatie tussen de blaas en de urethra tijdens de zogenaamde mictiecyclus. In deze cyclus worden twee fasen onderscheiden: de opslagfase, waarin de blaas langzaam voller wordt, en de ledigingsfase waarin de blaas wordt geleegd.

In de opslagfase is het belangrijk dat de urethra goed dicht zit, om lekkage te voorkomen. Dit wordt gerealiseerd door het spierweefsel van de urethra aan te spannen, er is dan ook wel sprake van een hoge urethrale druk. Aan de andere kant is het van belang dat de blaasdruk in deze fase zo laag mogelijk blijft; deze spier moet vooral niet worden aangespannen.
In de ledigingsfase wordt de urine uit de blaas geperst doordat deze samentrekt. Tevens wordt in deze fase de urethra ontspannen om zo het wegstromen van de urine mogelijk te maken. Er is dan dus sprake van een hoge blaasdruk en een lage urethrale druk. De urine kan naar buiten stromen zodra de blaasdruk hoger is dan de urethrale druk.

De coördinatie die nodig is voor het overschakelen van de ene naar de andere fase, wordt uitgeoefend door het centrale zenuwstelsel. Zowel de blaas als de urethra ontvangen besturingssignalen van het centrale zenuwstelsel via zogenaamde efferente zenuwvezels. Om een efficiente besturing mogelijk te maken, krijgt het centrale zenuwstelsel informatie over de toestand waarin beide organen zich bevinden. Dit gebeurt door sensorische zenuwen die registreren hoe vol de blaas is, en of er vloeistof door de urethra stroomt. Als alles goed werkt, coördineert het centrale zenuwstelsel op basis van deze informatie de samenwerking tussen blaas en urethra.

Soms is er echter een defect in het systeem. Als bijvoorbeeld de zenuwen die door het ruggenmerg lopen beschadigd raken, krijgt het centrale zenuwstelsel de benodigde informatie niet meer, of vallen de zenuwsignalen weg waarmee de organen worden bestuurd. Dit kan ernstige gevolgen hebben voor de betrokkene. Vaak vinden er geen samentrekkingen van de blaas meer plaats, of ontspant de urethra onvoldoende zodat de blaas niet geleegd kan worden. Als de blaas heel erg vol wordt, en de druk in de blaas te hoog, kan de urine van de blaas terug stromen naar de nieren, wat uiteindelijk zelfs de dood tot gevolg kan hebben.

Toch werken de organen in een dergelijke situatie vaak nog prima: er worden zenuwsignalen afgegeven die de toestand van de blaas en urethra beschrijven en er is nog reactie op zenuwsignalen die van het centrale zenuwstelsel komen. De coördinatie tussen beide ontbreekt echter. Het is niet ondenkbaar dat als al deze signalen in voldoende detail bekend zouden zijn, zou de coördinerende taak van het centrale zenuwstelsel overgenomen kan worden door een computer. Deze kan wellicht de blaas en de urethra besturen op basis van de informatie in de zenuwsignalen die deze organen afgeven. Het geheel van zenuwsignalen dat tussen de lage urinewegen en het centrale zenuwstelsel loopt, is een uiterst complex systeem. Daarom wordt vaak gewerkt met een model, een overzichtelijke, vereenvoudigde beschrijving van de werkelijkheid.

In dit proefschrift is een model opgesteld dat in algemene termen beschrijft hoe de toestand van de blaas en urethra wordt vertaald in zenuwsignalen die het centrale zenuwstelsel van informatie voorzien. Ook legt het relaties tussen de besturingssignalen die van het centrale zenuwstelsel komen, en de mate van samentrekking of juist ontspanning van de spieren in de blaas en urethra. Bovendien is er gekeken naar de manier waarop het centrale zenuwstelsel de signalen van de blaas en de urethra verwerkt.
Alle gevonden relaties zijn gebaseerd op experimenten met ratten, en de ontwikkelde modellen hoeven dus niet per se te gelden voor mensen. Wel zijn er grote overeenkomsten tussen de lage urinewegen van mens en rat, die het waarschijnlijk maken dat een eventueel licht aangepast ratten model ook van toepassing is op de mens.
Bij mens en rat spelen drie grote zenuwen een belangrijke rol. Er lopen zenuwsignalen van en naar de blaas via de nervus pelvicus en de nervus hypogastricus. De n. pudendus vormt de belangrijkste verbinding tussen de urethra en het ruggenmerg, maar waarschijnlijk spelen ook de n. pelvicus en de n. hypogastricus hierbij een rol.

Er is een model gemaakt van de blaas, waarin zenuwactiviteit die is gemeten in zenuwtakjes die naar de blaas lopen, wordt gerelateerd aan de druk in de blaas. Deze zenuw takjes zijn waarschijnlijk afsplitsingen van de nervus pelvicus. Het blijkt dat de druk in de blaas toeneemt als het besturingssignaal vanuit het centrale zenuwstelsel toeneemt. De relatie tussen het besturingssignaal en de blaasdruk kan worden vergeleken met de werking van een elektronisch *laag-doorlaat* filter. Dit betekent dat langzame veranderingen (*lage* frekwenties) in het stuursignaal worden doorgelaten terwijl snelle veranderingen worden uitgefilterd. Dat wil niet zeggen dat een snelle, blijvende verhoging van het stuursignaal geen effect heeft op de blaasdruk, de blaasdruk reageert alleen langzamer, alsof het een langzame verhoging betreft. De blaas heeft ongeveer 3 seconden nodig om de blaasdruk aan te passen aan een plotselinge verhoging of verlaging in het besturingssignaal. Als het besturingssignaal wegvalt, neemt de blaasdruk weer langzaam af tot zijn uitgangswaarde.
De toestand van de blaas wordt aan het centrale zenuwstelsel doorgegeven door sensorische zenuwen. De activiteit in deze zenuwen bleek recht evenredig met de blaasdruk: Hoe hoger de druk, hoe hoger de zenuwactiviteit. Het besturingssignaal dat naar de blaas loopt en het

sensorische signaal dat van de blaas komt, lopen door dezelfde zenuwbundels. Als er in een intacte blaaszenuw wordt gemeten, meet je de optelsom van het sensorische (het afferente signaal) en het besturingssignaal (het efferente signaal).

Ook de urethra geeft een sensorisch signaal af, dat afhangt van de druk in de urethra. In tegenstelling tot het sensorische signaal van de blaas, daalt dat van de urethra echter weer snel als de druk niet verder verandert. Toch reageren de sensoren in de urethra niet alleen op veranderingen in de druk. Een plotselinge, blijvende verhoging van de urethrale druk heeft een blijvende toename in de sensorische zenuwactiviteit tot gevolg, maar deze stabiele verhoging is kleiner dan de direkte reactie op een plotselinge verandering. Het sensorische signaal van de urethra is dus het grootst bij een plotselinge grote drukverhoging in de urethra.

Uit analyse van blaaszenuw signalen bleek dat het centrale zenuwstelsel besturingssignalen naar de blaas gaat sturen op het moment dat de sensorische zenuwactiviteit van de blaas een bepaalde, vaste drempel overschrijdt. Er kon ook worden geconcludeerd dat de sensorische informatie van de urethra geen rol speelt bij het op gang komen van een blaascontractie, omdat de contractie al begint als de urethrale zenuwen nog niets doen. De gedachte in dit model is dus dat als de sensorische zenuwactiviteit boven de drempel komt, het centrale zenuwstelsel stuursignalen naar de blaas stuurt. Deze zorgen voor blaascontractie, waardoor de blaasdruk toeneemt. Doordat de sensorische signalen afhangen van de druk stijgen ze nog verder.

Hier liep het model spaak, omdat het geen verklaring geeft voor het beëindigen van de besturingssignalen naar de blaas. Daarom is nauwkeuriger gekeken naar de mechanische grootheden die mogelijk de sensorische signalen van de blaas bepalen. In de metingen waarop het eerste model was gebaseerd, waren er maar kleine variaties in het blaasvolume Hierdoor kon de eventuele invloed van het blaasvolume op de sensorische signalen nauwelijks worden waargenomen. Uit metingen waarbij het blaasvolume flink varieerde, bleek dat sensorische zenuwactiviteit van de blaas beter correleerde met de wandspanning dan met druk. De wandspanning is bij benadering evenredig met blaasdruk × volume. Als nu de blaas langzaam voller wordt, neemt de sensorische zenuwactiviteit langzaam toe met het blaasvolume. Bovendien stijgt de blaasdruk licht. Als een drempelwaarde (de startdrempel) wordt overschreden, stuurt het centrale zenuwstelsel stuursignalen naar de blaas die voor contractie zorgen. Hierdoor neemt de blaasdruk, en daarmee de sensorische zenuwactiviteit toe. Als er nu vloeistof uit de blaas wordt geperst, neemt de sensorische zenuwactiviteit af doordat blaasvolume daalt, totdat het weer onder een bepaalde waarde (de stopdrempel) komt. Dan stopt het stuursignaal en dus ook de contractie, waardoor de druk afneemt. Hierdoor daalt het sensorische signaal nog verder. Dit model is dus bi-stabiel: een kleine overschrijding van de drempel zorgt in beide richtingen voor een definitieve verandering. De beide drempels bleken zeer nauwkeurig vast te liggen. In 30% van de ratten lag de stopdrempel iets hoger dan de startdrempel. In de overige beestjes was er geen verschil.

De zenuwen en mechanische grootheden die tot zover zijn beschreven vertonen grote overeenkomsten in de mens en de rat. Een aspect dat bij de rat echt anders is dan bij de mens is het optreden van ritmische contracties (circa 10 per seconde) in de urethra tijdens de ledigingsfase. Dit is een eigenaardig verschijnsel omdat dit de weerstand van de urethra lijkt te verhogen; tijdens een urethrale contractie kan de urine er niet doorheen. De urethra bestaat uit 2 typen spiercellen: glad en dwarsgestreept. Dwarsgestreept spierweefsel is het type waaruit ook de meeste skeletspieren bestaan, terwijl glad spierweefsel meestal voorkomt in organen als de darmen, bloedvaten of de blaas. Een kenmerkend verschil tussen deze spiertypen is dat dwarsgestreept spierweefsel veel sneller kan aan- en ontspannen. De ritmische contracties van de urethra worden dan ook veroorzaakt door urethraal dwarsgestreept spierweefsel. Het centrale zenuwstelsel stuurt hiervoor een gepulseerd zenuwsignaal via de nervus pudendus naar de urethra.
Bij iedere puls in dit stuursignaal komt er aan het uiteinde van de zenuw een stof vrij (acetylcholine) die ervoor zorgt dat de dwarsgestreepte spiercellen samentrekken. Deze stof blijkt er echter ook voor te zorgen dat de gladde spiercellen in de urethra ontspannen. Het netto effect op de urethra hangt af van het aantal contracties per seconde. Bij 10 contracties per seconde is de ontspanning van de gladde spiercellen zo groot dat het netto effect maximale urethrale ontspanning oplevert.

Hoewel ritmische urethrale contracties bij de mens niet voorkomen in de ledigingsfase, kunnen deze toch ook voor de mens belangrijk zijn. Er is een behandelingsmethode die bij mensen toegepast wordt, waarbij de nervus pudendus elektrisch wordt gestimuleerd met 10 pulsen per seconde. Deze methode wordt toegepast om instabiele blaascontracties tegen te gaan, en heeft een tot nu toe onverklaard bij-effect: verlaging van de urethrale weerstand. Er is nog geen onderzoek gedaan naar dit mechanisme, maar het zou op een soortgelijke manier kunnen werken als bij ratten.

Hoewel het in dit proefschrift ontwikkelde model van de lage urinewegen nog niet compleet is, brengt het de mogelijkheid de besturingstaak van het centrale zenuwstelsel door een computer uit te laten voeren een stuk dichterbij. Ook mensen met een beschadigd ruggenmerg zouden dan weer kunnen genieten van een normaal functionerende blaas en urethra. Er zijn echter nog wel een aantal vragen die daarvoor beantwoord moeten worden. Het oplossen van deze neurofysiologische puzzel brengt wellicht verlichting voor hen die zich in hun bacchanaal gedrag geremd weten door fysieke beperkingen van hun lage urinewegen.

CURRICULUM VITAE

Ik werd op 15 maart 1967 in Veldhoven geboren. In 1985 haalde ik mijn atheneum-B diploma aan het Christelijk Lyceum in Almelo, waarna ik elektrotechniek ging studeren in Delft. Mijn ingenieursdiploma behaalde ik in 1991 bij de vakgroep Informatietheorie, na een afstudeeronderzoek bij de afdeling Algemene Heelkunde van het Academisch Ziekenhuis Rotterdam, Dijkzigt.
In 1992 en 1993 werkte ik als onderzoeker mee aan een vervolgproject op mijn afstudeeronderzoek, nu in dienst van de Erasmus Universiteit Rotterdam. Het betrof hier een door het Astma Fonds gefinancierd onderzoek naar de ontwikkeling, validatie en bruikbaarheid van de Forced Oscillation Technique om ademmechanica te meten tijdens beademing. Dit onderzoek omvatte meettechnische aspecten en modelvorming.
In 1994 begon ik met een tijdelijke aanstelling voor 1 jaar bij de afdeling Urologie-Urodynamica van de Erasmus Universiteit Rotterdam aan het onderzoek dat zou uitmonden in dit proeschrift. Dit kon worden afgerond binnen een door de Nierstichting gefinancierde hernieuwde aanstelling in de periode 1995-2000.
Vanaf 1 mei 2000 werk ik bij de faculteit Wiskunde en Natuurwetenschappen van de Rijks Universiteit Groningen aan een door NWO gefinancierd onderzoek getiteld "Requirements Engineering for Realtime Medical Support Systems", naar de toepasbaarheid van kunstmatige intelligentie in de anaesthesie.

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