Neurophysiological modeling of voiding in rats: urethral nerve response to urethral pressure and flow
Joost Le Feber, Els Van Asselt and Ron Van Mastrigt


You might find this additional information useful...

This article cites 20 articles, 7 of which you can access free at:
http://ajpregu.physiology.org/cgi/content/full/274/5/R1473#BIBL

This article has been cited by 2 other HighWire hosted articles:

Pudendal nerve stimulation induces urethral contraction and relaxation
J. le Feber and E. van Asselt
Am J Physiol Regulatory Integrative Comp Physiol, November 1, 1999; 277 (5): R1368-R1375. [Abstract] [Full Text] [PDF]

Threshold for efferent bladder nerve firing in the rat
E. van Asselt, J. le Feber and R. van Mastrigt
Am J Physiol Regulatory Integrative Comp Physiol, June 1, 1999; 276 (6): R1819-R1824. [Abstract] [Full Text] [PDF]

Medline items on this article’s topics can be found at http://highwire.stanford.edu/lists/artbytopic.dtl on the following topics:
Veterinary Science .. Ganglia
Physiology .. Nerves
Physiology .. Urethra
Physiology .. Rats

Updated information and services including high-resolution figures, can be found at:
http://ajpregu.physiology.org/cgi/content/full/274/5/R1473

Additional material and information about American Journal of Physiology - Regulatory, Integrative and Comparative Physiology can be found at:
http://www.the-aps.org/publications/ajpregu

This information is current as of December 12, 2006.
Neurophysiological modeling of voiding in rats: urethral nerve response to urethral pressure and flow

JOOST LE FEBER, ELS VAN ASSELT, AND RON VAN MASTRIGT

Department of Urology-Urodynamics, Erasmus University Rotterdam, 3000 DR Rotterdam, The Netherlands

Le Febet, Joost, ELS van Asselt, and Ron van Mastriget. Neurophysiological modeling of voiding in rats: urethral nerve response to urethral pressure and flow. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1473–R1481, 1998.—In male urethan-anesthetized 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 (t1) was inversely proportional to the perfusion rate. After infusion of a fixed volume, the urethra opened and the NA decreased with a constant time constant (−1.8 s) to an elevated level (NA_left). NA_left 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_left, and t1 showed very good reproducibility (SD ~19% of mean). The measured activity was most likely afferent and conducted to the major pelvic ganglion.

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 relationships between neural activity and system dynamics (i.e., bladder pressure and urethral flow).

In a previous study, we quantitatively described the relationships 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 by the nerves leading through the major pelvic ganglion (2, 10, 13). Figure 1 shows a schematic drawing of this configuration.

In the present study, the relationships 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 urethan (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; Ref. 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 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 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 SP 1400 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 ~0.5 mm 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 amplifier (amplification range 10,000–200,000) and band pass filtered using a Krohn-Hite model 3944 (Bessel 500–2,500 Hz, 4th order filter). The combination of the symmetrical electrode and an amplifier with a very high common mode rejection ratio (>100 dB) 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 was 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 was calculated as the mean value of the rectified nerve signal in 100-ms intervals

\[
NA(t) = \frac{\sum_{i=1}^{2,500} \text{ENG}(i)}{2,500}
\]

where ENG(i) (electroneuromgram) represents the ith sample of the nerve signal in each 100-ms interval containing 2,500 points (1, 7, 27).

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 relationships between urethral nerve activity and urethral pressure and flow, and penile clamp measurements were done 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).

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 10 V, stimulation frequency 10 or 100 Hz, pulse width 50 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. Stimulation (100 Hz) was used to investigate urethral pressure changes in response to the stimulation. Because 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.

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) an initial phase of no flow through the urethra, 2) a phase when the pump was switched on and there was a constant fluid current through the urethra, and 3) a phase when the pump was switched off.

The signal-to-noise ratio (SNR) of each measurement was estimated as

\[
\text{SNR}_{\text{est}} = \frac{\max(\text{NA}) - \min(\text{NA})}{\min(\text{NA})}
\]

To derive a stable measure for SNR_{est}, averages of the 10 lowest and the 10 highest values were taken for minimum [min(NA)] and maximum [max(NA)] nerve activity. 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, Eqs 5 and 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

\[
\text{Fit error} = \left| \sum \frac{\text{NA}_{\text{est}} - \text{NA}_{\text{meas}}}{\sum \text{NA}_{\text{meas}}} \right| \times 100\%
\]

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

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 the insertion or withdrawal of 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 five flow rate settings were each applied once. In mode II, nerve activity was measured during five consecutive maneuvers in which the urethral pressure was...
manually varied. Modes 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\textsubscript{level}; see Eq. 6) was calculated as a function of the steady-state pressure (mean pressure in the last 5 s of perfusion). This relationship was described by a linear function. In the mode II measurements, the relationship between pressure and nerve activity was described by a linear relationship as well and offset and slope of both relationships were compared.

Because we often found deterioration in the nerve activity over the course of an experiment, especially during the first 10–15 min after connection of the electrode to the nerve, a stabilization period of 15 min was introduced before the measurements were started.

Lateral transection. 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. Because 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 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 means ± SD.

RESULTS

Urethral nerve activity was successfully measured in 15 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\textsubscript{ura}, just beyond the bladder neck) were measured at five different flow rate settings (1.1, 2.2, 5.4, 10.8, and 21.4 ml/min).

In each rat, dividing by P\textsubscript{ura} at 5.4 ml/min normalized the measured pressures, and the resulting fractions were averaged for all rats. In Fig. 2 the relative P\textsubscript{ura} are multiplied by the mean P\textsubscript{ura} at 5.4 ml/min (79 ± 24 hPa), which gives the average P-Q relationship

\[
P\textsubscript{ura} = 3.1 \cdot Q + 59 \quad (4)
\]

where Q is the perfusion rate.

Standard deviations in Fig. 2 refer to differences between rats.

Major nerve stimulation. In the major nerve stimulation measurements, the nerves that innervate the lower urinary tract were electrically stimulated with increasing amplitude (max 10 V), 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 an ~25-ms delay in four of five rats. Figure 3 shows five 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\textsubscript{ura} and thus, because a constant flow rate was applied, urethral resistance. Stimulation of pelvic, cavernous, or hypogastric nerve generally did not change the urethral resistance.

Urethral perfusion. 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) a phase at the start of the measurement when there was no flow through the urethra, 2) a phase when there was a stepwise change to the selected flow rate, which was maintained for ~30 s, and 3) a phase when the flow was suddenly set to zero again by switching off the pump. P\textsubscript{ura} and urethral nerve activity usually showed a pattern as in Fig. 4.

In phase 1 (t < t\textsubscript{1}), there was only noise and possibly some baseline activity (NA\textsubscript{0}). In phase 2 (t\textsubscript{1} < t < t\textsubscript{2}), the neural activity was modeled by an increase during a period δt, until NA\textsubscript{max} was reached. Then the nerve activity decreased with a certain time constant θ\textsuperscript{-1} to NA\textsubscript{level} (see Fig. 5). This can be mathematically formulated as in Eqs. 5 and 6

\[
NA(t) = NA\textsubscript{0} + [(NA\textsubscript{max} - NA\textsubscript{0}/6\delta t] \cdot (t - t\textsubscript{1}) \quad \forall t\textsubscript{1} < t \leq t\textsubscript{1} + \delta t \quad (5)
\]

\[
NA(t) = NA\textsubscript{level} + (NA\textsubscript{max} - NA\textsubscript{level}) \cdot \exp[-θ\cdot(t - (t\textsubscript{1} + \delta t))] \quad \forall t\textsubscript{1} + \delta t < t \leq t\textsubscript{2} (6)
\]
In these relationships, \( NA_0 \) 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 Fig. 4). During phase 3 \((t > t_2)\), when there was no flow, the nerve activity returned to \( NA_0 \) and the pressure decreased to a level at which the urethra closed \((56 \pm 22 \text{ hPa})\). This pressure did not depend on the applied flow rate and showed excellent reproducibility in each rat (average coefficient of variation \( 2.9 \pm 1.9\% \)).

The model parameters \( \delta t, NA_{\text{max}}, \psi, \) and \( NA_{\text{level}} \) (Eqs. 5 and 6) were estimated in each rat by minimizing the sum of squared deviations [average fit error (Eq. 3): \( 5.2 \pm 1.4\% \)]. Some of the measurements \( (7.6\%) \) were excluded, because SNR was \( < 0.3 \).

One-way ANOVA (see Table 1) showed that \( NA_{\text{max}} \) and \( \delta t \) were significantly dependent on the applied flow rate \((P < 0.05)\) in 100 and 75\% of the animals, respectively. The flow dependency of \( NA_{\text{level}} \) and \( \psi \) 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 12 rats and then multiplied by the average parameter value at 5.4 ml/min. The model parameter \( NA_{\text{max}} \) was found to increase with the flow rate and could be described by a two-parameter function (Fig. 6)

\[
NA_{\text{max}} = 0.32 \cdot Q^{0.24}
\]

In many measurements, particularly at low flow rates, \( \psi \) could not accurately be determined because of the poor SNR. This resulted in a large coefficient of variation \((\text{SD as a percentage of mean} \sim 59\%)\). We found a slight increase with \( Q \), but this was significant in only 2 of 12 animals. We therefore modeled \( \psi \) by a constant \((\psi = 0.56, \text{Table 2})\).

\( \delta t \) decreased with \( Q \) (Fig. 6) and was described by

\[
\delta t = 15.9/Q
\]

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 \( NA_{\text{max}} \) was reached \((0.27 \text{ ml})\). When this volume was separately calculated at each flow rate in each rat and the mean volumes were averaged, a volume of \( 0.26 \pm 0.19 \text{ ml} \) was found. The average coefficient of variation of these volumes, however, was only 29\% (Table 2). The relationship between \( Q \) and \( NA_{\text{level}} \) could be described by a linear function (Fig. 6)

\[
NA_{\text{level}} = 2.9 \cdot 10^{-3} \cdot Q + 0.29
\]

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

The model parameter \( NA_{\text{max}} \) was also modeled as a function of the pressure at \( t_1 + \delta t \), \( P_{\text{ura}}(t_1 + \delta t) \). There was an average difference of \( 5.8 \pm 5.1 \text{ hPa} \) between \( P_{\text{ura}}(t_1 + \delta t) \) 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 \( NA_{\text{max}} \) and \( P_{\text{ura}}(t_1 + \delta t) \) 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 \( NA_{\text{max}} \) had to be normalized before averaging all rats. Because pressure readings were not exactly equal, this normalization could not be made to the value of \( NA_{\text{max}} \) at a certain pressure. We therefore fitted a linear function to the data of each rat and calculated the value of \( NA_{\text{max}} \) at 100 hPa, which was used as a calibration factor. All \( NA_{\text{max}} \) values were scaled by this calibration factor. Overall we thus found a linear relationship between \( NA_{\text{max}} \) and \( P_{\text{ura}}(t_1 + \delta t) \) (Fig. 7)

\[
NA_{\text{max}} = 0.0039 \cdot P_{\text{ura}}(t_1 + \delta t) - 0.02
\]
Nerve activity usually decreased with time. Therefore, we estimated the degeneration of the nerve activity by plotting the relative values of $NA_{\text{max}}$, $NA_{\text{level}}$ (mode I), and nerve activity at 140 hPa (mode II) versus time. On average, the measured nerve activity decreased 5.7 ± 9.9% with time in these measurements (range 220 to 110%). The mean ratios of parameters $a$ and $b$ that described the relationship 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 Fig. 10. On average, there were no differences in the evoked nerve activity between the two modes.

Lateral transection. Lateral transection measurements were done in four 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 with 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 four t-tests, and none of them were 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, $NA_{\text{max}}$ and $\delta t$ depended significantly on the perfusion rate $Q$ ($P < 0.01$) but not on cut (intact vs. transected, $P > 0.3$). $NA_{\text{level}}$ and $\psi$ did not significantly depend on either $Q$ or cut ($P > 0.15$, see Table 3).

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 was assessed by one-way analysis of variance. Dependencies were considered significant if $P < 0.05$; $\psi$ not significant. $NA_{\text{max}}$, maximum nerve activity; $\psi$, time constant of decreasing nerve activity; $NA_{\text{level}}$, level to which the nerve activity decreased during perfusion; $\delta t$, period of increasing nerve activity.

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

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>$NA_{\text{max}}$</th>
<th>$\delta t$</th>
<th>$NA_{\text{level}}$</th>
<th>$\psi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;0.003</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>&lt;0.003</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>3</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>4</td>
<td>&lt;0.002</td>
<td>&lt;0.003</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>5</td>
<td>&lt;0.001</td>
<td>&lt;0.04</td>
<td>&lt;0.01</td>
<td>NS</td>
</tr>
<tr>
<td>6</td>
<td>&lt;0.02</td>
<td>&lt;0.004</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>7</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>8</td>
<td>&lt;0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>NS</td>
</tr>
<tr>
<td>9</td>
<td>&lt;0.001</td>
<td>&lt;0.02</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>10</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>11</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>12</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

% of animals significant: 100% 75% 33% 16%
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 \( dt \), until \( NA_{\text{max}} \) was reached, followed by a decrease with a time constant \( w^2 \) down to \( NA_{\text{level}} \). \( NA_{\text{level}} \) depended linearly on the amplitude of the step change in flow rate (Eq. 10), and \( dt \) decreased inversely proportional to it (Eq. 9). \( NA_{\text{max}} \) increased at a decreasing rate with \( Q \) (Eq. 8).

The values of the parameters \( NA_{\text{max}}, dt, \) and \( NA_{\text{level}} \) showed good reproducibility (mean coefficient of variation \( \sim 18\% \), Table 2). This means that, as in postganglionic bladder nerves (12), a quantitative model of nerve activity and urethral dynamics is possible. One-way ANOVA showed that the flow rate dependency of the parameters was significant (\( P < 0.05 \)) in 100, 75, and 33% of the animals (\( NA_{\text{max}}, dt, \) and \( NA_{\text{level}} \)). Because \( w \) depended significantly on the flow rate in only 2 of 12 animals, it was modeled by a constant value \( w = 0.56 \) s\(^{-1}\). The model described the data very well (fit error \( \sim 5.3\% \)).

Figure 2 shows that, in our measurements, urethral flow and \( P_{\text{ura}} \) were linearly related. Others obtained Fig. 6. Model presented in Fig. 5 and Eqs. 5 and 6 was fitted to data obtained from measurements at 5 different perfusion rates \( Q \). Values of \( NA_{\text{max}} \) (A), \( NA_{\text{level}} \) (B), and \( dt \) (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: \( NA_{\text{max}} = 0.32 \cdot Q_{0.24}, NA_{\text{level}} = 0.0029 \cdot Q + 0.29, dt = 15.9/Q \).

**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 \( dt \), until \( NA_{\text{max}} \) was reached, followed by a decrease with a time constant \( \varphi^{-1} \) down to \( NA_{\text{level}} \). \( NA_{\text{level}} \) depended linearly on the amplitude of the step change in flow rate (Eq. 10), and \( dt \) decreased inversely proportional to it (Eq. 9). \( NA_{\text{max}} \) increased at a decreasing rate with \( Q \) (Eq. 8).

The values of the parameters \( NA_{\text{max}}, dt, \) and \( NA_{\text{level}} \) showed good reproducibility (mean coefficient of variation \( \sim 18\% \), Table 2). This means that, as in postganglionic bladder nerves (12), a quantitative model of nerve activity and urethral dynamics is possible. One-way ANOVA showed that the flow rate dependency of the parameters was significant (\( P < 0.05 \)) in 100, 75, and 33% of the animals (\( NA_{\text{max}}, dt, \) and \( NA_{\text{level}} \)). Because \( \varphi \) depended significantly on the flow rate in only 2 of 12 animals, it was modeled by a constant value \( \varphi = 0.56 \) s\(^{-1}\). The model described the data very well (fit error \( \sim 5.3\% \)).

Figure 2 shows that, in our measurements, urethral flow and \( P_{\text{ura}} \) were linearly related. Others obtained...
were calculated. The average ratios are shown.

Fig. 10. Relationship between P_{ura} and nerve activity was described and/or traction on the nerve. However, the decrease may be caused by the dissection from the underlying tissue as well (18). The deterioration may 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

\[ NA_{level} = 9.4 \cdot 10^{-4} \cdot P + 0.23 \quad (11) \]

Due to transient effects, Eq. 4 is probably not valid at \( t = t_0 + \delta t \) (Fig. 5). We therefore directly compared \( NA_{\text{max}} \) to the instantaneous pressure at \( t_0 + \delta t \). Equation 10 shows that the transient nerve activity was also proportional to the pressure. A noticeable difference between Eq. 10 and Eq. 11 is the nonzero offset in Eq. 11. This is probably caused by the nonlinear 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 (12).

Between \( t_0 \) and \( t_0 + \delta t \), urethral nerve activity increased linearly with time and thus with the volume in the urethra \( V_{ura}(t) = (t - t_0) \cdot Q/60 \). The inverse proportional relationship between Q and \( \delta t \) suggests that \( NA_{\text{max}} \) was always found at a constant injected volume (0.26 ± 0.19 ml). This volume showed very good reproducibility in each rat (average coefficient of variation ~29%). Because 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.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rat 2</th>
<th>Rat 5</th>
<th>Rat 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cut</td>
<td>Q</td>
<td>Cut</td>
</tr>
<tr>
<td>( NA_{\text{max}} )</td>
<td>NS</td>
<td>&lt;0.005</td>
<td>NS</td>
</tr>
<tr>
<td>( NA_{\text{level}} )</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>( \delta t )</td>
<td>NS</td>
<td>&lt;0.005</td>
<td>NS</td>
</tr>
<tr>
<td>( \varphi )</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

In 3 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 \( P < 0.05 \).
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 $−0.04 \mu V$. 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 (∼30 s vs. <3 s) (24). Because $NA_{ave}$ 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 Eqs. 10 and 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 $NA_{ave}$. 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 ($P_{ura\ close} = 56 ± 22 hPa$). This is in very good agreement with the data in Fig. 2, where extrapolation of the pressure flow curve leads to $P_{ura\ close} = 59 hPa$. In guinea pigs, $P_{ura\ close}$ was reportedly independent of the state of urethral relaxation (6). In our measurements, however, this can hardly be the case, because 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 nerve was transsected 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). Because 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, although 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, because 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 hindleg 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 dorsal 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 dorsal 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 dorsal nerve of the penis or the pudendal nerve (8 vs. 18 ms) may be explained by the longer pathway through the dorsal 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, because 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 two 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 in-
duced 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 \( \sim 30 \text{ m/s} \) (2). In our experiments, the distance between stimulation and recording electrode was \( \sim 10 \text{ mm} \), which would result in a 0.3-ms latency. In our measurements, such a response would coincide with the stimulus artifact, which lasted \( \sim 2 \text{ ms} \). 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 \( \sim 5.3\% \)). This model consisted of a linearly increasing nerve activity during \( \Delta t \), until \( N_{A_{\text{max}}} \) was reached. Then the activity decreased with a time constant \( \tau \) to a steady-state level. \( N_{A_{\text{max}}}, \delta \), and \( N_{A_{\text{level}}} \) depended significantly on the perfusion rate in a large number of animals (70 \( \pm 36\% \)). Evidence has been provided though, that the nerve activity was generated by pressure sensors in the urethra, rather than flow rate sensors. \( N_{A_{\text{level}}} \) increased linearly with the steady-state pressure, and the \( N_{A_{\text{max}}} \) was proportional to the instantaneous pressure after \( \Delta t \) (at \( t = t_1 + \delta t \)). \( \delta \) 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 \( \varphi^{-1} \) (1.8 s).

This study was supported by the Dutch Kidney Foundation (Grant C95.1429) and by the Foundations “Stichting Urologisch Wetenschappelijk Onderzoek” and “Vereniging Trustfonds Erasmus Universiteit Rotterdam” in The Netherlands.

Address for reprint requests: J. le Feber, Dept. of Urology-Urodynamic, Rm. EE 1630, Erasmus Univ. Rotterdam, PO Box 1738, 3000 DR Rotterdam, The Netherlands.

Received 12 September 1997; accepted in final form 27 January 1998.

REFERENCES


