Functional modelling of voiding

Modelvorming van de urinelozing

Proefschrift

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
prof.dr. P.W.C. Akkermans, M.A.
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 17 april 1996 om 13.45 uur

door

Jan Groen

geboren te Zuidveen

Promotiecommissie

Promotor:	Prof.dr. F.H. Schröder
Co-promotor:	Dr.ir. R. van Mastrigt
Overige leden:	Prof.dr.ir. N. Bom Prof.dr. P.D. Verdouw Prof.dr. A. Versprille

The investigations presented in this thesis were performed at the Department of Urology (Division Urodynamics), Medical Faculty, Erasmus University Rotterdam.

Voor Wilma,
voor Hettie

Contents

Chapter 1

Introduction

The function of the lower urinary tract

Urine is continuously produced in the kidneys. It is the function of the lower urinary tract to temporarily store the urine and expel it under appropriate circumstances.[1] A normal bladder has the ability to hold an increasing amount of urine without a significant increase in intravesical pressure, up to a certain volume. The concept of compliance was introduced to quantify this ability. The bladder neck and the urethra remain closed during the storage phase to maintain continence. As bladder filling progresses, a sensation of fullness gradually develops and an appropriate place to empty the bladder will be looked for. Voiding is initiated by relaxation of the pelvic floor, followed by an intravesical pressure rise and opening of the bladder neck and the urethra. The pressure rise is sustained until the bladder is empty.[1]

The function of the lower urinary tract can be evaluated in a cystometric study.[2] In such a study, intravesical pressure is measured during controlled bladder filling and during voiding. The pressure that represents bladder function is that generated by the detrusor muscle. This so-called detrusor pressure is estimated by subtraction of the rectal pressure, which is used as a measure of intra-abdominal pressure, from the intravesical pressure. The flow rate during voiding is measured with a flowmeter. Additionally, pelvic floor activity is usually registered by means of electromyography.

Neurology of the micturition cycle

Despite the fact that the function of the lower urinary tract can, apart from the pelvic floor activity and the degree of continence, be evaluated in simple mechanical terms: pressure, flow rate and volume, the underlying neurologic mechanisms are complicated and not completely understood.[3] The spinal segments Th10-L2 and S2-S4 play a role in the micturition cycle.[1] The sympathetic hypogastric nerves and the parasympathetic pelvic nerves originate from these two groups of segments and innervate the bladder and the urethra. Ascending and descending pathways connect the segments with several higher structures in the central nervous system.[1]

Basic properties that define lower urinary tract function in the storage phase of the micturition cycle are compliance and continence. It is uncertain if the compliance of a bladder can entirely be ascribed to the viscoelastic properties of bladder wall structures or if the sympathetic nervous system exerts some additional relaxing influence.[3] Continence is normally maintained at the bladder neck, which is innervated both sympathetically and parasympathetically.[4] In the male, a smooth muscle structure, known as the internal sphincter, can anatomically be recognized in this part of the urinary tract. In the female such a structure is not found. In both sexes the urethra contains within its walls the rhabdosphincter, also known as the external sphincter. The striated muscle fibres which constitute this sphincter are different from those in the pelvic floor, among other things

because they are all of the slow twitch type. The sphincter is thus capable of sustained contraction over relatively long periods of time and contributes to the maintenance of continence.[4] The somatic innervation of the rhabdosphincter is currently under discussion.[4] According to the classical description it is innervated by the pudendal nerves. There is also evidence, however, that the innervation occurs via the pelvic nerves.[4] The medial (periurethral) parts of the levator ani provide an additional occlusive force on the urethral wall.[5] This muscle, which is innervated by the pudendal nerves, consists of an admixture of fast and slow twitch fibres and, unlike the rhabdosphincter, possesses the morphologic features of typical voluntary muscles. The slow twitch fibres are responsible for the maintenance of tone of the pelvic floor. The fast twitch fibres are mainly activated during events which cause intra-abdominal pressure elevation, such as coughing.[5]

During the voiding phase of the micturition cycle the function of the constituting parts of the lower urinary tract is reversed: the bladder wall generates pressure and the structures normally providing continence relax to allow urinary flow. This reversal is achieved by a coordination of reflexes.[3] In the cat, Barrington distinguished seven reflexes associated with micturition. Not all of these could be identified in man. The reflexes differ in the stimulus involved (bladder or urethral distension, flow through the urethra), the nervous pathway and the elicited response (bladder contraction or urethral relaxation). In urodynamic studies in anaesthetized animals micturition is usually provoked by filling the bladder. According to Barrington's first, fifth and sixth reflex, this induces bladder contraction, rhabdosphincter relaxation and relaxation of urethral smooth muscle, respectively. Normally, micturition is initiated voluntarily, emphasizing the role of the cerebral cortex.

The course of micturition

In a simple mechanistic approach, micturition can be considered as the result of modulation of the mechanical system consisting of the bladder and the urinary outflow tract by its neurogenic control mechanisms. The course of micturition is determined by the properties of the mechanical system, which can conceptually be described in terms of bladder contractility and urethral resistance, and the extent to which these properties depend on the intensity of neurogenic stimulation. By definition, the contractility of a muscle is its ability to develop force (or pressure) and/or to shorten. As a consequence, bladder contractility can be expressed in terms of detrusor pressure and/or flow rate or, alternatively, shortening velocity of the bladder circumference. Urethral resistance is reflected by the pressure head needed to drive a given flow rate through the urethra. Because, as stated, bladder contractility and urethral resistance determine the course of micturition and these two concepts both involve detrusor pressure and urinary flow rate, it follows that the course of a given voiding in a urodynamic examination can best be

studied by analysis of the resulting pressure/flow relationship.[6] Information on the properties of bladder and urethra, i.e., contractility and resistance, however, cannot straightforwardly be derived from this relationship. Methods have therefore been developed to estimate parameters that quantify these properties from the measured pressure and flow rate data.[6]

Aim of the present study

The mechanical properties of the lower urinary tract have been studied extensively and are well-known.[6] Those of particular importance to the voiding phase of the micturition cycle are bladder contractility and urethral resistance. As described in the preceding sections, these two properties are modulated by neurologic factors. For a complete understanding of micturition it is therefore necessary to know the time course of the nerve signals controlling bladder contractility and urethral resistance and how these signals affect those properties. It was the aim of the present study to examine the latter, i.e. the dependence of bladder contractility and urethral resistance on neurogenic stimulation. The guinea pig was used as a model. This animal is considered a good model in urodynamic studies, even under anaesthesia.[7] Part of the experiments was done in vitro. Electrical stimulation was then used to mimic neurogenic stimulation.

References

1. *Mundy, A.R. and Thomas, P.J.: Clinical physiology of the bladder, urethra and pelvic floor. In: Urodynamics: principles, practice and application, 2nd edn. Edited by A.R. Mundy, T.P. Stephenson and A.J. Wein. New York: Churchill Livingstone, chapt.2, 1994.*
2. *Blaivas, J.G.: Techniques of evaluation. In: Neurourology and Urodynamics: principles and practice. Edited by S.V. Yalla, E.J. McGuire, A. Elbadawi and J.G. Blaivas. New York: Macmillan Publishing Company, chapt.10, 1988.*
3. *Morrison, J.F.B.: Reflex control of the lower urinary tract. In: The physiology of the lower urinary tract. Edited by M. Torrens and J.F.B. Morrison. London: Springer-Verlag, chapt.7, 1987.*
4. *Dixon, J. and Gosling, J.: Structure and innervation in the human. In: The physiology of the lower urinary tract. Edited by M. Torrens and J.F.B. Morrison. London: Springer-Verlag, chapt.1, 1987.*
5. *Dixon, J.S. and Gosling, J.A.: The anatomy of the bladder, urethra and pelvic floor. In: Urodynamics: principles, practice and application, 2nd edn. Edited by A.R. Mundy, T.P. Stephenson and A.J. Wein. New York: Churchill Livingstone, chapt.1, 1994.*

6. *Griffiths, D.J.: Hydrodynamics and mechanics of the bladder and urethra. In: Urodynamics: principles, practice and application, 2nd edn. Edited by A.R. Mundy, T.P. Stephenson and A.J. Wein. New York: Churchill Livingstone, chapt.5, 1994.*
7. *Mostwin, J.L., Karim, O.M.A., van Koeveringe, G. and Seki, N.: Guinea pig as an animal model for the study of urinary bladder function in the normal and obstructed state. Neurourol.Urodyn. 13: 137-145, 1994.*

Chapter 2

Comparison of detrusor contractility of guinea pig bladders in situ and strips from these in vitro

J. Groen, E. van Asselt, R. van Mastrigt, R. Bosch, G.A. van Koeveringe

The Journal of Urology 150: 1002-1006, 1993

Abstract

To study the relative importance of neurogenic factors in detrusor contractility, active bladder wall stress values were compared in situ and in vitro. Eight male guinea pigs were used. The active stress in the bladder wall in spontaneous micturition contractions was calculated from the results of urodynamic examinations and compared with the active stress developed in response to optimum electrical stimulation in full thickness bladder wall strips taken from the same bladders. The results indicated that, in normal micturition, the detrusor muscle is not fully stimulated, and the rate of pressure development is not determined by mechanical factors.

To identify topological variations of detrusor contractility, the strips were taken from three different locations. It was found that strips from the posterior wall contracted more forcefully than those from the anterior wall.

Introduction

Factors determining the course of micturition include the contractile properties of the detrusor muscle. In the literature, myogenic and neurogenic aspects of contractility are discriminated.[1] Most often, these terms are used in the context of stimulatory pathways: neurogenic pathways involve neurotransmitters, whereas myogenic pathways are supposed to bypass receptor mechanisms. Experimentally both pathways are usually discriminated using tetrodotoxin (TTX). Eventually both pathways converge to the same contractile elements in the smooth muscle cells, producing mechanical contraction. Conceptually, therefore, the pathways can be modelled as in Fig.1.

Clinical methods developed to quantify bladder contractility[2-5] are based on detrusor contractions, thus involving the neurogenic pathway. These methods therefore measure a combination of properties related to the neurotransmitters, receptors and so forth and (mechanical) properties of the smooth muscle cells. This combination of (mechanical) properties of the detrusor muscle and properties of the neurogenic pathway, as determined in in vivo bladder contractions, will be called neurogenically modulated contractility. The

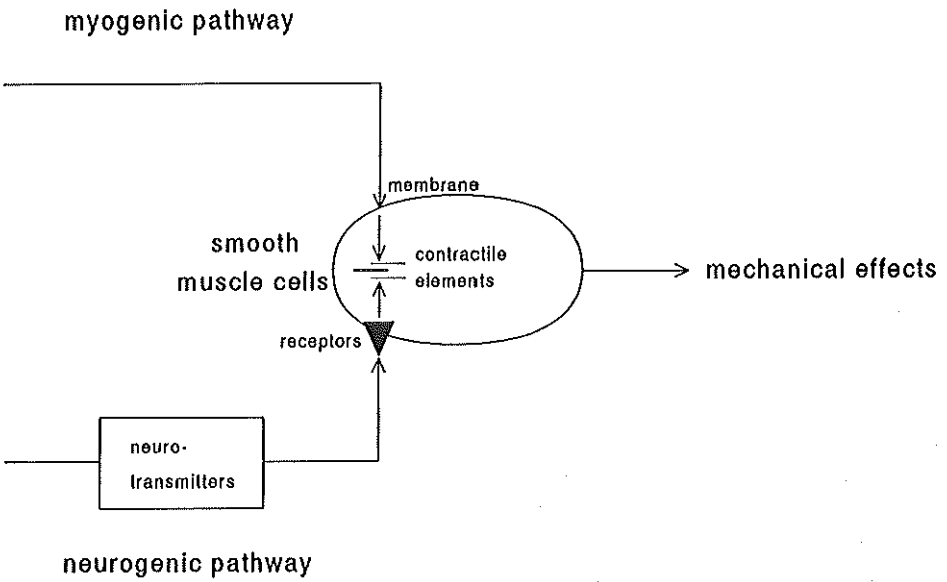


Figure 1. Schematic representation of stimulatory pathways. The contractile properties of the smooth muscle cells, when fully stimulated (via either pathway), are referred to as mechanical contractility. In vivo, only the neurogenic pathway is involved. Contractile properties measured in vivo are therefore referred to as neurogenically modulated contractility.

properties of the maximally activated smooth muscle cells, as determined in vitro, are considered to represent the mechanical properties of these cells and will be called mechanical contractility. As it is unknown to what extent the detrusor smooth muscle cells are stimulated in a normal micturition contraction, it is impossible to draw conclusions about the mechanical contractility of the detrusor muscle from clinical measurements. A way to relate neurogenically modulated to mechanical contractile properties is to compare the results of urodynamic examinations of patients with the results of force and/or contraction velocity measurements on bladder biopsies. At present this invasive procedure does not seem to be justified. In animal studies, however, it is possible to compare measures of the contractility of the bladder in a micturition contraction with measures of the contractility of full thickness bladder wall strips under conditions of optimum stimulation. The comparison of the (neurogenically modulated) contractility measured in vivo with the contractility thus measured in vitro permits estimation of the intensity of the neurogenic stimulation, and forms the subject of the present study. Maximum active stress was taken as a measure of contractility.

Ghoniem et al.[6] showed topological variations of the maximum active stress in rabbit bladders. Such variations might exist in the guinea pig bladder as well. For an appropriate comparison it was necessary, therefore, to use and compare measurements from strips of different parts of the bladder.

Materials and Methods

Eight male guinea pigs with a mean weight of 992 g (range: 784 to 1335 g) were used. Each animal was anaesthetized with urethane (1.2 g/kg intraperitoneally). A small incision was made in the abdominal wall to localize the bladder, into which a 24 G angiocatheter was inserted. Subsequently, the abdomen was loosely closed with silk sutures. Body temperature was maintained by means of a heating pad.

A Harvard infusion pump was used to fill the bladder via the catheter with room temperature saline at a rate of 0.53 ml/min. Pressure was measured via the same catheter using a Statham/Gould pressure transducer (Gould Inc., Statham Instruments Division, Oxnard, California). The pressure signal was recorded on a strip chart recorder and, in 7 animals, it was sampled at a rate of 10 Hz by a PDP 11 type computer using the program CLIM.[7] Episodes of 25 seconds of intravesical pressure signal just prior to the moment of spontaneous micturition were stored. The start of micturition was defined as the moment when urine emerged from the urethra. Residual urine after voiding was removed with a syringe attached to the catheter. The micturition cycle was repeated 5 to 7 times, with intervals of 10 to 15 minutes.[8,9] In each animal the maximum active pressure (defined as the maximum pressure in a micturition contraction minus the pressure just before that contraction) in consecutive voiding cycles was normalized to the value found in the first micturition contraction. The same was done for the bladder

volume. The largest bladder volume in each animal at which micturition occurred was taken as its cystometric capacity.

Assuming a spherical shape of the bladder[10] maximum active stress σ in the bladder wall due to the micturition contraction was calculated from the maximum active pressure P_{mc}, the bladder volume V at the onset of micturition and the tissue volume V_t of the bladder, using the formula $\sigma=P_{mc}/((1+V_t/V)^{2/3}-1)$. The derivation of this formula is based on a balance of forces on two hypothetical hemispheres. $P_{mc}\pi r_i^2$, the active force trying to separate these hemispheres, is balanced by the active force $\sigma\pi(r_o^2-r_i^2)$ produced by the bladder wall; r_i and r_o are the inner and the outer radius of the filled bladder, respectively.

To prepare strips for the in vitro measurements the bladder was filled once more with a volume corresponding to the mean of the previously infused volumes. At three locations, the mid-ventral part of the anterior wall, the part of the posterior wall near the bladder dome and the part of the posterior wall just above the trigone, longitudinal strips of equal length were marked with 0.7 metric silk suture. After ligating both ureters and the urethra, the bladder was excised and kept at room temperature in a buffer solution of the following composition: NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.0; $NaHCO_3$ 8.0; HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) 10.5 and glucose 5.1 mmol/l; NaOH was added to adjust the pH to 7.4.

Full thickness bladder wall strips were dissected from the three previously marked locations. After the unloaded length had been measured, the strips were suspended in one organ bath containing 15 ml of the same buffer solution, which was equilibrated with 95% O_2 - 5% CO_2 at a temperature of 37°C. The pH was in the range 7.25 to 7.45 and the osmolality was close to 300 mOsm. The solution could be refreshed within a few seconds from a heated and aerated storage container; this was done after each stimulation. Each strip was attached at one end to a glass rod fixed to the bottom of the bath, and at the other end to a Grass force transducer (Grass Instruments, Quincy, Massachusetts) (compliance 0.002 m/N) with 0.7 metric silk thread. The strips were prestretched to their in situ length and allowed to accommodate for one hour before the onset of the experiments.

Subsequently, the strips were stimulated electrically for 4 seconds at intervals of 10 to 15 minutes by field stimulation, using two parallel platinum electrodes, one on each side of the set of three strips. To avoid electrolysis of the buffer solution, bidirectional pulse stimulation was used. All isometric contractions were sampled at a rate of 10 Hz with the PDP 11 computer and stored for further analysis. Phase plots, that is plots of the rate of change of force as a function of force, were made. From these the parameters F_{iso}, the maximum extrapolated force, and C, the time constant for isometric force development,[11] were derived, as well as the maximum rate of change of force.

To find optimum stimulus conditions, the pulse height, frequency and width were varied, starting from 10 V, 100 Hz and 5 ms, respectively, values based on earlier work in our laboratory.[12] In four sets of strips the applied voltage was alternated between 10 V

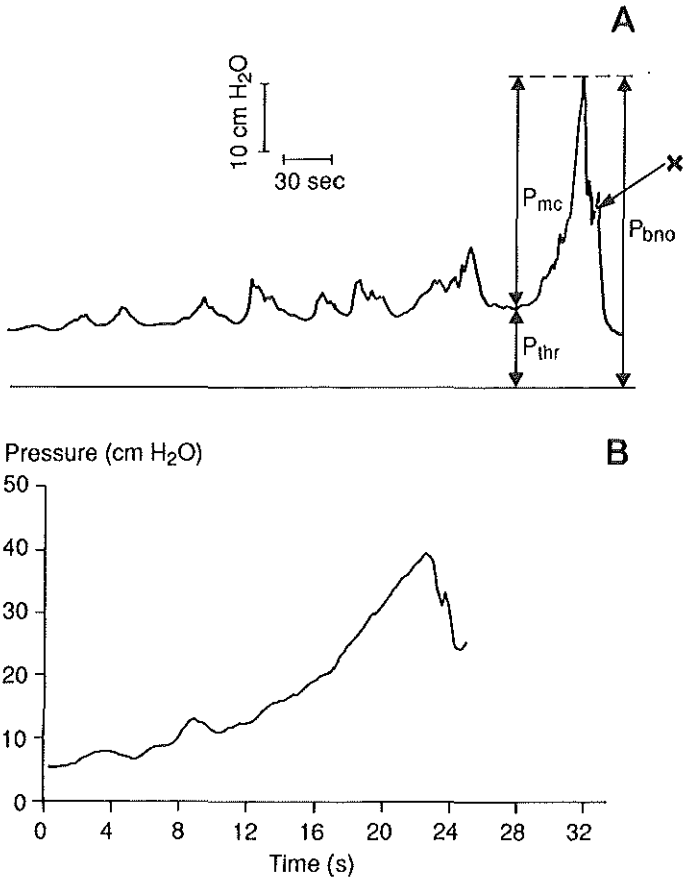


Figure 2. A: Typical cystometrogram tracing from a urethane-anaesthetized guinea pig. P_{thr} : intravesical pressure corresponding to the threshold level of the micturition reflex contraction. P_{mc} : active pressure rise due to this contraction. P_{bno} : bladder neck opening pressure. $P_{bno}=P_{thr}+P_{mc}$. At the point marked X micturition started. B: Corresponding example of the intravesical pressure signal as a function of time in a 25 second episode preceding micturition as stored by the computer. The time interval between the maximum pressure and the onset of micturition probably reflects the flow of urine through the urethra. Note the different time and pressure scales.

and 5, 7.5, 12.5, 15 or 20 V in a random order; the frequency and width were held constant. In three sets of strips the frequency was alternated between 100 Hz and 10, 25, 50, 200, 400 or 1000 Hz; the pulse height was held at 10 V. The pulse width was adapted to the frequency to attain a duty cycle of 50%. Previous experiments have shown that the contraction force in pig urinary bladder muscle strips was maximal at this value.[12] The F_{iso} values found at the various parameter settings were normalized by dividing them by the value measured at 10 V, 100 Hz, 5 ms. Measurements taken at the optimum parameter settings were used for further analysis.

After the experiments the strips were blotted and weighed. The cross-sectional area at the in situ length, that is, the length marked with sutures, was calculated, assuming a density of 1.0 g/ml. Maximum active stress, defined as the maximum stress in an electrically induced contraction minus the resting level stress, was calculated by dividing F_{iso} by the cross-sectional area.

The results from the in vivo experiments were compared statistically with the in vitro experiments using the two-tailed t test. The differences between the strips taken from the three locations were checked for significance using repeated measures analysis of variance and the Scheffé multiple comparison test.

Results

Micturition occurred in 7 animals. The measured cystometrograms (Fig.2A is an example), mostly showing a series of low-amplitude pressure waves preceding micturition and a biphasic pattern of the micturition contraction itself, were comparable to those described in the literature.[8,9] P_{thr} is the intravesical pressure corresponding to the threshold level of the micturition reflex contraction. Upon exceeding this threshold, detrusor contraction was triggered, and detrusor pressure rose with P_{mc} until the bladder neck opening pressure P_{bno} was reached and evacuation of the bladder started. An example of the intravesical pressure signal in the episode of 25 seconds just prior to micturition is shown in Fig.2B. The time difference between the maximum pressure and the onset of micturition probably resulted from the passage of urine (or saline) through the urethra.

From Fig.3 it appears there is a trend to lower bladder volumes during consecutive micturition cycles. The Spearman rank correlation coefficient between the normalized volume and the number of the micturition cycle was -0.89 ($p=0.003$). For the active pressure there also was a trend: $r=-0.75$ ($p=0.026$). The significance of these trends was assessed using the paired t test. For the active pressure there were no significant differences between any two micturition cycles at the 0.05 level. For the bladder volume in only one case a significant difference was found. These results indicate that the used time interval of 10 to 15 minutes was sufficient to obtain reproducible cystometric data. This observation is in agreement with data published by other investigators.[8,9]

In none of the animals was evacuation of the bladder complete. Neglecting diuresis,

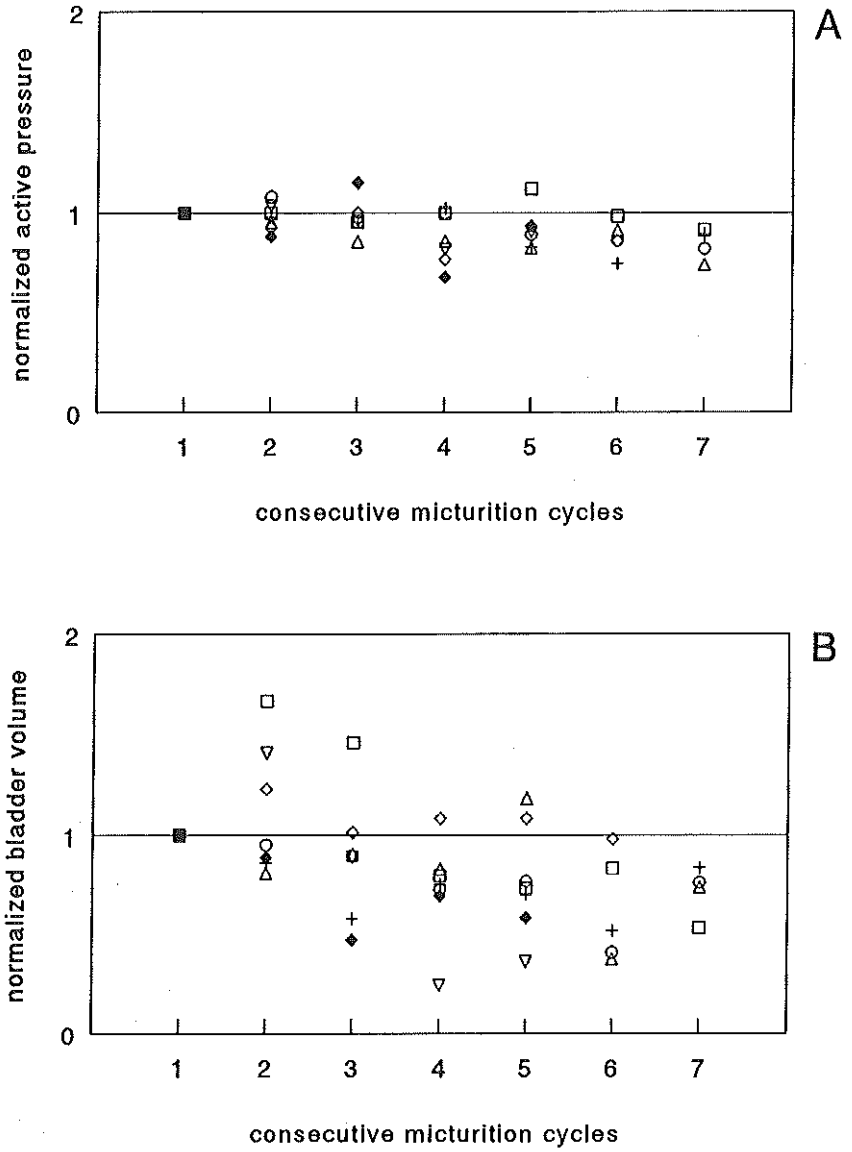


Figure 3. Maximum active pressure (A) and bladder volume (B) in consecutive micturition cycles in each guinea pig. For each separate animal both parameters were normalized to the value found in the first micturition contraction. Each symbol represents the results from one animal.

the residual volume was expressed as a percentage of the infused volume. In addition to the bladder weights, the results from the measurements in vivo are given in Table 1. For the maximum active pressure, the generated active stress in the bladder wall and the maximum rate of change of active stress during a micturition contraction, the three contractions in which the highest stress was achieved were used.

Table 1. The bladder weight of the eight animals and the results from the cystometries (N=7).

Bladder wet weight (mg)	779 ± 35
Cystometric capacity (ml)	3.6 ± 0.3
Residual volume (%)	48 ± 5
P_{mc} (cm H_2O)	23 ± 3
σ (N/cm^2)	1.50 ± 0.19
$(d\sigma/dt)_{max}$ (N/cm^2/s)	0.30 ± 0.04

The cystometric capacity of each animal was defined as the largest bladder volume at which spontaneous micturition occurred. The residual volume, given as a percentage of the infused volume, is the mean value of all micturitions. P_{mc} (the maximum active intravesical pressure), σ (the maximum active stress in the bladder wall) and $(d\sigma/dt)_{max}$ (the maximum rate of change of active stress; N=6: in one animal the pressure signal was not stored by computer) are the averaged results from the three contractions with the highest active stress. Mean values and standard errors of the means are given.

From Fig.4, which shows the pulse height and pulse frequency dependence of F_{iso} and hence of the active stress in the strips, it was concluded that 10 V, 100 Hz, 5 ms corresponded to optimum stimulus conditions.

During the first hour of measurement, the force exerted by the strips increased with repeated stimulations. Only after one hour of stimulation were reproducible values for the force obtained. To verify this, multiple application of the paired t test was carried out after the strips had been attached for more than two hours. The smallest p value obtained was 0.082, indicating that the force developed by each strip was reproducible. Therefore, the parameters studied were taken from the averaged values of the stimulations after more than two hours.

The characteristics of the strips and the results from the in vitro measurements are listed in Table 2. The p values given resulted from repeated measures analysis of variance and are the significance levels of the differences in the variables with respect to the bladder location. The Scheffé multiple comparison test was performed to identify the variables which are significantly different from the others. Both the maximum active

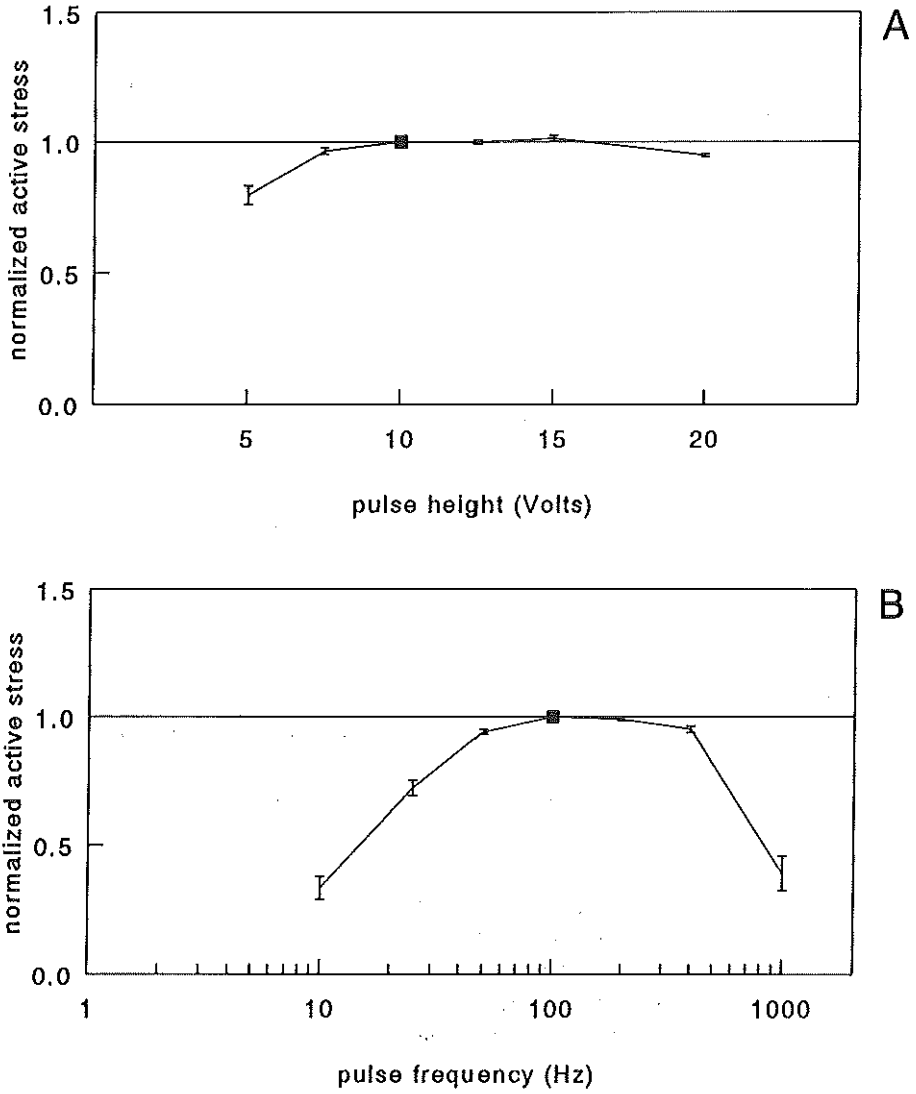


Figure 4. Optimization of parameter settings for electrical stimulation. A: Pulse height dependence of active stress. The active stress was normalized to the value at 10 V. The pulse frequency was 100 Hz; the pulse width was 5 ms (duty cycle 50%). Averaged results from 12 strips taken from 4 animals. B: Pulse frequency dependence of active stress. The active stress was normalized to the value at 100 Hz. The pulse height was 10 V; the duty cycle was 50%. Averaged results from 9 strips taken from 3 animals. The error bars represent the standard errors of the mean.

stress developed by a strip and the maximum rate of change of active stress were dependent on the location the strip was taken from.

Table 2. The characteristics of the bladder wall strips and the results from the electrical stimulation experiments (N=8).

	Anterior Bladder Wall Strip	$Posterior_{dome}$ Bladder Wall Strip	$Posterior_{trig}$ Bladder Wall Strip	p
Weight (mg)	49 ± 7	26 ± 5	23 ± 5	0.001*
L (mm)	8.6 ± 0.5	8.0 ± 0.4	8.2 ± 0.5	0.38
L_0 (mm)	6.1 ± 0.2	5.4 ± 0.2	4.7 ± 0.3	0.008**
L_0/L	0.72 ± 0.05	0.69 ± 0.04	0.58 ± 0.03	0.023
F_{iso} (mN)	75 ± 16	74 ± 8	88 ± 13	0.39
C (s)	0.83 ± 0.06	0.80 ± 0.04	0.93 ± 0.06	0.093
σ (N/cm^2)	1.50 ± 0.30	2.50 ± 0.25	3.69 ± 0.52	<0.0005**
$(d\sigma/dt)_{max}$ ($N/cm^2/s$)	0.96 ± 0.17	1.69 ± 0.17	2.11 ± 0.34	0.002**

*L and L_0 are the in situ length and the unloaded length of the strips, respectively. F_{iso} and C are the maximum extrapolated force and the time constant for isometric force development as derived from phase plots. The maximum active stress and the maximum rate of change of active stress are denoted by σ and $(d\sigma/dt)_{max}$. Strips from the mid-ventral part of the anterior wall, the part of the posterior wall near the bladder dome and the part of the posterior wall just above the trigone are denoted by anterior, $posterior_{dome}$ and $posterior_{trig}$, respectively. Mean values and standard errors of the mean are given; the p values resulted from repeated measures analysis of variance, and represent the significance levels of the differences in the variables in relation to the bladder location. In addition to this statistical procedure the Scheffé multiple comparison test was performed. The symbols * and ** denote the results.*

* *Parameter of the anterior wall strips is significantly different from parameters of the strips from both posterior wall locations.*

** *Parameter of the anterior wall strips is significantly different from parameter of the strips from the location above the trigone.*

The maximum active stress generated in the bladder wall during a micturition contraction and its maximum rate of change are compared with the same variables measured in the bladder wall strips in response to optimum electrical stimulation in Table 3. The p values resulted from the two-tailed t test. The differences between the rates of

change of active stress were larger than the differences between the active stress values themselves.

Table 3. Comparison of the maximum active stress (N=7) and the maximum rate of change of active stress (N=6) in the bladder wall in a micturition contraction with the same variables in strips from the same bladders.

	Bladder Wall in Situ	Anterior Bladder Wall Strip	$Posterior_{dome}$ Bladder Wall Strip	$Posterior_{trig}$ Bladder Wall Strip
σ (N/cm^2)	1.50 ± 0.19	1.32 ± 0.29 p=0.697	2.54 ± 0.28 p=0.062	3.60 ± 0.59 p=0.020
$(d\sigma/dt)_{max}$ ($N/cm^2/s$)	0.30 ± 0.04	0.97 ± 0.20 p=0.029	1.78 ± 0.21 p=0.002	2.31 ± 0.38 p=0.005

Anterior, $posterior_{dome}$ and $posterior_{trig}$ denote the same as in Table 2. Mean values and standard errors of the mean are given. The p values resulted from the two-tailed t test, and give the significance of the difference between the value measured in situ with that measured in vitro.

Discussion

In seven of the eight animals used, spontaneous micturition occurred. In some animals micturition did not occur until the abdominal wall was (loosely) closed again. It is hypothesized that in an open abdomen the bladder is shifted ventrally, thereby pinching off the bladder neck and proximal urethra. The closing of the abdomen restores the geometry, making a micturition reflex possible. In the one animal that did not void this may have been achieved to an insufficient degree, or alternatively its bladder was perhaps not filled to capacity.

Evacuation of the bladder started when the intravesical pressure equaled the bladder neck opening pressure, that is, when $P_{thr}+P_{mc}=P_{bno}$. So apart from the term related to the threshold of the micturition reflex, the maximum intravesical pressure rise measured equaled the bladder neck opening pressure. As there seems to be no reason for the bladder outlet resistance to change in consecutive micturition contractions, this might explain why the pressure rise reproduced fairly well. Large individual differences between the animals were found in the parameters determined from the cystometries. The maximum active pressure even varied by a factor of 3. Such large differences in urodynamic parameters were also found in patients in whom no abnormalities were

detected during urodynamic examinations.[13]

Because the active isometric force developed by strips is length dependent,[12,14,15] strips were prestretched to the in situ length to permit an appropriate comparison of the generated stress. The active stress values shown in Table 2 are in agreement with those found by Uvelius.[10] In strips from 4 female guinea pigs weighing 550 to 600 g, he measured a maximum active stress of 1.67 $\pm$ 0.15 (SEM) N/cm^2 on chemical stimulation at optimum length. These strips were taken from unspecified bladder locations.

Table 2 shows that the active stress measured at different locations in the bladder differed significantly; the stress in the anterior wall was smaller than the one in the posterior wall. One reason for this could be a lower density of smooth muscle tissue in the anterior wall. A provisional microscopic study of cross-sections of used strips did not show this. A different ratio of contractile elements to noncontractile elements in different parts of the bladder body is not known to us either. Also, the far greater mean weight of the anterior wall strips cannot explain the smaller stress values as, for a constant length, stress, unlike force, is independent of strip weight. Perhaps the topological variation of active stress can be explained by the fact, demonstrated by Gabella, that the posterior wall of the guinea pig bladder contains more neurons than the anterior wall[16]: previous experiments showed that at pulse widths of 5 ms a large part of the electrically induced response can be blocked by atropine, indicating the importance of neurotransmitters (acetylcholine).[11]

Although there were no significant differences in the in situ lengths L, that is, the prestretched lengths, of the strips, such differences did occur in the lengths L_0 of the unloaded strips. Because the unloaded length is partly determined by spontaneous contractions during dissection prior to mounting of the strips, this might reflect differences in the contractile properties of the different parts of the bladder. Ghoniem et al. found a topological variation of the active stress in rabbit bladder.[6] In rings selected from three different bladder zones, the upper body, the lower body and the base, a decrease in the active stress was observed in the direction from the upper body towards the base. The authors suggested that this attribute of the bladder contributes to its evacuating function. The fact that in the guinea pig bladder the location dependence of the active stress seems to run in another direction could perhaps be associated with the different bladder form: the guinea pig bladder is almost spherical, whereas the rabbit bladder is slightly elongated.[10] It is, however, hard to imagine how in this case it would contribute to the evacuating function.

Table 3 shows that the maximum active stress measured in the bladder in situ was comparable in magnitude with the maximum active stress in strips from the anterior wall. Compared with the stress in strips from the posterior wall, maximum active stress in vivo was rather small; for strips from the location just above the trigone the difference was significant. In comparing in vivo and in vitro stress values, it should be kept in mind that a micturition contraction is not an isometric contraction. In such a contraction the stress would have been higher: as no active shortening of the detrusor muscle takes place the

exerted force is higher in accordance with the force-velocity curve. Furthermore, in a micturition contraction the maximum intravesical pressure equals the bladder neck opening pressure; higher pressure values are not attained. These considerations are confirmed by the results of Peterson et al.[9]: in guinea pigs with tied urethral catheters a significantly higher maximum intravesical pressure rise was measured than in animals in whom urethral catheters were allowed to leak. Consequently, the stress value given in Table 3 for the bladder wall in vivo represents the lower margin of the value that could have been attained. (The drawback of studying isometric contractions is the possible inhibition of the micturition reflex,[17] causing unknown effects on the cystometric data. Stop-flow tests constitute an alternative. However, in such tests the pressure is measured at volumes smaller than capacity).

As described in the introduction, the maximum active stress was considered a measure of the neurogenically modulated contractility of the detrusor muscle, that is, it is influenced by both neurogenic and myogenic factors. In vitro, contractile responses were measured in response to electrical stimulation. Optimum electrical stimulation parameters for the strips were in general agreement with those found by van Mastrigt and Glerum on pig bladder strips.[12] With optimum parameters, electrically induced contractile responses are comparable in magnitude with potassium chloride induced responses and thus can be considered to represent maximal (mechanical) contractility.[11]

From Table 3 it can be concluded that, in a micturition contraction, detrusor contractility is smaller than in vitro; in other words, during micturition the bladder is not fully stimulated. A possible explanation for this difference is the fact that the in vivo results were measured in urethane-anaesthetized animals. However, Peterson et al. observed that the maximum active pressure was not significantly different in urethane-anaesthetized and conscious animals.[9] They also observed that, in conscious animals, bladder capacity was significantly smaller. This shows that the micturition cycle is affected by urethane, despite the fact that this anaesthetic is known to impair the micturition reflex only marginally.[18] The use of urethane might explain the large, probably nonphysiological amount of residual urine. As the micturition contractions were short-lived (see Fig.2), the residue is probably caused by an insufficient duration of the micturition contraction rather than by insufficient urethral relaxation.

Apart from the difference in intensity of stimulation, the development of active stress in time was also much slower in the bladder wall in vivo than in the electrically stimulated bladder wall strips (Table 3). In the strips, the time constant that characterizes the rate of rise of force was approximately 0.85 seconds (Table 2). From the cystometrograms it was estimated that the same time constant in vivo was 3 to 5 seconds. This large difference indicates that the rate of pressure development in vivo is not determined by mechanical factors.

It is concluded that, during micturition, the guinea pig detrusor muscle is not fully stimulated. Contractile force rises much slower in vivo than in electrically induced responses in vitro. Our results support the hypothesis that, in the patient without

neurogenic abnormalities, clinical methods for deriving contractility parameters from pressure-flow rate data estimate a combination of properties of the neurogenic stimulation and mechanical properties of the detrusor muscle. Comparative studies using whole bladder preparations, in which functional integrity is conserved, are indicated for further investigation. There is a significant topological variation in the contractility of the guinea pig bladder wall; in the posterior wall it is higher than in the anterior wall.

References

1. *Brading, A.F., Mostwin, J.L., Sibley, G.N.A. and Speakman, M.J.: The role of smooth muscle and its possible involvement in diseases of the lower urinary tract. Clin.Sci., suppl.14, 70: 7s-13s, 1986.*
2. *Griffiths, D.J., Constantinou, C.E. and van Mastrigt, R.: Urinary bladder function and its control in healthy females. Am.J.Physiol. 251: R225-R230, 1986.*
3. *Kaplan, S.A., Blaivas, J.G., Brown, W.C. and Schuessler, G.: Parameters of detrusor contractility: The effect of bladder volume and outlet resistance on Qmax, power, and work in an in vitro whole rabbit model. Neurourol.Urodyn. 8: 375-376, 1989.*
4. *van Mastrigt, R.: Application of animal and physical models to human cystometry. In: Micturition. Edited by J.O. Drife, P. Hilton and S.L. Stanton. London: Springer-Verlag, chapt.5, pp.59-79, 1990.*
5. *van Mastrigt, R.: Estimation of contractility parameters for the urinary bladder. Neurourol.Urodyn. 10: 37-45, 1991.*
6. *Ghoniem, G.M., Regnier, C.H., Biancani, P., Johnson, L. and Susset, J.G.: Effect of vesical outlet obstruction on detrusor contractility and passive properties in rabbits. J.Urol. 135: 1284-1289, 1986.*
7. *van Mastrigt, R.: A computer program for on-line measurement, storage, analysis and retrieval of urodynamic data. Comp.Prog.Biomed. 18: 109-117, 1984.*
8. *Maggi, C.A., Meli, A. and Santicioli, P.: Neuroeffector mechanisms in the voiding cycle of the guinea-pig urinary bladder. J.Auton.Pharmacol. 7: 295-308, 1987.*
9. *Peterson, J.S., Hanson, R.C. and Noronha-Blob, L.: In vivo cystometrogram studies in urethane-anesthetized and conscious guinea pigs. J.Pharmacol.Methods 21: 231-241, 1989.*
10. *Uvelius, B.: Relation between mechanical and morphological characteristics in urinary bladder smooth muscle. Acta Physiol.Scand., suppl. 483: 5-51, 1980.*
11. *van Koeveringe, G.A. and van Mastrigt, R.: Excitatory pathways in smooth muscle investigated by phase-plot analysis of isometric force development. Am.J.Physiol. 261: R138-R144, 1991.*
12. *van Mastrigt, R. and Glerum, J.J.: Electrical stimulation of smooth muscle strips from the urinary bladder of the pig. J.Biomed.Eng. 7: 2-8, 1985.*

13. *van Venrooij, G.E.P.M., Boon, T.A. and Vervest, H.A.M.: Urodynamic findings in the lower urinary tract: I. Stress-incontinent vs. -continent women. Neurourol. Urodyn. 9: 97-107, 1990.*
14. *Griffiths, D.J., van Mastrigt, R., van Duyl, W.A. and Coolsaet, B.L.R.A.: Active mechanical properties of the smooth muscle of the urinary bladder. Med.Biol.Eng. Comput. 17: 281-290, 1979.*
15. *van Mastrigt, R.: The length dependence of the series elasticity of pig bladder smooth muscle. J.Muscle Res.Cell Motil. 9: 525-532, 1988.*
16. *Gabella, G.: Intramural neurons in the urinary bladder of the guinea-pig. Cell Tissue Res. 261: 231-237, 1990.*
17. *Morrison, J.F.B.: Reflex control of the lower urinary tract. In: The physiology of the lower urinary tract. Edited by M. Torrens and J.F.B. Morrison. London: Springer-Verlag, chapt.7, pp.209-212, 1987.*
18. *Maggi, C.A., Santicioli, P. and Meli, A.: The nonstop transvesical cystometrogram in urethane-anesthetized rats. J.Pharmacol.Methods 15: 157-167, 1986.*

Chapter 3

Contractility of the guinea pig bladder measured in situ and in vitro

J. Groen, R. van Mastrigt, R. Bosch

Neurourology and Urodynamics 13: 587-595, 1994

Abstract

To study the relative importance of neurogenic factors in detrusor contractility and to relate a total bladder in vitro contractility model to a previously described bladder wall strip model, active intravesical pressure values were compared in situ and in vitro in eight male guinea pigs. In situ, the active pressure was measured in spontaneous isometric and non-isometric micturition contractions. In vitro, the active pressure was measured in isometric contractions of the same bladders, developed in response to optimal electrical stimulation. The volume dependence of the active pressure generated by the bladder was measured in vitro in order to relate bladder capacity to the volume where the generated force is maximal and to determine the optimal volume at which to study detrusor contractility. The results indicated that in normal micturition the detrusor muscle was not fully stimulated: active pressure in isometric contractions in vivo was about 60% of the pressure values attained in vitro at the same bladder volume. Most micturitions occurred at a volume where the active pressure generated in vitro was about 80% of the maximal pressure. The active pressure-bladder volume relationship complied with the sliding filament-cross bridge theory. In whole bladder preparations active stress was about twice as high as in strips.

Introduction

In a previous study we compared the contractility of guinea pig bladders in situ with the contractility of full thickness bladder wall strips taken from the same bladders.[1] The in situ contractions were non-isometric. As these contractions were evoked via a neurogenic pathway, contractility in situ was called neurogenically modulated. In vitro, contractility was evaluated from isometric contractions, induced by optimal electrical stimulation. Maximum active stress was taken as a measure of contractility. It was concluded that in a micturition contraction the bladder is not fully stimulated, i.e., contractility was smaller in the bladder in situ than in the strips. This conclusion was based on the assumptions that the active stress generated by a longitudinal strip corresponds to the active stress generated by the complete bladder and that the intravesical pressure in non-isometric contractions is not significantly smaller than the pressure in isometric contractions.

In this study these assumptions are verified, and measurements are reported on total bladders in vitro under conditions of optimal electrical stimulation. The results are compared with the earlier described results on strips to assess the feasibility of the total bladder in vitro contractility model as opposed to the strip model. A comparison of maximum active pressures in isometric contractions in vivo and in vitro enabled the estimation of the intensity of the neurogenic stimulation in vivo, thereby providing our earlier conclusion with a quantitative basis.

In an in vivo study on guinea pig bladders, Uvelius and Gabella found that the active intravesical pressure, induced by pelvic nerve stimulation, was maximum at volumes which were small with respect to capacity.[2] To verify the myogenic basis of this pattern, the active pressure-bladder volume relationship was determined in four bladders in vitro, thus also yielding the optimum volume at which to study bladder contractility. The resulting data were interpreted in terms of a force-length relationship in accordance with the sliding filament-cross bridge theory of contraction.[3]

Materials and Methods

Eight male guinea pigs with a mean weight of 905 g (range: 710-1145 g) were used. Each animal was anaesthetized with urethane (1.2 g/kg, i.p.). A small incision was made in the abdominal wall to localize the bladder, into which a 24 G angiocatheter was inserted. Subsequently, the abdomen was closed again loosely with silk sutures. Body temperature was maintained by means of a heating pad.

A Harvard infusion pump was used to fill the bladder via the catheter with room temperature saline at a rate of 0.53 ml/min. Pressure was measured via the same catheter using a disposable pressure transducer. The pressure signal was recorded on a strip chart recorder. Each bladder was filled 6 to 10 times, with intervals of 10 to 15 minutes.[4,5] In

alternating filling cycles, the urethra was allowed to leak or occluded in the bulbar part with 3.5 metric silk suture. Thus two kinds of micturition contractions were measured: voiding contractions (when the urethra was allowed to leak) and isometric contractions (when it was occluded). The voided volume was collected and measured. Residual urine after voiding and following an isometric contraction was removed with a syringe attached to the catheter. For the voiding contractions, the residual volume was expressed as a percentage of the volume at the onset of micturition. The largest bladder volume in each animal at which a micturition contraction occurred was taken as its cystometric capacity. In each micturition contraction the maximum active pressure (defined as the maximum pressure during the contraction minus the pressure just before) was measured.

Maximum active stress σ in the bladder wall due to the micturition contraction was calculated from the maximum active pressure P_{mc}, the bladder volume V at the onset of micturition, and the tissue volume V_t of the bladder as $\sigma=P_{mc}/((1+V_t/V)^{2/3}-1)$. The derivation of this formula was described previously.[1]

Following the in vivo experiments, the puncture site in the bladder induced by the catheter was closed by means of a 0.7 metric purse string suture. Both ureters were ligated. The bladder was excised and kept at room temperature in a buffer solution of the following composition: NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.0; $NaHCO_3$ 8.0; HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) 10.5 and glucose 5.1 mmol/l; NaOH was added to adjust the pH to 7.4. After two 5 F catheters had been inserted via the remaining part of the urethra, the bladder was suspended in an organ bath containing 85 ml of the same buffer solution, which was equilibrated with 95% O_2 - 5% CO_2 at a temperature of 37°C. The pH was in the range 7.3 to 7.4 and the osmolality was close to 300 mOsm. The solution could be refreshed within a few seconds from a heated and aerated storage container; this was done after every second stimulation. One catheter was connected to a Hospal infusion pump, the other one to a disposable pressure transducer. The bladder was filled to a volume corresponding to the mean of the previously infused volumes and allowed to accommodate for one hour before the onset of the experiments. Subsequently, the bladder was stimulated electrically for 4 seconds at intervals of 7 to 10 minutes by field stimulation, using two parallel platinum electrodes (20 x 30 mm), one on each side of the bladder. In order to avoid electrolysis of the buffer solution, biphasic pulse stimulation was used. All isometric contractions were sampled at a rate of 10 Hz with a PDP 11 type computer and stored for further analysis. Phase plots, i.e., plots of the rate of change of pressure as a function of pressure, were made. From these the parameters P_{iso}, the maximum extrapolated active pressure, and C, the time constant for isometric pressure development, were derived.[6]

To find optimum stimulus conditions, the pulse height, frequency and width were varied in four bladders, starting from 12.5 V, 100 Hz and 5 ms, respectively, values based on the previously described measurements on strips. First, the applied voltage was semi-randomly varied between 5, 7.5, 10, 12.5, 15, 20, 25 and 30 V; the pulse frequency and width were kept constant. Subsequently, the frequency was varied between

25, 50, 100, 200, 400 and 800 Hz; the pulse height was kept at 12.5 V. The pulse width was adapted to the frequency to attain a duty cycle of 50%. Previous experiments have shown that the contraction force in pig urinary bladder muscle strips was maximum at this value.[7] The P_{iso} values found at the various parameter settings were normalized by dividing them by the value measured at 12.5 V, 100 Hz, 5 ms. Measurements taken at the optimum parameter settings were used for further analysis.

In the remaining four bladders active pressure was measured at optimum parameter settings, while the bladder volume was varied. The active pressure-volume relationship was transformed into an active force-length relationship, taking the bladder inner circumference as the length of the bladder muscle and assuming a spherical shape.[2] Force was calculated by multiplying the pressure by the area enclosed by the inner circumference.

Following the experiments the bladder was blotted and weighed. The volume was calculated, assuming a density of 1.0 g/ml. Maximum active stress values were calculated similarly as in vivo.

In each bladder, active pressure was thus measured under three different circumstances: in voiding contractions in vivo, in isometric contractions in vivo, and in isometric contractions in vitro under conditions of optimal electrical stimulation. The results were compared statistically using repeated measures analysis of variance and the paired t test. Taking into account a possible volume dependence of the measured pressure, only those results were used where all three pressure values in a bladder had been measured at approximately the same volume.

The results of the in vitro experiments were compared with the previously described results of in vitro experiments on bladder wall strips. To this end, the values of parameters measured on the strips were averaged for each bladder. The mean value and the standard error of the mean were calculated from these averaged values. Differences between the parameters determined on strips and on whole bladders were checked for significance using the unpaired two-tailed t test.

Results

The measured cystometrograms were similar to those described previously.[1] In Table 1 parameter values of both studies are compared.

During the first hour of in vitro measurement, the pressure generated by the bladders increased with repeated stimulations. Therefore, the parameters studied were taken from measurements after reproducible pressure values were obtained. Fig.1 shows the pulse height and pulse frequency dependence of P_{iso}. It was concluded that 10 V, 200 Hz, 2.5 ms corresponded with optimum stimulus conditions.

In six bladders active pressure was measured under the three different circumstances (voiding contractions in vivo, isometric contractions in vivo, isometric contractions in

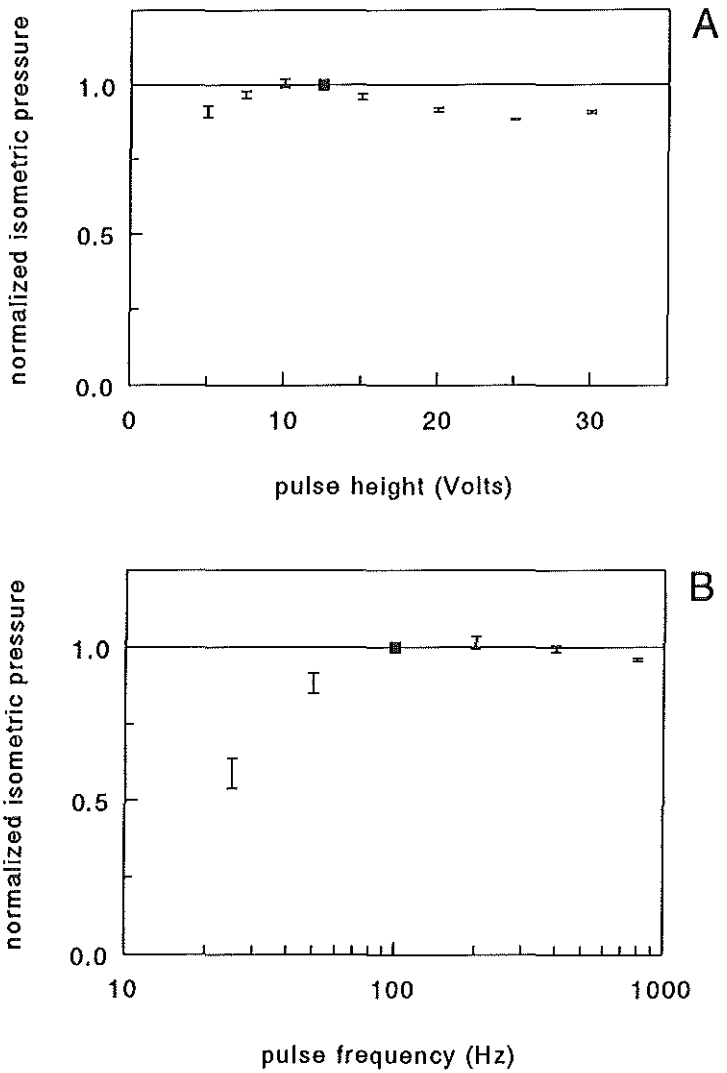


Figure 1. Optimization of parameter settings for electrical stimulation of total guinea pig bladders in vitro. Averaged results of 4 bladders and the corresponding standard errors of the mean. A: Pulse height dependence of extrapolated active pressure. The pressures were normalized to the value at 12.5 V. Pulse frequency was 100 Hz; pulse width was 5 ms (duty cycle 50%). Biphasic pulses were used. B: Pulse frequency dependence of extrapolated active pressure. The pressures were normalized to the value at 100 Hz. Pulse height was 12.5 V; duty cycle was 50%.

Table 1. The bladder weights of the guinea pigs used and the results of cystometries for both the present study and a former study.

	Present study (N=8)	Former study (N=7)
Bladder wet weight (mg)	572 ± 34	779 ± 35
Cystometric capacity (ml)	4.1 ± 0.3	3.6 ± 0.3
Residual volume (%)	38 ± 8	48 ± 5
P_{mc} (cm H_2O)	19 ± 1	23 ± 3
σ (N/cm^2)	1.54 ± 0.24	1.50 ± 0.19

The cystometric capacity of each animal was defined as the largest bladder volume at which a micturition reflex contraction occurred. The residual volume, given as a percentage of the volume at the moment micturition started, is the mean value of all voidings in each animal. P_{mc} (the maximum active intravesical pressure) and σ (the maximum active stress in the bladder wall) are the averaged results from the two voiding contractions with the highest active stress (in the former study P_{mc} and σ were taken from three contractions: in that study more voiding contractions were measured in each animal). Mean values and standard errors of the means are given.

vitro) at similar volumes. Table 2 shows that the pressure values were significantly different, while the corresponding volumes were not.

Table 2. Active pressures at the shown bladder volumes, measured in six bladders under three different circumstances: in voiding contractions in vivo, in isometric contractions in vivo, and in isometric contractions in vitro under conditions of optimum electrical stimulation.

	Voiding contraction in vivo	Isometric contraction in vivo	Isometric contraction in vitro	p
Active pressure (cm H_2O)	19.5 ± 1.0	39.3 ± 2.8	63.2 ± 1.8	<0.0005
Bladder volume (ml)	2.5 ± 0.3	2.8 ± 0.3	2.8 ± 0.2	0.53

Mean values and standard errors of the means are given. The p values resulted from repeated measures analysis of variance. Pairwise application of the t test showed that all three pressure values were significantly different from each other ($p \leq 0.001$).

Table 3 shows the results of the in vitro measurements, taken at volumes similar to the in vivo volumes. It also shows the results from the previously published measurements on bladder wall strips. It can be seen that in the whole bladder preparations maximum active stress exceeded the stress in the strips by almost exactly a factor of 2.

Table 3. The results of electrical stimulation experiments on eight guinea pig bladders in vitro compared with previously described results on strips from eight other bladders.

	Whole bladders	Bladder wall strips	p
V (ml)	2.8 ± 0.2	3.2 ± 0.2	0.27
P_{iso} (cm H_2O)	63.0 ± 1.7	—	—
C (s)	0.91 ± 0.09	0.85 ± 0.04	0.61
σ (N/cm^2)	5.03 ± 0.35	2.56 ± 0.30	<0.0005

In the whole bladder preparations V is the bladder volume; in the strips it is the volume corresponding with the stretched length of the strips. (In these experiments the in situ bladder was filled to the volume V and strips were marked; in vitro the strips were stretched to the marked length). P_{iso} and C are the maximum extrapolated pressure and the time constant for isometric pressure or force development as derived from phase plots. The maximum active stress is denoted by σ. Mean values and standard errors of the means are given; the p values resulted from the unpaired two-tailed t test.

The active pressure, generated by a bladder upon electrical stimulation, was strongly dependent on its volume (Fig.2A). The force-length relationship derived from this data (Fig.2B) shows that active force was maximally 1.60 N at a bladder circumference of approximately 6.5 cm. This corresponds to a volume of 4.6 ml, which is considerably larger than the volume of 2.5 to 2.8 ml at which most micturition contractions in vivo occurred (Table 2).

Discussion

Micturition reflex contractions were studied under two different circumstances: with an unmanipulated urethra and with an occluded urethra. In the first situation the bladder emptied; these micturition contractions were called voiding contractions. When the urethra was occluded, the contractions were called isometric. As in these contractions no shortening of the detrusor muscle took place, higher active pressure values were expected

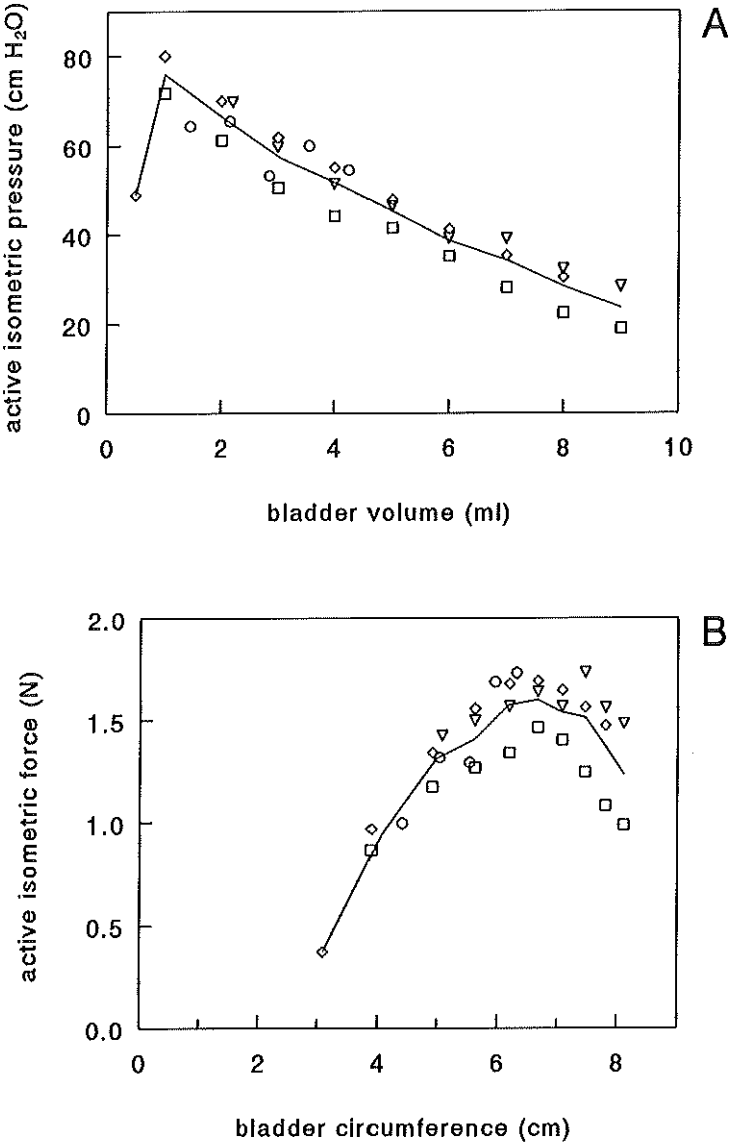


Figure 2. A: Bladder volume dependence of the extrapolated active isometric pressure, measured in vitro under conditions of optimal electrical stimulation. Results of four bladders, represented by four different symbols. B: The relationship between active force and bladder circumference, calculated from the data in A. The solid lines connect the mean values of clusters of data.

in accordance with the force-velocity relation. Table 2 shows that the difference in active pressure in voiding contractions and in isometric contractions was indeed quite large. This result is in agreement with the observations of Peterson et al.: also in urethane-anaesthetized guinea pigs, these investigators measured active pressure values of 22 ± 4 and 39 ± 3 cm H_2O in voiding contractions and in isometric contractions.[5]

As described in the introduction, maximum active pressure attained during isometric contractions in vivo was considered a measure of the neurogenically modulated contractility of the detrusor muscle, i.e., it is influenced by both neurogenic and myogenic factors. It can be directly compared with the maximum active pressure developed in vitro under conditions of optimal stimulation. In this study, electrical stimulation was used. With optimum parameters electrically induced contractile responses are comparable in magnitude with potassium chloride induced responses[6] and thus can be considered to represent maximal contractility. The stimulus optimization curves (Fig.1) and optimum stimulus parameters were similar to those found for the strips. Tests with tetrodotoxin were not done in the present study because it was our aim to measure maximal contractility irrespective of the stimulatory pathway involved.

Table 2 confirms our earlier conclusion that in a micturition contraction the neurogenically modulated contractility of the detrusor muscle is smaller than its maximal contractility, i.e., in micturition the bladder is not fully stimulated. In vitro studies by Levin et al. showed that complete bladder emptying can occur at stimulation intensities at which the generated pressure is submaximal.[8] This phenomenon was interpreted as a "reserve capacity to empty".

In our earlier study on full thickness bladder wall strips, maximum active stress was taken as the measure of contractility. The difference between the in vivo and in vitro contractility was less convincing than in the present study. Therefore, it is of importance to compare the measurements on strips with those on whole bladder preparations. This was previously done by Levin et al.[8] Using variable stimulation intensities, these authors showed that the contractile response of bladder strips was proportional to the contractile response of whole bladder preparations. When comparing the maximum active stress measured in both preparations, the structure of the muscle wall of the bladder should be kept in mind. It is considered to consist of three ill-defined layers, an outer and an inner longitudinal layer and an intermediate circular layer, which is usually the thickest.[9] Each layer seems to contain a number of muscle bundles. As the direction of these bundles is not uniform, the adjectives "longitudinal" and "circular" only express their general direction in the respective layers. In whole bladder preparations all bundles of the detrusor muscle are involved in the development of force (pressure), while in strips only the longitudinal bundles are involved; the contraction of the bundles cut transversally is not recorded by the force transducers. If all three layers were equally thick and all muscle bundles ran in the longitudinal and circular direction perfectly, a ratio of the maximum active stress in whole bladders and in strips of 3:2 would be expected. In the other extreme situation, where in each muscle layer all bundles run in randomly

distributed directions, a ratio of 2:1 is expected. The results shown in Table 3 fit in with both situations described.

The in vitro measured active pressure decreased almost linearly with increasing volume after an initial maximum had been reached (Fig.2A). Although in this case the maximum is based on one measurement only, similar relationships were found by Uvelius and Gabella in in vivo measurements on the guinea pig and by Kaplan et al. in in vitro rabbit bladders.[2,10] The force-length relationship was calculated from the data in Fig.2A. The results (Fig.2B) were in agreement with those reported for pig bladder strips[7,11,12] and for strips from the guinea pig bladder[13] (although in the latter case the decrease of force beyond the optimal length was reported not to be significant). This force-length relationship, and therefore the pressure-volume relationship, is as predicted by the sliding filament-cross bridge theory.[3]

In the present study micturition contractions occurred at volumes well below the volume at which the active force in vitro was maximal, i.e., at volumes corresponding to the rising part of the force-length relationship (Fig.2). As a result of the fact that forces rise steeply with bladder circumference in this part of the force-length characteristic, the decrease of isometric pressure with bladder volume, caused by geometrical factors, is only moderate at mean capacity. This enables the bladder to empty over a wide range of volumes. At mean capacity, active isometric pressure in vitro was approximately 80% of maximum.

Conclusions

During micturition the guinea pig detrusor muscle is not fully stimulated. In our data the isometric pressure during a micturition contraction amounted on average to 60% of the pressure attained during electrical stimulation of the same bladder in vitro at the same volume. In addition to that, micturition usually occurred at a volume where the pressure developed by the detrusor in vitro was about 80% of its maximum value. The in vitro pressure is volume dependent and complies with the sliding filament-cross bridge theory. At optimal electrical stimulation, the stress induced in the wall of a whole bladder preparation is about twice as high as the stress induced in a longitudinal full thickness bladder wall strip. This difference can be understood from the structure of the bladder wall.

References

1. *Groen, J., van Asselt, E., van Mastrigt, R., Bosch, R. and van Koeveringe, G.A.: Comparison of detrusor contractility of guinea pig bladders in situ and strips from these in vitro. J.Urol. 150: 1002-1006, 1993.*

2. *Uvelius, B. and Gabella, G.: Relation between cell length and force production in urinary bladder smooth muscle. Acta Physiol.Scand. 110: 357-365, 1980.*
3. *Huxley, A.F.: Muscle structure and theories of contraction. Prog.Biophys.Biophys. Chem. 7: 255-318, 1957.*
4. *Maggi, C.A., Meli, A. and Santicioli, P.: Neuroeffector mechanisms in the voiding cycle of the guinea-pig urinary bladder. J.Auton.Pharmac. 7: 295-308, 1987.*
5. *Peterson, J.S., Hanson, R.C. and Noronha-Blob, L.: In vivo cystometrogram studies in urethane-anesthetized and conscious guinea pigs. J.Pharmacol.Methods 21: 231-241, 1989.*
6. *van Koeveringe, G.A. and van Mastrigt, R.: Excitatory pathways in smooth muscle investigated by phase-plot analysis of isometric force development. Am.J.Physiol. 261: R138-R144, 1991.*
7. *van Mastrigt, R. and Glerum, J.J.: Electrical stimulation of smooth muscle strips from the urinary bladder of the pig. J.Biomed.Eng. 7: 2-8, 1985.*
8. *Levin, R.M., Kato, K., Lin, A.T., Whitmore, K. and Wein, A.J.: Correlation of in vitro pressure generation with urinary bladder function. Neurourol.Urodyn. 10: 185-192, 1991.*
9. *Uvelius, B.: Relation between mechanical and morphological characteristics in urinary bladder smooth muscle. Acta Physiol.Scand., suppl. 483: 5-51, 1980.*
10. *Kaplan, S.A., Blaivas, J.G., Brown, W.C. and Levin, R.M.: Parameters of detrusor contractility: I: The effects of electrical stimulation, hysteresis, and bladder volume in an in vitro whole rabbit bladder model. Neurourol.Urodyn. 10: 53-59, 1991.*
11. *Griffiths, D.J., van Mastrigt, R., van Duyl, W.A. and Coolsaet, B.L.R.A.: Active mechanical properties of the smooth muscle of the urinary bladder. Med.Biol.Eng. Comput. 17: 281-290, 1979.*
12. *van Mastrigt, R.: The length dependence of the series elasticity of pig bladder smooth muscle. J.Muscle Res.Cell Motil. 9: 525-532, 1988.*
13. *Longhurst, P.A., Kang, J., Wein, A.J. and Levin, R.M.: Comparative length-tension relationship of urinary bladder strips from hamsters, rats, guinea pigs, rabbits and cats. Comp.Biochem.Physiol. 96A: 221-225, 1990.*

Chapter 4

Contractility parameters of the guinea pig bladder in situ: similarity to human bladder contractility

J. Groen, R. van Mastrigt, E. van Asselt, G.A. van Koeveringe, R. Bosch

The Journal of Urology 151: 1405-1410, 1994

Abstract

The parameters P_{isv} (active isovolumetric detrusor pressure) and v_{max} (maximum shortening velocity), which characterize the contractility of the detrusor muscle, were determined in guinea pigs. To this end it was necessary to develop a method of measuring flow rates in these small animals. The values found were used to calculate the contractility parameter W_{max}. Thirteen animals were used. The results found for P_{isv} and v_{max} were 43.0 $\pm$ 3.7 cm H_2O and 20.2 $\pm$ 3.7 mm/s, respectively. The latter corresponded to about 0.38 muscle lengths/s, which is similar to values reported for bladder strips from other species. Previous work showed that in vitro P_{isv} decreased with increasing bladder volume over a wide range of volumes. In vivo P_{isv} seemed to be independent of bladder volume. This suggests that neurogenic stimulation intensifies as volume increases. v_{max} also was independent of volume. W_{max} appeared to be suitable for detecting differences in the contractility of the bladders of different animals. Values were not significantly different in isovolumetric and nonisovolumetric contractions. Normalized to the size of the bladder, the W_{max} values indicated that the power generated by the guinea pig bladder is similar to the power generated by the human bladder.

Introduction

In a micturition contraction the detrusor pressure rises above the (passive) pressure determined by the viscoelastic properties of the bladder wall to the amount of P_{det}, the active detrusor pressure. The relation between P_{det} and the shortening velocity v of the bladder circumference can be modelled by the equation[1]

$$(P_{det}/P_{isv} + a/P_{isv}).(v + b) = (1 + a/P_{isv}).b \qquad (1)$$

where P_{isv} is the active detrusor pressure in an isovolumetric contraction and a and b are constants (Fig.1). It has been shown experimentally that a/P_{isv} is approximately 0.25.[1] By definition, $b=(a/P_{isv}).v_{max}$, where v_{max} is the potential maximum shortening velocity (at zero pressure), and consequently $b=v_{max}/4$.

The above means that the contractility of a given bladder can be completely characterized by the constants P_{isv} and v_{max}. Not all micturitions result in pressure/flow rate plots from which good estimations of these parameters can be derived.[2] Also, the use of two parameters hampers comparison of the contractility of different bladders. Several methods have therefore been developed to quantify bladder contractility in patients.[3-8] The parameter W_{max} is considered to give a good estimation of the contraction strength of a given detrusor in the general population.[5] This parameter is based on the measurement of pressure and flow rate in a nonmanipulated micturition and gives an approximated value for the maximum power generated by the detrusor per bladder unit surface area.

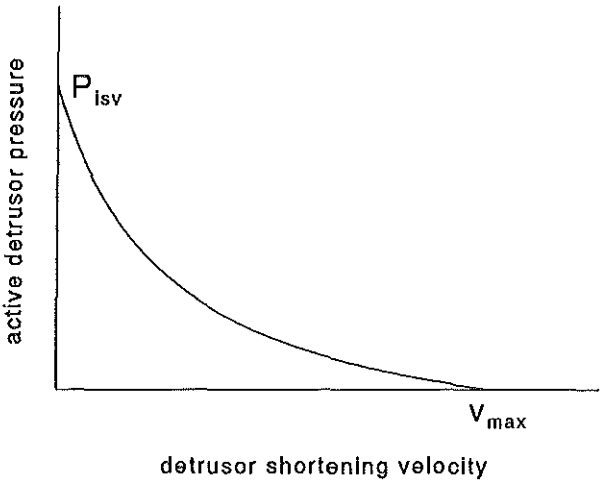


Figure 1. Curve showing the theoretical relation between the active detrusor pressure and the detrusor shortening velocity. Assuming a constant "curvature" of the curve, this relation is completely described by the intercepts P_{isv} and v_{max} on both axes.

To study the physiological basis and relevance of the contractility parameters P_{isv}, v_{max} and the combined parameter W_{max}, pressure/flow rate studies were done in guinea pigs. The values derived for these parameters were compared with values from clinical studies.

Because the volumes voided by the guinea pig are small, a special method of flow rate measurement had to be developed.

Materials and Methods

Thirteen male guinea pigs with a mean weight of 930 g (range: 710 to 1335 g) were used. Each animal was anaesthetized with urethane (1.2 g/kg intraperitoneally). A small incision was made in the abdominal wall to localize the bladder, into which a 24 G angiocatheter was inserted. Subsequently, the abdomen was loosely closed with silk sutures. Body temperature was maintained by means of a heating pad.

A Harvard infusion pump was used to fill the bladder via the catheter with room temperature saline at a rate of 0.53 ml/min until a micturition contraction occurred. Pressure was measured via the same catheter using a disposable pressure transducer. The pressure signal was sampled with a PDP 11 type computer at a rate of 10 Hz. Each bladder was filled 4 to 10 times, with intervals of 10 to 15 minutes.[9] In six animals, the urethra was alternately allowed to leak and occluded in the bulbar part with a 3.5 metric silk suture. In these animals two kinds of micturition contractions were distinguished: voiding contractions (when the urethra was allowed to leak) and isovolumetric contractions (when it was occluded). In two animals isovolumetric contractions alone were measured, and in five animals only voiding contractions were measured. Via a cone-shaped funnel, the voided volume was collected in a glass tube (diameter 4.7 mm) equipped with a central glass rod (diameter 1.5 mm) to prevent the formation of bubbles. The tube was connected to a disposable pressure transducer. The volume signal thus measured was also sampled with the computer. The total voided volume was determined using a measuring glass. Residual urine after voiding and after an isovolumetric contraction was removed with a syringe attached to the catheter. In the voiding contractions, the residual volume was expressed as a percentage of the bladder volume at the onset of micturition. The largest bladder volume in each animal at which a micturition contraction was measured was taken as the cystometric capacity. (Occasionally, after an isovolumetric contraction the bladder was filled further without emptying it until the next contraction occurred. The corresponding volume was not considered to represent capacity). Compliance was calculated from the bladder volume at the start of micturition and the difference between the pressure at the start of filling and at the start of the micturition contraction.

Following the experiments the bladders were blotted and weighed. The volumes were calculated, assuming a density of 1.0 g/ml.

The voided volume signal, as stored by the computer, was filtered (low pass filter 1 Hz) and subsequently differentiated to derive a flow rate signal. For each data sample the instantaneous bladder volume was calculated from the volume at the beginning of the voiding contraction and the integrated flow rate signal. The shortening velocity v of the bladder circumference was derived from the instantaneous volume and the flow rate Q, considering the bladder a thin-walled sphere:[1] $v=Q/2R^2$, where R is bladder radius. Because some amount of urine was left in the bladder in all micturitions, this caused no mathematical difficulties at the end of voiding.

The parameters P_{isv} and v_{max} were determined as follows: In each of the eight animals in which isovolumetric contractions were measured (2 to 6 in each animal), P_{isv} was calculated as the average of the maximum active pressures measured in these contractions. The mean value of the eight P_{isv} values was calculated and used for the five animals in which no isovolumetric contractions were measured. In each voiding contraction, eight consecutive samples were selected where shortening velocity was near maximum (Fig.2B). The mean value of the shortening velocities of these samples was taken as the maximum value of the shortening velocity attained during voiding. The corresponding intravesical pressure was derived likewise. The time interval by which the flow rate signal was delayed with respect to the pressure signal, as derived from calibration measurements, was taken into account. Using these pressure/velocity data and using the appropriate value for P_{isv}, v_{max} was derived algebraically from equation (1) for each micturition contraction. One v_{max} value per animal was calculated. The mean v_{max} values found in the separate animals were averaged. In addition, the v_{max} values were normalized for the initial (preloaded) length L_0 of the contracting tissue. The mean of the outer and inner circumference of the bladder wall was taken for L_0.

The mean values of P_{isv} and v_{max} were introduced into a computer program to determine the contractility parameter W_{max} from the filtered pressure and flow rate signals measured during the voiding contractions, as follows: For each data sample the instantaneous detrusor contraction strength W was calculated according to:[5]

$$W = [(P_{det}+P_{isv}/4).(v+v_{max}/4)-P_{isv}.v_{max}/16]/2\pi \qquad (2)$$

The maximum of W was taken as W_{max}. In the isovolumetric contractions W_{max} was calculated manually, using the formula $W_{max}=(\text{maximum of } P_{det}).v_{max}/8\pi$. This was derived from equation (2) by substituting $v=0$.

Several aspects of the P_{isv}, v_{max} and W_{max} values found were examined. A systematic increase or decrease of the parameters in the course of the experiment was studied by comparing the first result in each animal with the last result, using the paired t test. The separate P_{isv}, v_{max} and W_{max} values found in each animal were plotted against the bladder volume in the corresponding micturition contraction in order to investigate the volume dependence of these parameters. To examine the reproducibility of P_{isv}, v_{max} and W_{max} within the animals and the differences between the distinct animals, the within-animal standard deviation was compared with the between-animal standard deviation; analysis of

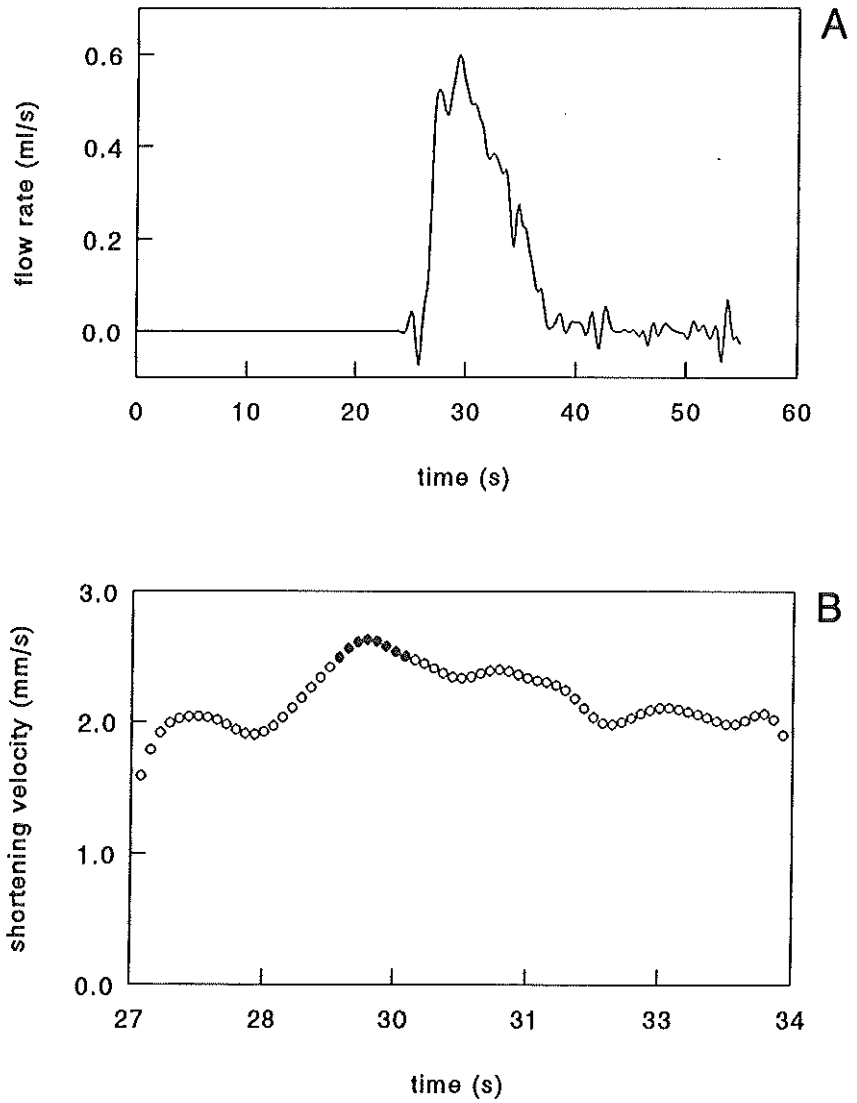


Figure 2. A: Flow rate signal, derived from the volume signal in Fig.3 by filtering and differentiation. The irregularity in the beginning of the signal is due to the filtering procedure. B: Bladder circumference shortening velocity as derived from the bladder volume and the flow rate data in A in a selected time interval. The marked samples were used to calculate the maximum shortening velocity attained during voiding.

variance was used for this. As the number of appropriate contractions (isovolumetric contractions for P_{isv}, voiding contractions for v_{max}) was not equal in all animals, there was no unique way of doing this. The first two contractions were used. In the six animals in which isovolumetric as well as voiding contractions were measured, the mean W_{max} values in both kinds of contractions were compared, again using the paired t test. Finally, the dependence of W_{max} on bladder compliance was examined. This was done by determining the Pearson correlation coefficient between the mean values of W_{max} in the animals and the mean values of compliance.

Results

The measured cystometrograms were similar to those described previously.[9] Relevant cystometric data are given in the Table. An example of a recording of intravesical pressure and voided volume during a voiding contraction is shown in Fig.3. Fig.2A shows the flow rate signal derived from the volume signal in Fig.3. Typical values of the maximum flow rate were 0.3 to 0.5 ml/s.

The mean value found for P_{isv} was 43.0 ± 3.7 (SEM) cm H_2O. Fig.4A shows that the separate values of P_{isv}, found in each animal, were fairly reproducible. This was

Table. The bladder weight of the 13 animals and the results of the cystometries.

Bladder wet weight (mg)	642 ± 39
Cystometric capacity (ml)	3.7 ± 0.3
Mean capacity (ml)	2.9 ± 0.3
Residual volume (%)	38 ± 7
Compliance (ml/cm H_2O)	0.54 ± 0.08
Premicturition pressure (cm H_2O)	10 ± 1
Maximum intravesical pressure in voiding contractions (cm H_2O)	28 ± 2
Maximum intravesical pressure in isovolumetric contractions (cm H_2O)	50 ± 3

The cystometric capacity of each animal was defined as the largest bladder volume at which a micturition reflex contraction occurred, while mean capacity was derived from all micturition contractions in each animal. The residual volume, given as a percentage of the volume at the onset of micturition, is the mean value of all voidings in each animal. Compliance was calculated as the mean value in all fillings. The pressures shown were derived from all appropriate contractions. Mean values and standard errors of the mean are given.

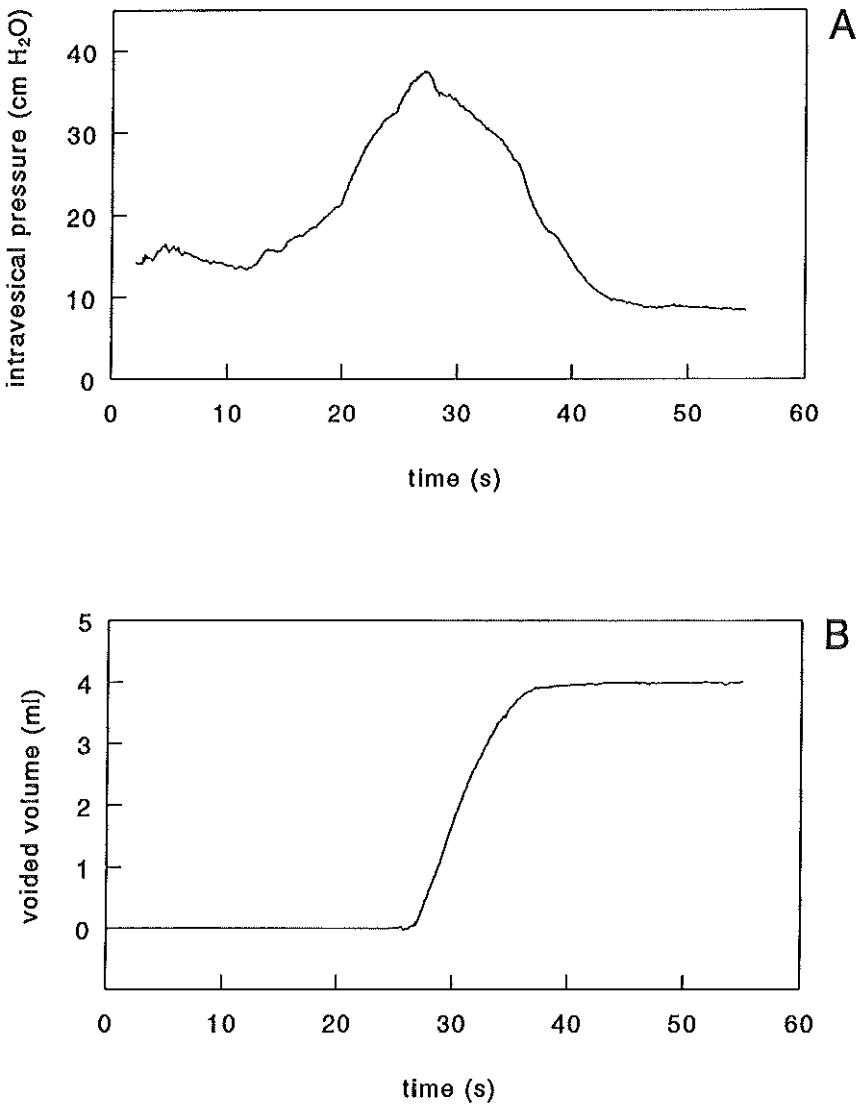


Figure 3. Example of intravesical pressure (A) and voided volume (B) as measured during voiding of a guinea pig.

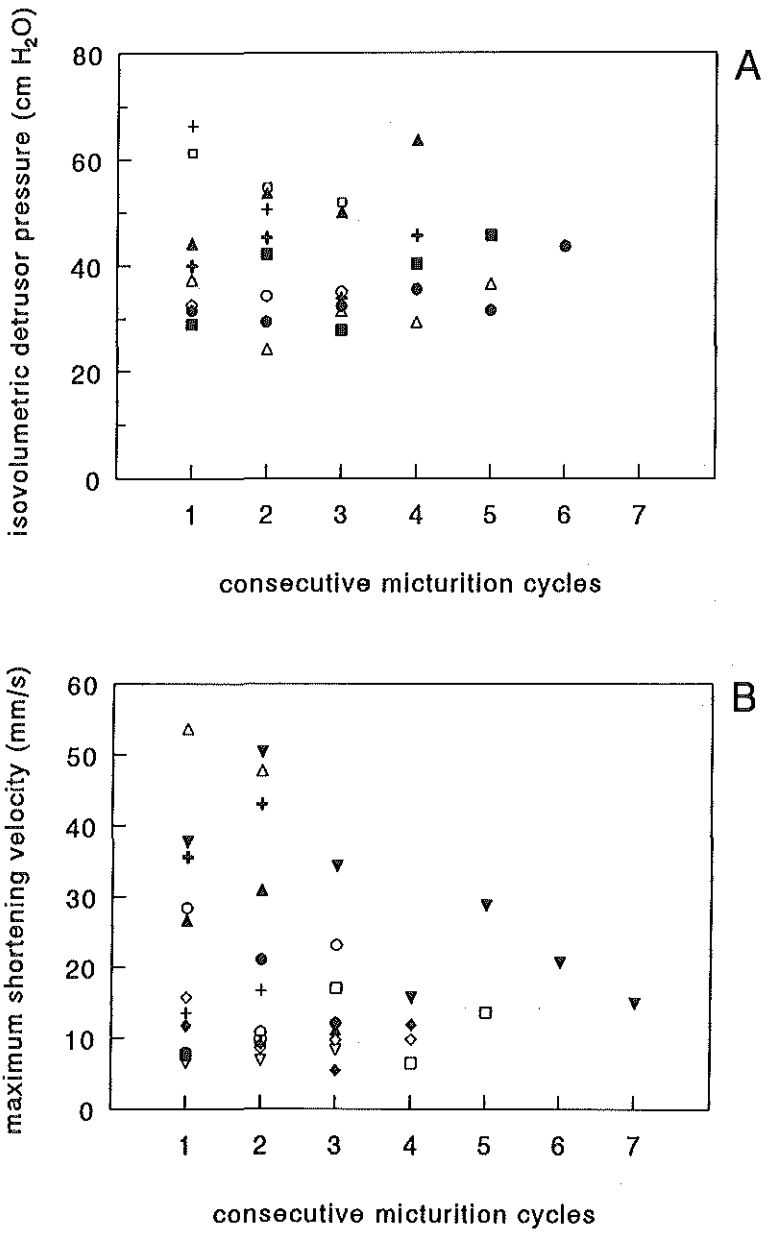


Figure 4. The calculated values of the parameters P_{iso} (A) and v_{max} (B) in consecutive isovolumetric and voiding contractions, respectively. Each symbol represents the results of one guinea pig.

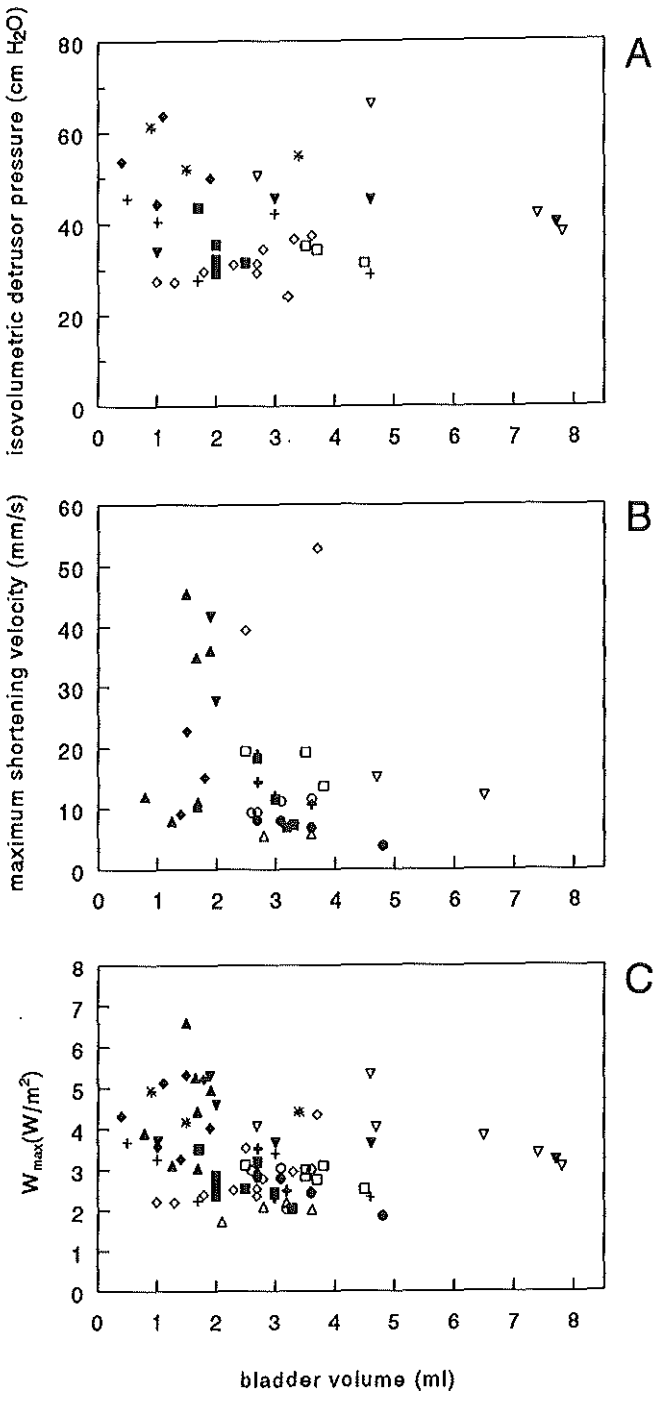
confirmed by the paired t test: the first and the last result in each animal were not significantly different ($p=0.40$). The mean value found for v_{max} was 20.2 $\pm$ 3.7 mm/s. From Fig.4B it can be seen that large differences in v_{max} within one animal were occasionally found. As the first and the last results within the animals were not significantly different ($p=0.31$ in the paired t test), it can be concluded that v_{max} did not systematically change in the course of the experiment.

Considering only the first two appropriate contractions, for P_{isv} as well as for v_{max}, the within-animal standard deviation was significantly smaller than the between-animal standard deviation: 1.7 versus 4.6 cm H_2O for P_{isv} ($p=0.0049$) and 5.2 versus 19.2 mm/s for v_{max} ($p=0.0001$). This means that these parameters were determined sufficiently accurately to detect differences between the different animals.

A scatter plot of all P_{isv} and v_{max} values as a function of the bladder volume is shown in Fig.5A,B. In a given animal, P_{isv} does not seem to be dependent on volume. At first sight, the parameter v_{max} seems to decrease with increasing volumes. However, in the eight animals in which it was possible to calculate the regression coefficient (those in which there were more than two measurements), in only one case did the 95% confidence interval not include the value 0 mm/s/ml. In the 11 animals in which voiding contractions were measured, the mean value of v_{max}/L_0 was 0.38 $\pm$ 0.08 (SEM) muscle lengths/s.

In six animals, voiding contractions as well as isovolumetric contractions were measured. The separate W_{max} values calculated from these contractions are shown in Fig.6. Comparison of the mean values from both kinds of contractions showed that these were not significantly different ($p=0.12$). It was concluded therefore that all W_{max} values could be pooled, irrespective of the kind of contraction they were derived from. The mean values found ranged from 2.0 to 4.5 W/m^2 and averaged 3.3 $\pm$ 0.2 W/m^2. As the W_{max} values in the last contraction in each animal were not significantly different from those in the first one ($p=0.88$), it was concluded that the reproducibility of this parameter is good. Fig.5C is a scatter plot of all W_{max} values as a function of the bladder volume. For all 13 animals the 95% confidence interval of the regression coefficient was calculated. In two cases this interval did not include the value 0 W/m^2/ml. One of these intervals contained only positive values, while the other contained negative values. In the first two contractions the within-animal standard deviation for W_{max} was significantly smaller than the between-animal standard deviation (0.50 versus 1.50 W/m^2; $p=0.0002$). The correlation coefficient between W_{max} and bladder compliance was 0.15, which was not significant ($p=0.33$).

Figure 5. Scatter plot of the values of the parameters P_{isv} in isovolumetric contractions (A), v_{max} in voiding contractions (B) and W_{max} (C) as a function of bladder volume. Each symbol represents the results of one animal. The larger volumes resulted from continuing filling after an isovolumetric contraction had occurred. In C isovolumetric and voiding contractions were pooled.


A
isovolumetric detrusor pressure (cm H2O)
0
20
40
60
80
0 1 2 3 4 5 6 7 8
B
maximum shortening velocity (mm/s)
0
10
20
30
40
50
60
0 1 2 3 4 5 6 7 8
C
Wmax(W/m2)
0
1
2
3
4
5
6
7
8
0 1 2 3 4 5 6 7 8
bladder volume (ml)

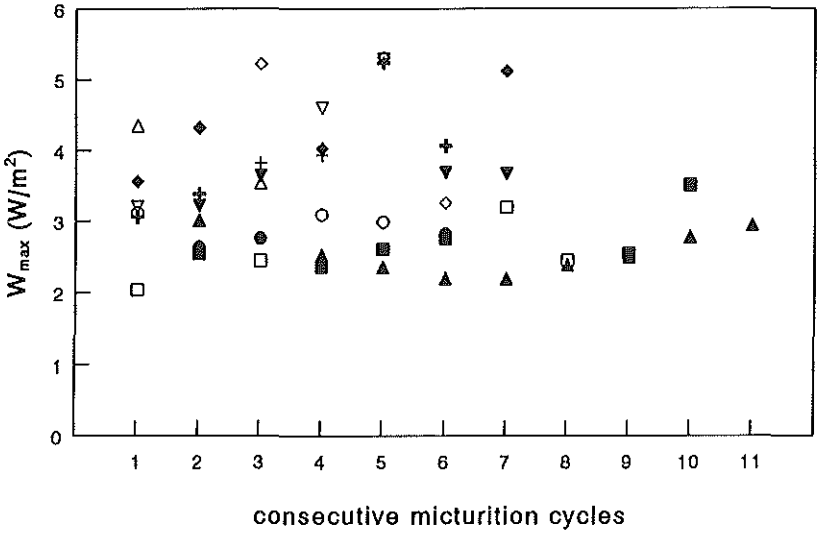


Figure 6. The calculated values of the parameter W_{max} in consecutive micturition contractions in the six animals where both isovolumetric and voiding contractions were measured. The open symbols represent the results of the voiding contractions, the filled symbols those of the isovolumetric contractions in the same animal.

Discussion

Assessment of detrusor contractility during voiding requires the measurement of urinary flow rate and detrusor pressure. In clinical urodynamics, detrusor pressure is taken as the difference between intravesical and rectal pressure. As the passive, viscoelastic contribution due to the filling of the bladder is usually small, it is usually neglected in the assessment of detrusor contractility. In our experiments rectal pressure was not measured, so that active detrusor pressure in a micturition contraction had to be derived from changes in intravesical pressure. Active pressure was defined as the instantaneous pressure minus the postmicturition pressure, rather than the premicturition pressure, to allow for the low compliance observed in some animals.

In previous experiments the bladder volume dependence of the isovolumetric detrusor pressure was examined under conditions of optimum electrical stimulation in vitro.[10] After an initial maximum had been reached, pressure decreased almost linearly with increasing volume in the range from 1 to 9 ml. This observation was in agreement with in vitro results in the rat and rabbit bladder.[11,12] In the present study, no clear volume dependence of isovolumetric detrusor pressure was observed in vivo (Fig.5A). Observations in humans are somewhat contradictory. Using the stop flow technique, Susset et al. found

that the maximum detrusor pressure was independent of the volume present in the bladder at the time flow was stopped.[13] Constantinou et al. asked healthy volunteers to attempt to void against an obstructed urethra at incremental bladder volumes and also found no volume dependence of isovolumetric pressure.[14] Griffiths and van Mastrigt on the other hand, again using the stop flow technique, noticed a negative association between isovolumetric detrusor pressure and bladder volume.[4] The clinical significance of this association, however, apparently was so small that these authors called isovolumetric pressure "relatively independent" of volume. The contrast between the evident volume dependence of isovolumetric pressure in vitro and the marginal dependence in vivo suggests the possibility of a neurogenic feedback mechanism that regulates the contractile response over a wide range of volumes. The weak (or absent) volume dependence of isovolumetric detrusor pressure justified its application in the calculation of the maximum shortening velocity, derived from voiding contractions at different volumes.

Occasionally a large variation in v_{max} values was found in one animal. This can partly be understood by realizing that active detrusor pressure during voiding was about one-half isovolumetric detrusor pressure (Table), implying that shortening velocity during voiding was about 1/6 of maximum shortening velocity (equation (1)). Small variations in experimental data thus led to larger variations in the calculated v_{max}. No volume dependence of maximum shortening velocity could be shown (Fig.5B), as was also found in patients.[4] This is in agreement with the sliding filament-cross bridge theory. In this theory, maximum shortening velocity (at zero load) is determined by the cross bridge cycling rate. This cycling rate, in turn, is primarily determined by the ATP (adenosin triphosphate)-hydrolysis rate[15] and is independent of the stretched muscle length.

The (relative) volume independence of the parameters P_{isv}, v_{max} and, in particular, W_{max} justifies their use in clinical practice. For all three parameters the within-animal standard deviation was significantly smaller than the between-animal standard deviation. This implies that these parameters provide a means of detecting differences in contractility of the bladders of different animals. The similarity of W_{max} values in isovolumetric and voiding contractions supports the validity of the W_{max} concept.

In this study, the parameter v_{max} was determined in a group of guinea pigs which were fairly homogeneous with respect to bladder volume (Table). When comparing the value of v_{max} with values measured in other experiments, a normalization for the initial length of the contracting tissue is needed: a greater length implies a larger number of cells coupled in series, their shortening velocities being added.[16] The normalized value of 0.38 ± 0.08 muscle lengths/s is similar to the values found by other investigators in in vitro studies on strips of pig and rabbit bladder.[17,18] In humans a mean value of 24 mm/s was found for v_{max}.[4] To our knowledge, values for the human bladder weight have not been reported in the literature. However, in our laboratory the mean weight of 15 pig bladders was 56 g.[19] These bladders contained amounts of urine similar to human capacity, so that an assumed value of 50 g for the human bladder seems reasonable. With this and an assumed bladder volume of 300 ml,[4] a normalized v_{max} value of 0.08 lengths/s

can be estimated, which is far less than the value found in guinea pigs. When comparing contractility parameters in isovolumetric contractions, bladder volume has to be taken into account. By taking active stress σ as a measure of contractility, force is normalized for the cross-sectional area of the contracting tissue. As was derived previously,[9] σ can be calculated as $P_{isv}/[(1+V_t/V)^{2/3}-1]$, where V and V_t are bladder volume and bladder tissue volume. P_{isv} is about 100 cm H_2O on an average in humans[4] and 43 cm H_2O in guinea pigs. Taking the above bladder weights and 300 ml and 2.9 ml for the volumes of the human bladder[4] and the guinea pig bladder (Table) at capacity, active stress values of 9.2 and 3.0 N/cm^2 are found for humans and for guinea pigs. The ratio of these values is close to the ratio of P_{isv} values. As the value of the parameter W_{max} depends on both the detrusor pressure and the detrusor shortening velocity, it is of interest to compare the power exerted by both kinds of bladder. W_{max} values reported for humans vary widely and can range from 5 to 30 W/m^2.[2,5,20] On average, W_{max} is about 12 W/m^2.[2,20] Because of the wide variation seen in humans, the range of W_{max} values found in guinea pigs (2.0 to 4.5 W/m^2) was expected. The values themselves are much smaller than in humans and average 3.3 W/m^2. As W_{max} gives the approximated maximum power generated by the bladder wall per unit surface area, the thickness of this wall should be taken into account when comparing W_{max} values. With the above values for bladder volumes and bladder weights, the thicknesses of both kinds of bladder can be estimated as 2.2 and 0.6 mm, a ratio of 3.7. The ratio of W_{max} values found for humans and guinea pigs is about the same. Assuming the same fraction of muscle tissue in the bladder wall, the similarity of normalized W_{max} values implies a comparable power developed at the cellular level for guinea pig and human bladder tissue.

In conclusion, a method was described to measure the urinary flow rate in guinea pigs. The contractility curve of the guinea pig bladder could be characterized by the parameters P_{isv} = 43 cm H_2O and v_{max} = 20 mm/s on average. The latter corresponds to about 0.38 muscle lengths/s. In contrast to measurements in vitro, the isovolumetric detrusor pressure in vivo was fairly constant over a wide range of bladder volumes. The bladder volume independence of the contractility parameter W_{max} and the similarity of values in isovolumetric and nonisovolumetric contractions support the applicability of this concept. W_{max} was fairly reproducible within a given animal and appeared to be suitable to assess the relative contractility of a given bladder. Normalized for bladder wall thickness, the power generated by the guinea pig bladder wall per unit surface area was in the same order of magnitude as is found for humans.

References

1. *van Mastrigt, R. and Griffiths, D.J.: Contractility of the urinary bladder. Urol.Int. 34: 410-420, 1979.*
2. *van Mastrigt, R.: Estimation of contractility parameters for the urinary bladder.*

Neurourol.Urodyn. 10: 37-45, 1991.

3. *Griffiths, D.J.: Urodynamic assessment of bladder function. Br.J.Urol. 49: 29-36, 1977.*
4. *Griffiths, D.J. and van Mastrigt, R.: The routine assessment of detrusor contraction strength. Neurourol.Urodyn. 4: 77-87, 1985.*
5. *Griffiths, D.J., Constantinou, C.E. and van Mastrigt, R.: Urinary bladder function and its control in healthy females. Am.J.Physiol. 251: R225-R230, 1986.*
6. *van Mastrigt, R. and Griffiths, D.J.: An evaluation of contractility parameters determined from isometric contractions and micturition studies. Urol.Res. 14: 45-52, 1986.*
7. *Schäfer, W.: Analysis of active detrusor function during voiding with the bladder working function. Neurourol.Urodyn. 10: 19-35, 1991.*
8. *Venegas, J.G., Sullivan, M.P., Yalla, S.V. and Cravalho, E.G.: Measurement of detrusor intrinsic resistance (DIR) in humans as a contractility parameter. Neurourol.Urodyn. 11: 317-318, 1992.*
9. *Groen, J., van Asselt, E., van Mastrigt, R., Bosch, R. and van Koeveringe, G.A.: Comparison of detrusor contractility of guinea pig bladders in situ and strips from these in vitro. J.Urol. 150: 1002-1006, 1993.*
10. *Groen, J., van Mastrigt, R. and Bosch, R.: Contractility of the guinea pig bladder measured in situ and in vitro. Neurourol.Urodyn. 13: 587-595, 1994.*
11. *Carpenter, F.G.: Motor responses of bladder smooth muscle in relation to elasticity and fiber length. Invest.Urol. 6: 273-283, 1968.*
12. *Kaplan, S.A., Blaivas, J.G., Brown, W.C. and Levin, R.M.: Parameters of detrusor contractility: I: The effects of electrical stimulation, hysteresis, and bladder volume in an in vitro whole rabbit bladder model. Neurourol.Urodyn. 10: 53-59, 1991.*
13. *Susset, J.G., Brissot, R.B. and Regnier, C.H.: The stop-flow technique: a way to measure detrusor strength. J.Urol. 127: 489-494, 1982.*
14. *Constantinou, C.E., Djurhuus, J.C., Silverman, D.E., Towns, A.B., Wong, L. and Govan, D.E.: Isometric detrusor pressure during bladder filling and its dependency on bladder volume and interruption to flow in control subjects. J.Urol. 131: 86-90, 1984.*
15. *Bárány, M.: ATPase activity of myosin correlated with speed of muscle shortening. J.Gen.Physiol. 50 (part 2): 197-218, 1967.*
16. *Uvelius, B. and Gabella, G.: Relation between cell length and force production in urinary bladder smooth muscle. Acta Physiol.Scand. 110: 357-365, 1980.*
17. *van Mastrigt, R. and Glerum, J.J.: In vitro comparison of isometric and stop-test contractility parameters for the urinary bladder. Urol.Res. 13: 11-17, 1985.*
18. *Uvelius, B. and Hellstrand, P.: Effects of phasic and tonic activation on contraction dynamics in smooth muscle. Acta Physiol.Scand. 109: 399-406, 1980.*
19. *Schot, R., van Asselt, E. and van Mastrigt, R.: A method for isolating smooth muscle cells from pig urinary bladder with low concentrations of collagenase and*

papain: the relation between calcium concentration and isolated cell length. Urol. Res. 21: 49-53, 1993.

20. *van Mastrigt, R.: Age dependence of urinary bladder contractility. Neurourol. Urodyn. 11: 315-317, 1992.*

Chapter 5

Neurogenic modulation of micturition: the relation between stimulation intensity and the maximum shortening velocity of the guinea pig detrusor muscle

J. Groen, R. van Mastrigt, R. Bosch

The Journal of Urology 153: 228-232, 1995

Abstract

The course of micturition depends on bladder contractility and urethral resistance. The former is determined by geometrical, muscular and neurogenic factors. The muscular aspects of bladder contractility can be characterized by the parameters P_{isv}, the isovolumetric detrusor pressure, and v_{max}, the maximum (unloaded) shortening velocity of the detrusor muscle. The neurogenic control system of the urinary tract modulates bladder contractility, which might effectively change the values of P_{isv} and v_{max}. These parameters (might) also depend on the instantaneous bladder volume. In previous work the dependence of P_{isv} on the intensity of stimulation and bladder volume was measured in guinea pig bladders in vivo and in vitro. In the present work v_{max} was derived in five guinea pig bladders in vitro, using electrical stimulation and the stop-flow technique. This technique implies that pressure values measured at a certain shortening velocity of the bladder circumference and in an isovolumetric contraction at the same volume are used to derive v_{max} mathematically from the Hill equation. v_{max} was independent of the bladder volume in the range of 0.6 to 6.1 ml, but it was significantly different for the two intensities of stimulation used. Therefore, it is concluded that the maximum shortening velocity of the guinea pig detrusor muscle depends on the intensity of stimulation. During submaximal stimulation the detrusor not only generates lower pressures, it also contracts more slowly. A possible explanation for this phenomenon is that the bladder is not uniformly stimulated.

The isovolumetric pressure measured in the stop-flow test was compared with the isovolumetric pressure measured at the same bladder volume some minutes later. It was observed that shortening had a depressant effect of approximately 33% on the isovolumetric pressure. This implies that the clinically employed stop-flow test might underestimate detrusor contraction strength.

Introduction

The course of micturition depends on the contraction strength of the bladder and the resistance offered to urinary flow by the urethra. The contractile properties of the bladder are determined by factors related to the detrusor muscle and factors related to the nervous system. Inadequate or insufficiently sustained neurogenic stimulation of the detrusor muscle is therefore one of the possible causes of inefficient voiding, causing residual urine. In a series of experiments we studied the modulation of bladder contractility by neurogenic factors.[1-3]

The muscular aspect of bladder contractility is defined by the relationship between two variables: the shortening velocity v of the bladder circumference and the active detrusor pressure P(v) at that shortening velocity. This relationship can be modelled by a hyperbolic equation:[4]

$$[P(v)/P_{isv} + 0.25] \times [4 \times v + v_{max}] = 1.25 \times v_{max} \qquad (1)$$

where P_{isv} is the active detrusor pressure in an isovolumetric contraction and v_{max} is the bladder circumference shortening velocity at zero load (Fig. 1). Active pressure is defined as the pressure exceeding the (passive) pressure resulting from the viscoelastic properties of the bladder wall.

According to equation (1), the contractility of a given bladder can be fully characterized by the parameters P_{isv} and v_{max}. The neurogenic control system of the urinary tract modulates bladder contractility, which might effectively change P_{isv} and v_{max}.

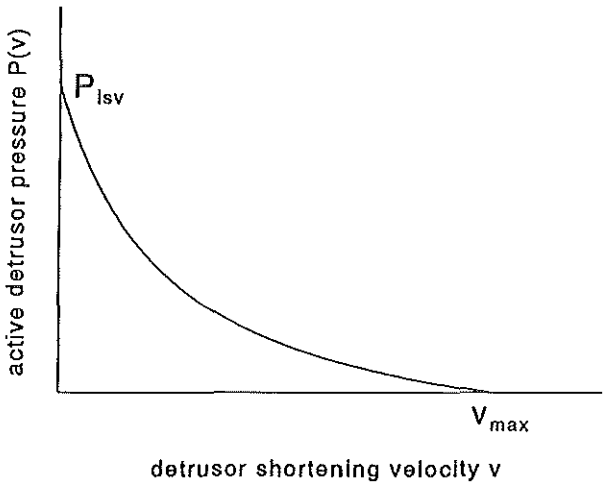


Figure 1. The theoretical relationship between the active detrusor pressure and the detrusor shortening velocity. According to equation (1), this relationship can be completely characterized by the intercepts P_{isv} and v_{max} on both axes.

Furthermore, these parameters (might) depend on the instantaneous bladder volume. In previous work P_{isv} and v_{max} were estimated in guinea pig bladders in vivo, while P_{isv} was also measured in vitro.[2,3] It was concluded that in vivo the bladder was not fully stimulated at normal capacity: the isovolumetric detrusor pressure was significantly lower than that attained in vitro under conditions of optimal electrical stimulation. Unlike the in vitro pressure values, pressure in vivo did not depend on bladder volume. Also the parameter v_{max} did not depend on bladder volume in vivo. In the present study the dependence of v_{max} on the intensity of electrical stimulation and the bladder volume was studied in vitro.

Materials and Methods

Five male guinea pigs weighing 895 to 1175 g were anaesthetized with urethane (1.2 g/kg intraperitoneally). The bladder was exposed via a midline incision in the abdominal wall. After ligation of both ureters the bladder was excised and kept at room temperature in a buffer solution of the following composition: NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.0; $NaHCO_3$ 8.0; HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) 10.5 and glucose 5.1 mmol/l; NaOH was added to adjust the pH to 7.4. Two catheters were inserted via the remaining part of the urethra: one 5 F catheter, which was connected to a disposable pressure transducer, and a stainless steel tube (inner diameter 1.7 mm), which was connected to a Harvard infusion pump via a low-compliance plastic tube. The bladder was suspended in an organ bath containing 85 ml of the above buffer solution, which was equilibrated with 95% O_2 - 5% CO_2 at a temperature of 37°C. The pH was in the range 7.3 to 7.4, and the osmolality was close to 300 mOsm. The solution could be refreshed within a few seconds from a heated and aerated storage container; this was done every 30 minutes. The bladder was initially filled with room temperature saline to a volume of 3 ml to accommodate for one hour before the start of the experiments.

Next, the bladder volume was changed to a start value in the range of 1.9 to 7.9 ml according to a scheme explained below. The bladder was stimulated electrically for 5.2 to 6.5 seconds at intervals of at least 5 minutes, using two parallel platinum electrodes (20 x 30 mm), one on each side of the bladder. Rectangular pulses were used. The applied voltage was 10 V, while the pulse frequencies used were 25 and 200 Hz. The pulse width was adapted to the frequency to attain a duty cycle of 50%. To avoid electrolysis of the buffer solution, biphasic pulse stimulation was used. Each measurement consisted of the following phases (Fig.2): During the first 2 seconds of stimulation the bladder contracted isovolumetrically at the start volume. During the following 1.2 to 2.5 seconds the bladder was emptied using the infusion pump at an imposed flow rate. During the last 2 seconds the bladder contracted isovolumetrically again at the resulting smaller volume. These three successive phases composed what will be called a stop-flow contraction. At least 5 minutes later an isovolumetric bladder contraction was induced once more by

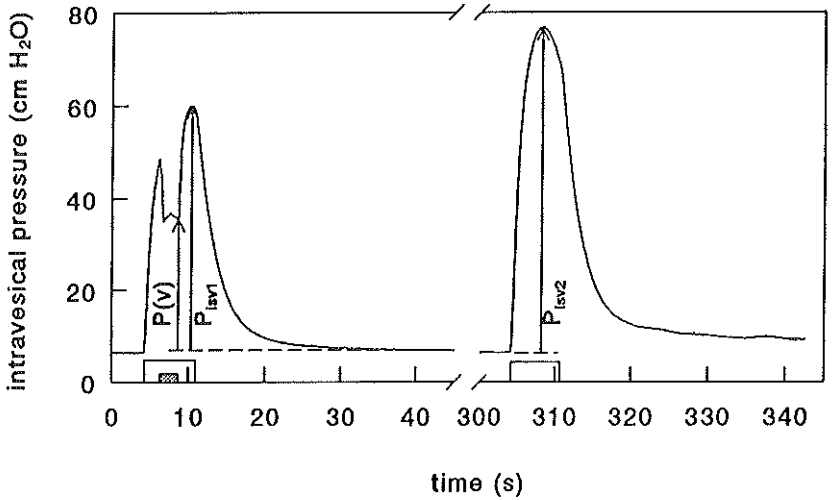


Figure 2. Schematic representation of the experimental procedure and the measured pressure signal in two consecutive contractions in response to electrical stimulation. A stop-flow contraction was followed by a completely isovolumetric contraction. During the time intervals indicated with the blank boxes, the bladder was stimulated electrically, while during the interval indicated with the shaded box the bladder was emptied using a pump. P(v) is the active detrusor pressure at the moment bladder emptying by the pump was stopped; P_{isv1} is the maximum active pressure in the isovolumetric contraction following detrusor shortening; P_{isv2} is the maximum active pressure in the second, completely isovolumetric contraction.

stimulating for 6.5 seconds at the last volume. All contractions were sampled at a rate of 10 Hz with a personal computer and stored for further analysis.

Two kinds of test measurements were performed to exclude artifacts. First the catheters were positioned at the bottom of the organ bath, which was subsequently emptied by the pump. The measured pressure was related to the volume of fluid displaced by the pump. In the second type of test measurements, the bladder experiments were repeated with the bladder replaced by a rubber balloon.

Bladder emptying during the contraction simulated micturition. Pressure was measured as a function of the continuously varying detrusor muscle length, or bladder circumference shortening velocity. This shortening velocity v was calculated from the instantaneous volume and the flow rate Q, considering the bladder a thin-walled sphere: $v=Q/2R^2$, where R is the bladder radius.[5] The settings used for Q on the infusion pump were 0.36, 0.71, 1.43 and 3.51 ml/s. The bladder start volumes and the duration of the bladder emptying phase, which ranged from 1.9 to 7.9 ml and from 1.2 to 2.5 seconds,

were adapted to Q to end bladder emptying at volumes ranging from 0.6 to 6.1 ml. The v values thus obtained ranged from 0.23 to 2.61 cm/s. The experimental procedure resulted in two active pressure values at the same volume: a value P(v) at the shortening velocity v caused by the pump and an isovolumetric value P_{isv1} (Fig.2). By dividing these two pressure values, data were obtained which were used to calculate v_{max} from equation (1). To study the volume dependence of v_{max}, this parameter was derived algebraically from equation (1) for each separate measurement. Next, equation (1) was fitted to the pooled pressure ratio/shortening velocity data for each bladder and for both stimulation frequencies, where v_{max} was the parameter to be changed. The Levenberg-Marquardt algorithm was used. The resulting v_{max} values found for both stimulation frequencies were compared using the paired t test.

Because it was found that (as in Fig.2) the second isovolumetric contraction was generally much higher than the isovolumetric part of the stop-flow contraction, the ratio P_{isv1}/P_{isv2} and its volume and shortening velocity dependence were also studied.

The pressure decay in the isovolumetric contractions (second part of Fig.2) was studied by dividing the active pressure P(6.5) at the end of the 6.5 second stimulation period by the maximum active pressure P_{isv2} attained in this period and plotting this ratio as a function of the bladder circumference. The bladder circumference (length) dependence of the ratio $P(6.5)/P_{isv2}$ was compared for both stimulation frequencies used.

In the above derivations, active pressure in the stop-flow contractions was defined as the instantaneous pressure minus the pressure after the contraction had subsided. In the completely isovolumetric contractions it was defined as the instantaneous pressure minus the pressure before the contraction.

Results

Examples of the test measurements in an empty organ bath and in a rubber balloon, performed at the highest flow rate used in the bladder experiments, are shown in Fig.3. It can be seen that the measured pressure faithfully reflected the pump volume and remained constant as soon as the pump stopped. No overshoot or bias during and after pumping was observed. Fig.2 shows a typical example of the measured intravesical pressure in two consecutive contractions, a stop-flow contraction with bladder emptying caused by the pump and a completely isovolumetric contraction. The effect of the flow rate on the measured pressure is obvious. The maximum pressure values in the two consecutive contractions at the same volume were generally considerably different, as in Fig.2: the mean value of the ratio P_{isv1}/P_{isv2} of all 114 measurements (Fig.4) was 0.67 ± 0.12 (SEM). For all 5 bladders and for both stimulation frequencies, the regression coefficient of this ratio on bladder volume and on shortening velocity was calculated. The 95% confidence interval of the regression coefficient on volume contained the value 0 ml^{-1} in 6 of the 10 cases; it contained only positive values in 3 cases and only negative

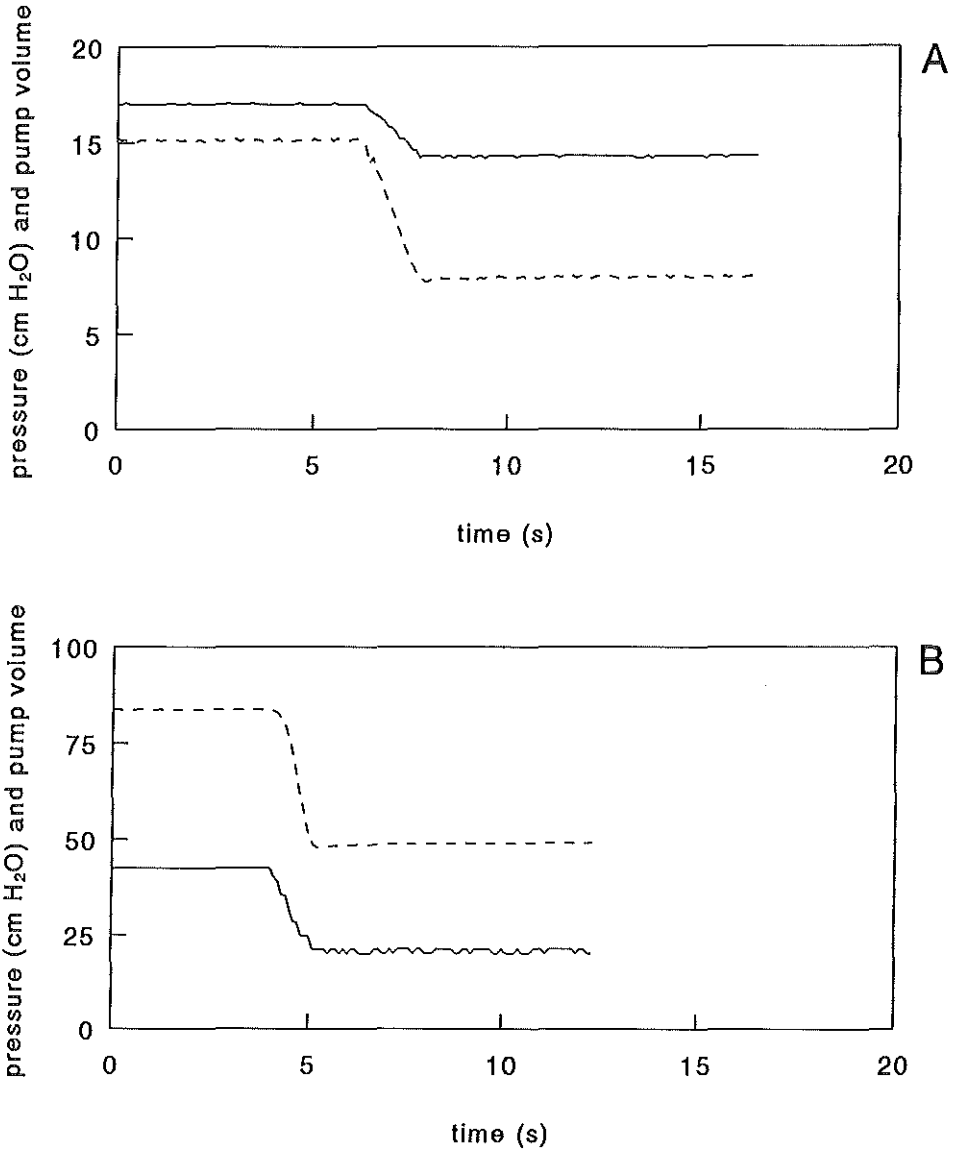


Figure 3. Results of test measurements at a flow rate of 3.51 ml/s. A: Catheters were at the bottom of the organ bath. B: Catheters were in a rubber balloon, situated in the bath. Solid lines: pump volume (arbitrary units). Broken lines: measured pressure.

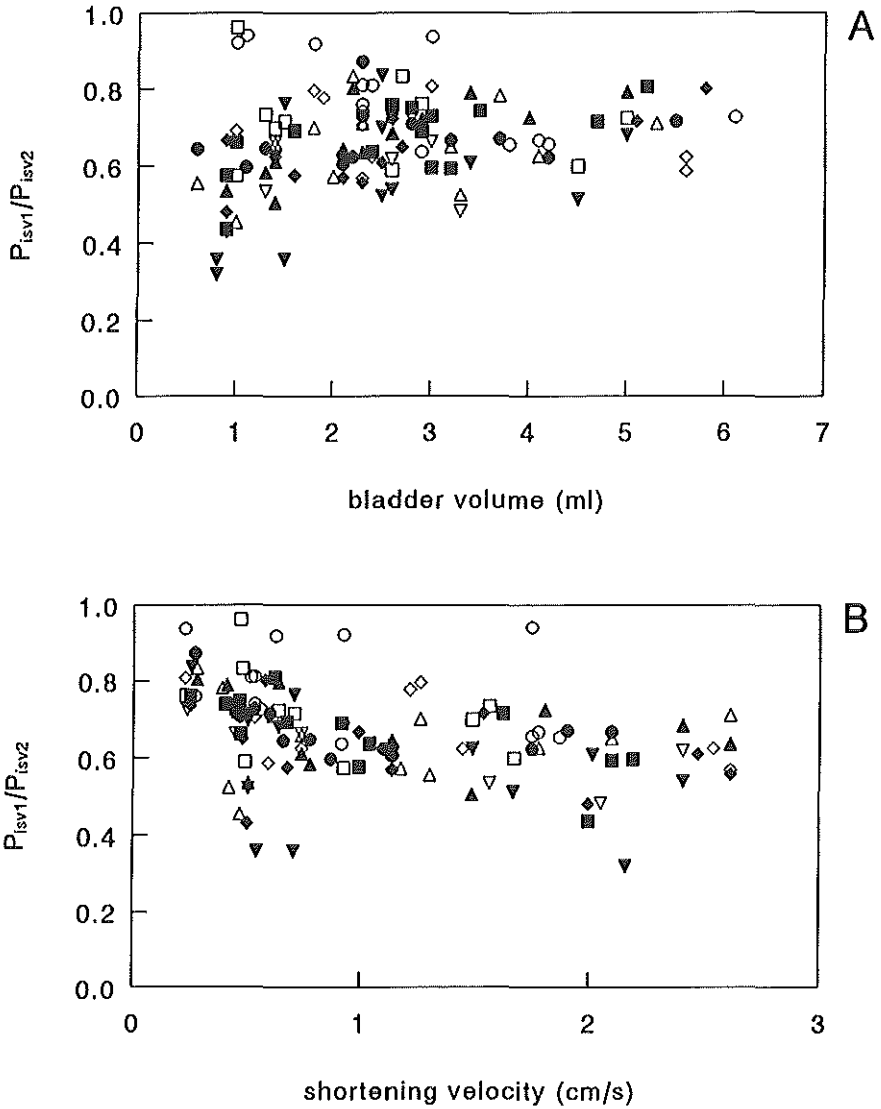


Figure 4. The ratio P_{isv1}/P_{isv2} of the maximum active pressure in the isovolumetric contraction immediately following detrusor shortening and in a completely isovolumetric contraction some minutes later as a function of the bladder volume (A) and the detrusor shortening velocity (B). Each symbol represents the results from one bladder. Filled and open symbols represent stimulation frequencies 200 and 25 Hz.

values in 1 case. The regression coefficient on shortening velocity was negative in all 10 cases, but in only one case was it significantly different from 0 $(cm/s)^{-1}$ at the 0.05 level. Fig.5 shows the algebraically derived v_{max} values as a function of volume for all 5 bladders and for both stimulation frequencies. Linear regression analysis showed that in only one of the 10 cases was the regression coefficient significantly different from 0 cm/s/ml at the 0.05 level. The result of fitting equation (1) to the pressure ratio / shortening velocity data of one bladder is shown in Fig.6. The five v_{max} values for the five different bladders, measured at stimulation frequencies 200 and 25 Hz, averaged 3.75 $\pm$ 0.08 (SEM) and 3.00 $\pm$ 0.22 cm/s. These values were significantly different (p=0.015).

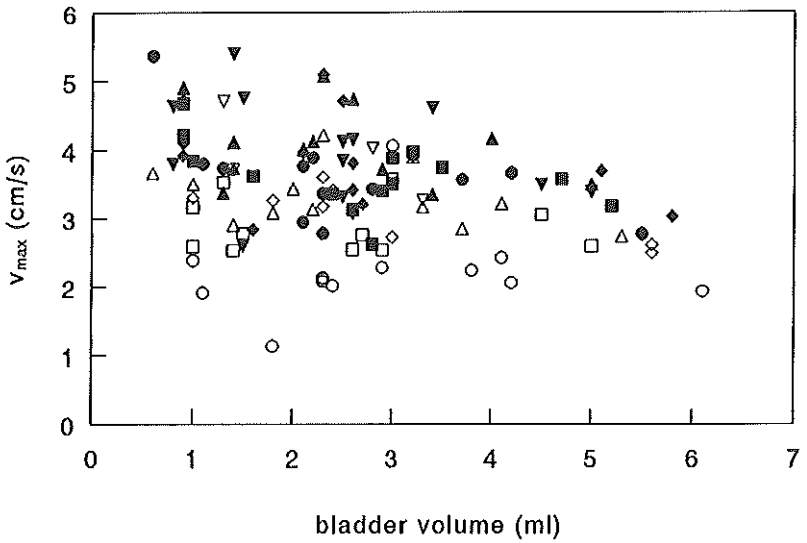


Figure 5. The v_{max} values as a function of bladder volume, as derived algebraically from equation (1), for all measurements in all five bladders at both stimulation frequencies. Different symbols represent the results from different bladders. Filled and open symbols represent stimulation frequencies 200 and 25 Hz.

In the second contraction in Fig.2 the pressure already started decreasing during stimulation. The decay in pressure was clearly volume dependent (Fig.7A). A straight line was fitted to all consecutive points where $P(6.5)/P_{isv2}$ was less than 0.95. The bladder circumference corresponding to the intersection with the line $P(6.5)/P_{isv2}=1$ was called cut-off circumference. It could be concluded that the cut-off circumferences at stimulation frequencies 200 and 25 Hz were not significantly different (p=0.62) and averaged to a

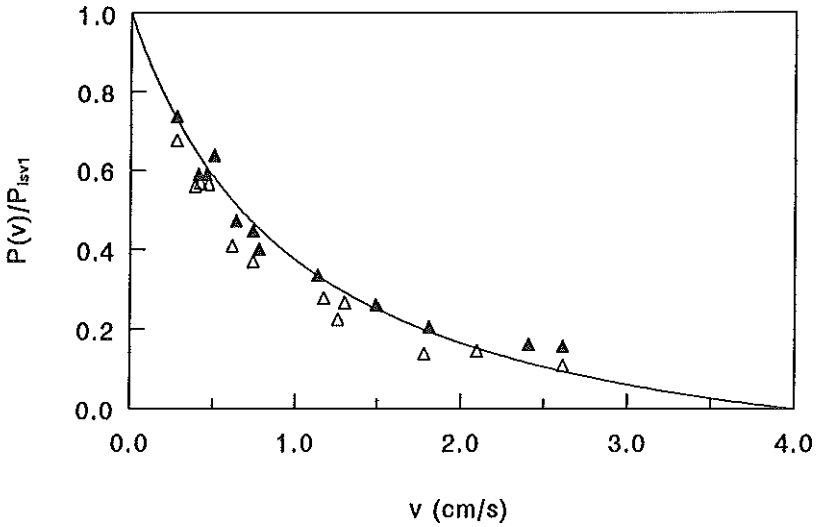


Figure 6. Pressure ratio/shortening velocity data, obtained in one bladder at both stimulation frequencies: 200 Hz (filled symbols) and 25 Hz (open symbols). The curve is the result of fitting equation (1) to the 200 Hz data.

value corresponding to a bladder volume of about 3.5 ml. In this small sample the cut-off circumference did not correlate with bladder weight ($p=0.58$). Fig.7B shows a plot of all $P(6.5)/P_{isv2}$ values in the 5 bladders as a function of bladder circumference, normalized for the cut-off circumference. The bladder circumference dependence of the ratio $P(6.5)/P_{isv2}$ was not significantly different for the two stimulation frequencies.

Discussion

Maximum shortening velocity

Values for v_{max}, the maximum (unloaded) shortening velocity of the detrusor muscle, were determined in vitro by simulating stop-flow tests. At a number of different flow rates or shortening velocity values, two pressure values were obtained at exactly the same bladder volume. The first of these was the pressure at the tested shortening velocity and the second the pressure at zero shortening velocity, or the isovolumetric pressure. To these values the Hill equation (1) was fitted, resulting in an estimate for v_{max} of the tested bladder at the used level of stimulation. The derivation of v_{max} from pressure values measured at imposed shortening velocities permitted the use of a sample frequency as low as 10 Hz. The limited number of flow rate settings of the infusion pump made it necessary to do the measurements at different volumes. This procedure is justified if v_{max}

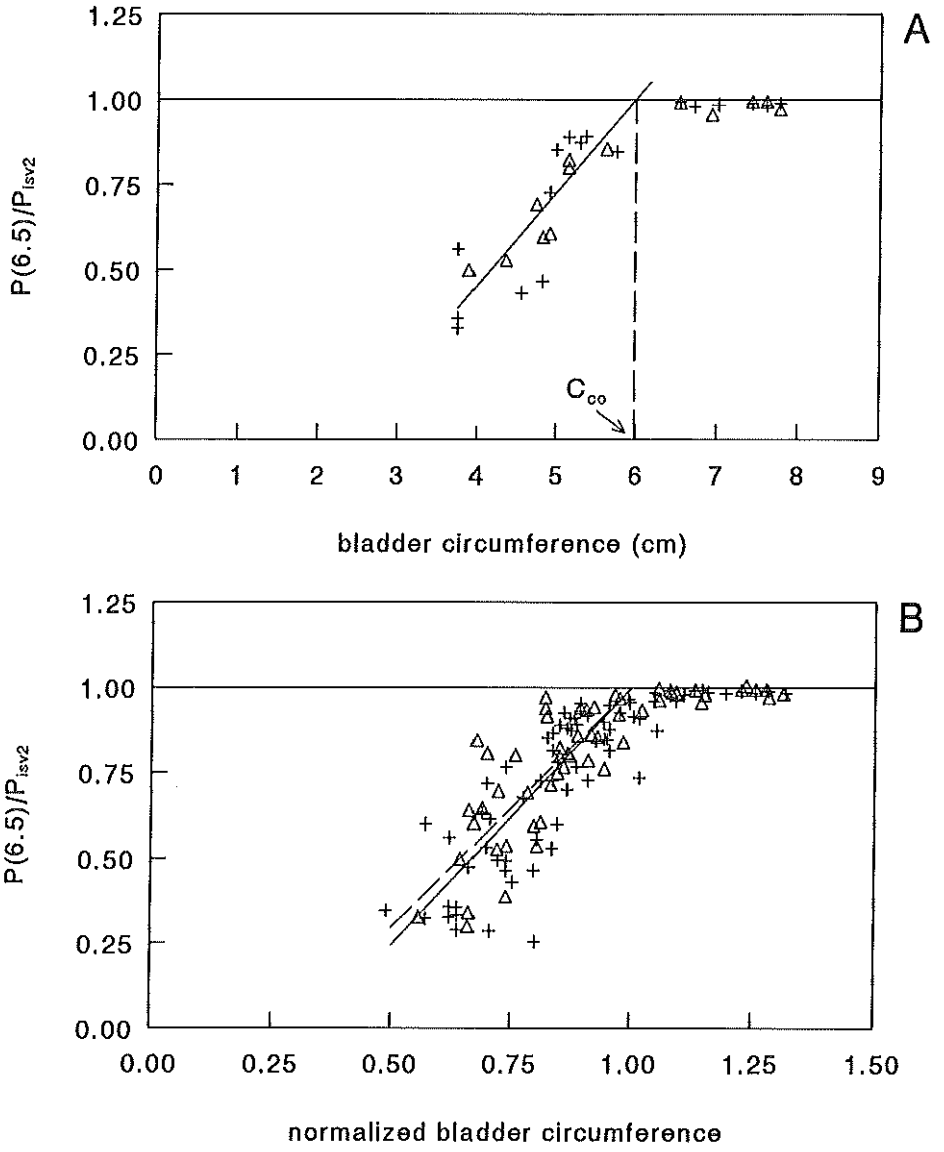


Figure 7. A: Pressure decay versus bladder circumference as measured in one bladder at stimulation frequencies 200 Hz (+) and 25 Hz (△). The straight line was fitted to the data for which $P(6.5)/P_{isv2} < 0.95$ at 200 Hz stimulation. C_{co} is the cut-off bladder circumference. B: Pooled data of pressure decay versus bladder circumference, the latter being normalized for the cut-off circumference. Symbols as in A. Solid and dotted lines correspond to 200 and 25 Hz data.

is volume independent. Theoretically the maximum shortening velocity of muscle is independent of the number of interacting cross-bridges and therefore of the stretched length.[6] The volume independence of v_{max} was previously observed in vivo[3] and was now confirmed in vitro by analysis of the volume dependence of the algebraically derived v_{max} values (Fig.5). The independence of v_{max} on bladder volume in the range studied permitted expression of v_{max} in terms of cm/s instead of muscle lengths/s; that is, no reference to the volume at which isometric force is maximal was needed. In the derivation of the (active) pressures P(v) during shortening and P_{isv1} during the subsequent isovolumetric part of the contraction, the pressure after the stop-flow contraction had subsided was taken as the passive pressure. This method implies that viscoelastic relaxation during shortening was disregarded, resulting in an underestimation of both the P(v) and the P_{isv1} values. Griffiths et al. showed that the viscoelastic effect is small compared with active forces (pressures).[7] Therefore, the neglect of this effect probably affected the v_{max} values only marginally.

Electrical stimulation was used to induce a bladder contraction. Previous work, performed at a bladder volume of 2.8 ml, showed that the stimulation parameters 10 V, 200 Hz, 2.5 ms resulted in optimum stimulation, that is, isovolumetric pressure was maximal, while at the setting 10 V, 25 Hz, 20 ms pressure was 58% of maximum.[2] The v_{max} values found at these settings in the present study, 3.75 $\pm$ 0.08 and 3.00 $\pm$ 0.22 cm/s, were significantly different as well. It can be concluded that, in our model, both parameters characterizing bladder contractility depended on the intensity of stimulation. The origin of this dependence cannot unequivocally be deduced from our experiments. Possibly the bladder is not uniformly stimulated at a submaximal intensity, implying that parts of the bladder do not contribute to contraction, despite the fact that the geometrical conditions were identical at both stimulation intensities used. In vivo, submaximal neurogenic stimulation might correspond to a situation in which neurotransmitters are not available to a sufficient degree to initiate a uniform contraction in the bladder.

Decay of pressure during stimulation

In the derivation of v_{max} the pressure P(v) at the moment flow stopped was compared with the maximum pressure P_{isv1} in the isovolumetric part of the same contraction. These two pressure values were separated in time by about 2 seconds. In the subsequently measured completely isovolumetric contractions, it was observed that, at small bladder volumes, pressure decreased quickly after a maximum had been reached, even while stimulation continued. Because the same phenomenon may have occurred in the stop-flow contractions where it could not be observed, it was carefully studied to evaluate the degree to which it might have affected the present results. This was done by examining the ratio $P(6.5)/P_{isv2}$, the quotient of the active pressure at the end of the 6.5 second stimulation period and the maximum active pressure attained in this period. Fig.7B shows that, for both stimulation frequencies, a cut-off circumference, above which pressure remained fairly constant in the time period studied, could be identified. A decay of

pressure after the maximum was described earlier in the pig and rabbit bladder,[7,8] although pressure decreased much more slowly in these bladders. The decrease in pressure while stimulation continues was previously shown to be volume dependent in the rabbit bladder.[9] It is of interest to realize that the two cut-off circumferences for the two stimulation frequencies were not significantly different. In addition, the degree of decrease in pressure was similar for both stimulation frequencies when bladder circumference was smaller than the cut-off circumference. These findings imply that the observed stimulation intensity dependence of v_{max} was not biased by a possible decrease in pressure. The independence of v_{max} on bladder volume (Fig.5) suggests that the v_{max} values themselves were not biased by such a decrease either.

Effect of preceding shortening on isovolumetric pressure

The two isovolumetric pressure values in each measurement were obtained at exactly the same volume. It was found that these values were considerably different. No dependence of the ratio of these values on bladder volume or stimulation intensity was seen. This suggests that the phenomenon is not due to macroscopic effects of bladder stimulation, but is related to muscle shortening itself. It is known that striated muscle fibres generate less isometric force immediately after active shortening to a certain length compared with an isometric contraction at the same length without preceding shortening.[10] This shortening-induced weakening of contraction has been called "shortening-induced deactivation". The phenomenon is ascribed to a reduced affinity of the binding sites for calcium on the thin filaments.[10] Morphologic evidence for deactivation at short lengths was first reported by Taylor and Rüdel.[11] Our data suggest that shortening-induced deactivation also occurs in the smooth muscle of the guinea pig urinary bladder. As the pressure values measured during shortening in the present experiments should be related to isovolumetric values at the same state of activation, the time delay by which these two pressures are measured should be minimized. Therefore the lower value of P_{isv1}, rather than P_{isv2}, was used in the derivation of v_{max}. The effect of shortening-induced deactivation measured in the guinea pig bladder, a reduction in isovolumetric pressure of $33 \pm 12\%$, was much larger than the reduction in isometric force of 4.0 to 7.5% measured by Edman in striated muscle fibers.[10] The difference cannot be explained by a possible decay in pressure in the stop-flow contraction, because it was also present at bladder volumes larger than 3.5 ml, where isovolumetric pressure remained nearly constant after the maximum (Fig.4A). The deactivation being shortening-induced, a negative regression coefficient of the ratio P_{isv1}/P_{isv2} on the shortening velocity was expected. Such a relationship was not found. Edman showed that the degree of force reduction was not markedly affected by shortening velocities in the range of v_{max} to 1/5 of v_{max}.[12]

The effect of shortening on the isometric force has been demonstrated in whole muscle in situ[12] and might also exist in the human bladder. The duration of the effect is unknown from our experiments. Unless this period is very short, it has implications for

the clinically employed stop-flow test. In this test the patient is asked to interrupt micturition. The isovolumetric pressure occurring afterwards is used to assess bladder contractility. Deactivation would underestimate it.

References

1. *Groen, J., van Asselt, E., van Mastrigt, R., Bosch, R. and van Koeveringe, G.A.: Comparison of detrusor contractility of guinea pig bladders in situ and strips from these in vitro. J.Urol. 150: 1002-1006, 1993.*
2. *Groen, J., van Mastrigt, R. and Bosch, R.: Contractility of the guinea pig bladder measured in situ and in vitro. Neurourol.Urodyn. 13: 587-595, 1994.*
3. *Groen, J., van Mastrigt, R., van Asselt, E., van Koeveringe, G.A. and Bosch, R.: Contractility parameters of the guinea pig bladder in situ: similarity to human bladder contractility. J.Urol. 151: 1405-1410, 1994.*
4. *Griffiths, D.J. and van Mastrigt, R.: The routine assessment of detrusor contraction strength. Neurourol.Urodyn. 4: 77-87, 1985.*
5. *van Mastrigt, R. and Griffiths, D.J.: Contractility of the urinary bladder. Urol.Int. 34: 410-420, 1979.*
6. *Huxley, A.F.: Muscle structure and theories of contraction. Prog.Biophys.Biophys. Chem. 7: 255-318, 1957.*
7. *Griffiths, D.J., van Mastrigt, R., van Duyl, W.A. and Coolsaet, B.L.R.A.: Active mechanical properties of the smooth muscle of the urinary bladder. Med.Biol.Eng. Comput. 17: 281-290, 1979.*
8. *Levin, R.M., Ruggieri, M.R., Gill, H.S., Haugaard, N. and Wein, A.J.: Studies on the biphasic nature of urinary bladder contraction and function. Neurourol.Urodyn. 6: 339-350, 1987.*
9. *Kaplan, S.A., Blaivas, J.G., Brown, W.C. and Levin, R.M.: Parameters of detrusor contractility: I: the effects of electrical stimulation, hysteresis, and bladder volume in an in vitro whole rabbit bladder model. Neurourol.Urodyn. 10: 53-59, 1991.*
10. *Edman, K.A.P.: Depression of mechanical performance by active shortening during twitch and tetanus of vertebrate muscle fibers. Acta Physiol.Scand. 109: 15-26, 1980.*
11. *Taylor, S.R. and Rüdel, R.: Striated muscle fibers: inactivation of contraction induced by shortening. Science 167: 882-884, 1970.*
12. *Edman, K.A.P.: Mechanical deactivation induced by active shortening in isolated muscle fibres of the frog. J.Physiol. 246: 255-275, 1975.*

Chapter 6

Shortening induced deactivation in the guinea pig urinary bladder

J. Groen, R. van Mastrigt, R. Bosch

Acta Physiologica Scandinavica, in press

Abstract

Shortening induced deactivation, the depressant effect of active muscle shortening on the subsequently measured isometric force, has been shown in smooth muscle strips and rings. The guinea pig bladder permits the investigation of this phenomenon in a whole organ preparation. Previous work in our laboratory showed that shortening of the in vitro guinea pig detrusor muscle had a depressant effect on the isovolumetric pressure that could be generated immediately afterwards. To test the hypothesis that this was caused by deactivation, the effects of active and passive detrusor shortening on the subsequently measured isovolumetric pressure were compared. The isovolumetric pressures measured after five minute periods of recovery were taken as control values.

It was found that the isovolumetric pressure after passive shortening was 7% smaller than the isovolumetric pressure without preceding shortening. This difference was ascribed to viscoelastic relaxation during shortening. Active shortening had an additional 8% depressant effect on isovolumetric pressure compared with passive shortening. The effects of active and passive shortening differed significantly. It was concluded that shortening induced deactivation in the guinea pig urinary bladder smooth muscle in toto can be considered proven.

The fact that deactivation is shown both by striated and smooth muscle preparations is in line with the assumption that it is caused by reduced actin-myosin interaction. The hypothesis that (in striated muscle) the latter is effected by a decrease in troponin-calcium binding, however, needs reconsideration.

Introduction

It has been known for a long time that active shortening of striated muscle has a depressant effect on isometric force, i.e. when a muscle is allowed to shorten to a certain length during stimulation, the isometric force that is generated after shortening is smaller than the force that would have been attained if the muscle had been kept at the shortened length throughout.[1] It is also smaller when compared with a situation where the muscle had shortened passively to that length. The phenomenon has been studied extensively by Edman, who called it "shortening induced deactivation".[2] Based on theoretical considerations and experiments, it is thought that the deactivation is caused by cross bridge cycling induced structural changes in the contractile system.[2,3] As shortening of smooth muscle is effected by cross bridge cycling as well, deactivation can also be expected in this type of muscle. Several authors indeed found a depressant effect of active shortening on isometric force in isotonic afterloaded and quick release experiments.[4,5] These experiments were done in muscle strips or rings. In the course of experiments in our laboratory on total guinea pig bladders in vitro it was noted that the isovolumetric pressure measured immediately after bladder emptying was much smaller than the pressure measured 5 minutes later in an isovolumetric contraction.[6] In these experiments, micturition was simulated by inducing a bladder contraction electrically and simultaneously emptying the bladder with an infusion pump. By suddenly stopping the pump while bladder stimulation continued, detrusor pressure rose to isovolumetric values. It was hypothesized that the noted difference in pressure values should also be ascribed to shortening induced deactivation. As this might have consequences for a clinically used bladder contractility test, this hypothesis was tested in the present study. Pressures measured after active and passive shortening were compared with the pressure in isovolumetric contractions without preceding shortening of the guinea pig detrusor. From previous work in our laboratory it is known that in isovolumetric contractions pressure decreases after a maximum has been reached, even while stimulation continues.[6] The degree of this pressure decay during stimulation depends on the bladder volume. Because the results of the present work might be biased by this decay, the experiments were performed at two values of the bladder volume to study the extent of this phenomenon.

Materials and Methods

Five male guinea pigs weighing 820 to 1280 g were urethane-anaesthetized (1.2 g/kg i.p.). The bladder was exposed via a midline incision in the abdominal wall. After ligation of both ureters the bladder was excised and kept at room temperature in a buffer solution of the following composition: NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.0; $NaHCO_3$ 8.0; HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) 10.5 and glucose 5.1 mmol/l; NaOH was added to adjust the pH to 7.4.

Two catheters were inserted via the remaining part of the urethra: one 5 F catheter, which was connected to a disposable pressure transducer, and a stainless steel tube (inner diameter 1.7 mm), which was connected to a Harvard Apparatus (Millis, Massachusetts) model 2202 infusion pump via a low-compliance plastic tube. The bladder was suspended in an organ bath containing 85 ml of the above buffer solution, which was equilibrated with 95% O_2 - 5% CO_2 at a temperature of 37°C. The pH was in the range 7.3-7.4 and the osmolality was close to 300 mOsm. The bladder was initially filled with room temperature saline to a volume of 3 ml to accommodate for one hour before the start of the experiments.

Next, the bladder volume was increased to a start volume, using the infusion pump at a speed of 2.1 ml/min. The actual values of the two start volumes used will be discussed below. The bladder was stimulated electrically for 8.0 seconds at intervals of 5 minutes, using two parallel platinum electrodes (20 x 30 mm), one on each side of the bladder. Rectangular pulses, generated by a Dytech Corporation (Santa Clara, California) model 801 pulse generator and amplified by a Kepco (Flushing, New York) model BOP 72-5 (M) bipolar operational power amplifier, were used. The stimulation parameters were set to optimum values determined in an earlier study, i.e., the pulse amplitude, frequency and width were 10 V, 200 Hz and 2.5 ms.[7] At this pulse width, the response to electrical stimulation is probably partly neurogenic and partly myogenic in nature.[8] A specially developed device inverting alternate pulses was used in order to minimize electrolysis of the buffer solution. Control experiments demonstrated that using this device gas bubbles could be observed at the electrodes only after about two minutes of continuous stimulation. With monophasic pulse stimulation, however, the bubbles developed within a few seconds. The buffer solution could be refreshed within a few seconds from a heated and aerated storage container; this was done every 30 minutes. With this procedure, reproducible results were obtained for several hours. Two kinds of measurements, called "active shortening sequences" and "passive shortening sequences", were performed. An active shortening sequence consisted of the following phases (Fig.1): During the first 2.0 seconds of stimulation the bladder contracted isovolumetrically at the start volume. During the following 2.5 seconds the bladder was emptied using the infusion pump at a flow rate of 0.71 ml/s. During the last 3.5 seconds the bladder contracted isovolumetrically at the resulting end volume. These three successive phases composed what will be called a "stop-flow contraction". After 5 minutes an isovolumetric bladder contraction was induced once more by stimulating for another 8.0 seconds at the end volume as a control measurement. In a passive shortening sequence (Fig.2) the bladder was first emptied for 2.5 seconds at a flow rate of 0.71 ml/s and immediately afterwards stimulated for 8.0 seconds. After 5 minutes another isovolumetric contraction was induced by stimulating the bladder for 8.0 seconds as a control measurement. In a previous study the isovolumetric pressure decreased after a maximum had been reached, even while stimulation continued.[6] The decrease in pressure in a 6.5 second stimulation period was less than 5% when the bladder volume was above 3.5 ml. Below this volume,

the pressure decrease was strongly volume dependent. For this reason, the start volumes used were 3.8±0.1 (SEM) and 5.9±0.1 ml, so that the end volumes were 2.0±0.1 and 4.1±0.1 ml. Active and passive shortening sequences were measured in alternating order. Approximately 8 active and 8 passive shortening sequences were measured in each bladder. All contractions were sampled at a rate of 10 Hz with a personal computer, using a Statham/Gould model SP1400 pressure monitor (Gould Inc., Statham Instruments Division, Oxnard, California).

The maximum active pressures P_{isv1} in the stop-flow contractions and P_{isv2} in the subsequent isovolumetric contractions were determined and compared. Active pressure was defined as the instantaneous pressure minus the pressure just before the isovolumetric contraction, as indicated in Figs.1 and 2. As illustrated in Fig.1, it was again observed that in the isovolumetric contractions pressure decreased after the initial maximum while stimulation continued. To account for a similar possible decay in pressure during the stop-flow contractions, where it cannot be observed, the following method was used (Fig.1). The time interval τ from the start of stimulation to the maximum in the stop-flow contraction was determined. Next, the active pressure $P_{\tau 2}$ in the subsequent isovolumetric contraction, τ seconds after stimulation started, was determined. P_{isv1} was compared with $P_{\tau 2}$ as well as with P_{isv2}.

Statistical significance was evaluated with analysis of variance and the t test.

Results

A total number of 43 active and 42 passive shortening sequences were measured in the 5 bladders. Figs.1 and 2 show typical examples of the measured intravesical pressure recordings in both kinds of measurements. The fast increase of pressure upon stimulation and the gradual return to the passive baseline level in the 5 minute rest period were similar in all measurements. The effect of detrusor shortening on the measured pressure is obvious in both the active and the passive shortening sequence. It can be seen that the maximum pressure after active shortening was much smaller than the maximum in the subsequent isovolumetric contraction. In the passive shortening sequences both maxima were more or less similar, although the paired t test, applied to the pooled data, showed that the first isovolumetric contraction was on average significantly smaller than the second one ($p < 10^{-11}$); the mean difference was about 7%. The values of the ratios P_{isv1}/P_{isv2} and $P_{isv1}/P_{\tau 2}$, measured at both volumes studied, are summarized in Table 1.

Analysis of variance showed that the P_{isv1}/P_{isv2} ratios depended significantly on the bladder number ($p < 0.0005$) and the nature of preceding shortening (active versus passive; $p < 0.0005$), but not on the bladder volume ($p = 0.65$). Using the $P_{isv1}/P_{\tau 2}$ instead of the P_{isv1}/P_{isv2} values when the muscle was shortening actively, the pressure ratios were additionally significantly different between both volumes ($p = 0.004$). However, applying analysis of variance to the subset of active shortening sequences only, i.e. deleting the

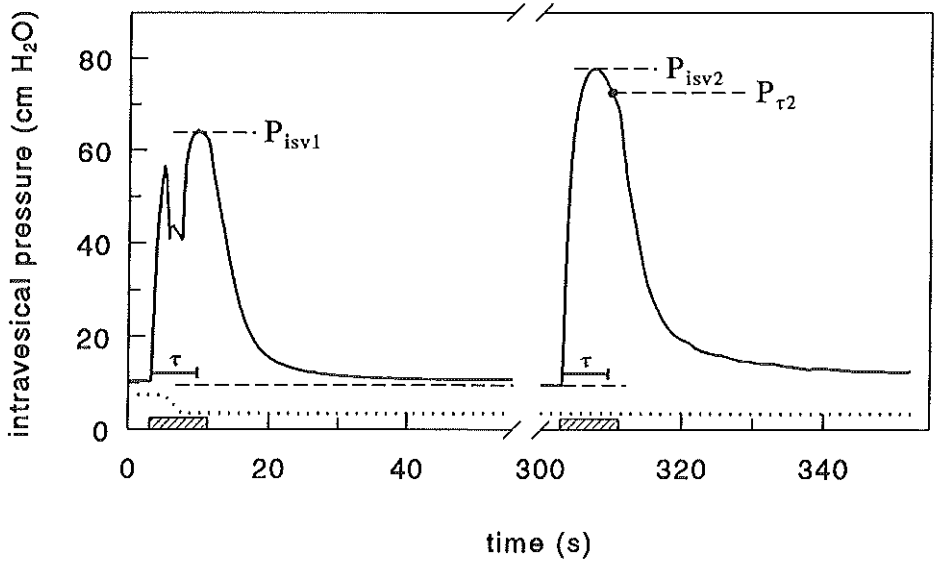


Figure 1. Schematic representation of the experimental procedure and the measured pressure signal in an active shortening sequence. A stop-flow contraction was followed by a completely isovolumetric contraction. During the time intervals indicated with the shaded boxes, the bladder was stimulated electrically. The bladder volume is indicated by the dotted line (arbitrary units). The start and the end volume were 3.6 and 1.8 ml. The dashed line indicates the applied zero level of active pressure; see text. For τ, P_{isv1}, P_{isv2} *and* $P_{\tau 2}$*: see text.*

nature of preceding shortening as an independent variable, the $P_{isv1}/P_{\tau 2}$ values depended significantly on the bladder number ($p<0.0005$), but not on the volume ($p=0.10$). It can be seen from Table 1 that the differences between the $P_{isv1}/P_{\tau 2}$ values at both volumes were small for all bladders.

Discussion

The depressant effect of active shortening on the subsequently measured isometric force has been demonstrated in smooth muscle strips and rings. As a seemingly similar effect was noted in earlier studies in our laboratory in guinea pig bladders in vitro, and this effect might have consequences for a clinically used test for urinary bladder contractility, the effects of active and passive shortening of the guinea pig detrusor muscle on isovolumetric pressure development were compared in the present study. The

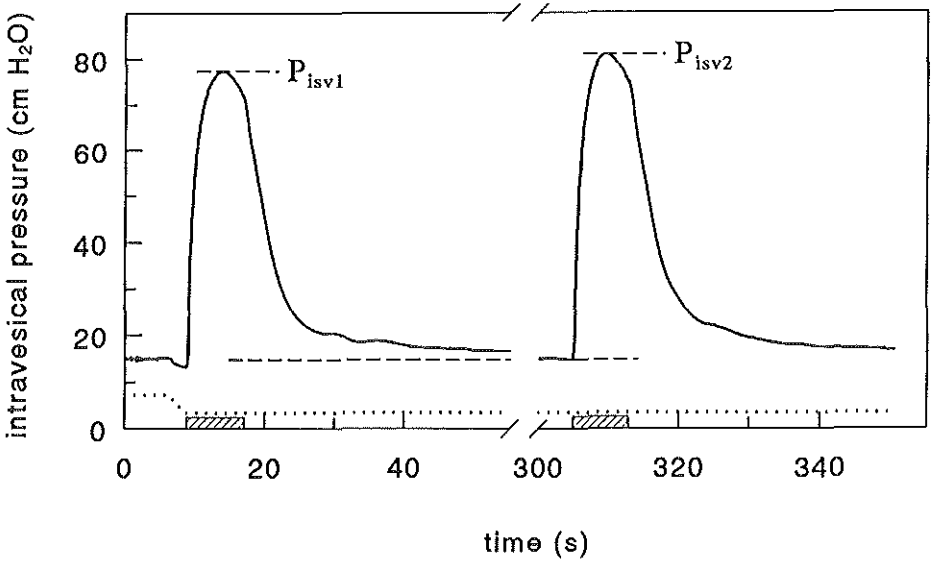


Figure 2. Schematic representation of the experimental procedure and the measured pressure signal in a passive shortening sequence. Immediately after decreasing the bladder volume from 3.6 ml to the end volume of 1.8 ml, an isovolumetric contraction was induced electrically. This contraction was followed by one without preceding bladder emptying. Symbols as in Fig.1.

isovolumetric pressure measured after 5 minutes was taken as a control value. A first finding was that the isovolumetric pressure measured after passive shortening was on average 7% smaller than the isovolumetric pressure in the control measurements. This is probably caused by viscoelastic relaxation during shortening.[9] As a consequence of this mechanism, passive pressure immediately after shortening is smaller than the steady state value, implying that the P_{isv1} values were underestimated in our analysis. As in first approximation passive and active pressures are independent and additive,[9] this viscoelastic relaxation most likely also occurred when the detrusor shortened actively, so that it may be assumed that it did not affect the comparison of the effects of passive and active shortening.

In the isovolumetric contractions, pressure decreased after a maximum had been reached, even while stimulation continued. Because such a decay might also have been present in the stop-flow contractions, where it cannot be observed, it is unclear if the maximum pressure in these contractions can reliably be related to the maximum in the isovolumetric measurements. Therefore, an additional method of analysis was used in which the possible decay of pressure in the stop-flow contractions was allowed for.

Table 1. Values of the ratio P_{isv1}/P_{isv2} in active and passive shortening sequences and of the ratio P_{isv1}/P_{r2} in active shortening sequences at both volumes studied.

	V = 2.0 ± 0.1 ml			V = 4.1 ± 0.1 ml		
bladder	active P_{isv1}/P_{isv2}	active P_{isv1}/P_{r2}	passive P_{isv1}/P_{isv2}	active P_{isv1}/P_{isv2}	active P_{isv1}/P_{r2}	passive P_{isv1}/P_{isv2}
1	0.81 [5] (0.77-0.87)	0.89 [5] (0.88-0.92)	0.97 [5] (0.91-1.02)	0.87 [5] (0.86-0.88)	0.90 [5] (0.89-0.90)	0.95 [5] (0.93-0.98)
2	0.80 [5] (0.79-0.82)	0.88 [5] (0.85-0.90)	0.96 [5] (0.92-1.00)	0.83 [5] (0.82-0.85)	0.86 [5] (0.84-0.88)	0.93 [5] (0.90-0.95)
3	0.82 [5] (0.80-0.84)	0.86 [5] (0.84-0.88)	0.95 [5] (0.92-0.98)	0.85 [5] (0.82-0.88)	0.87 [5] (0.84-0.90)	0.93 [5] (0.92-0.94)
4	0.76 [4] (0.73-0.79)	0.80 [4] (0.77-0.81)	0.90 [4] (0.84-0.94)	0.72 [3] (0.66-0.76)	0.78 [3] (0.77-0.80)	0.89 [3] (0.88-0.89)
5	0.83 [4] (0.82-0.85)	0.87 [4] (0.86-0.89)	0.94 [4] (0.92-0.95)	0.76 [2] (0.73-0.79)	0.82 [2] (0.78-0.85)	0.89 [1]
mean	0.80±0.03 [23]	0.86±0.04 [23]	0.94±0.04 [23]	0.82±0.06 [20]	0.85±0.04 [20]	0.93±0.03 [19]

Values given for the individual bladders are mean values and ranges. Values given in the last row are the mean values and the standard deviations of the pooled data in each column. The numbers in square brackets refer to the number of measurements composing the mean. In the bladders 4 and 5, the experiment was interrupted because of leakage at the attachment of the bladder to the catheters.

Previous work showed that the decrease in isovolumetric pressure in a 6.5 second stimulation period was less than 5% when the bladder volume was above 3.5 ml.[6] Additionally, it was noted that in the stop-flow contractions the maximum occurred within 6.5 seconds. As a result a difference of less than 5% was expected between the 4th and 5th column of Table 1. On average this was found. The difference between the 1st and 2nd column, however, was only slightly larger. This indicates that the time delay between the moments at which P_{isv2} and P_{r2} (Fig.1) were read was small in relation to the decrease in pressure.

Irrespective of the method of analysis used, the depressant effect of active detrusor muscle shortening on the subsequently measured isovolumetric pressure was significantly larger than the corresponding effect of passive shortening. Shortening induced deactivation in the smooth muscle of the guinea pig bladder can, therefore, be considered proven. The fact that this phenomenon occurs both in striated and smooth muscle may contribute to the discussion on the mechanisms by which it is caused. The involvement of calcium has been shown by Edman.[2] This author convincingly argued that deactivation must be explained by structural changes in the contractile system. These might decrease the affinity of the system for calcium and thus lead to a reduced actin-myosin interaction. Ekelund and Edman proposed that it is specifically the calcium affinity of troponin that changes as a result of filament sliding.[3] This hypothesis cannot universally explain the occurrence of shortening induced deactivation, because troponin is not found in smooth muscle.

No volume dependence of the depressant effect of active shortening could be shown. This corresponds with earlier work in which the P_{isv1}/P_{isv2} values were studied in the range from 0.6 to 6.1 ml.[6] The sarcomere length dependence of deactivation was studied by Edman in tetanic contractions of single frog skeletal muscle fibres in the range from 1.7 to 2.9 μm.[2] Maximum force after active shortening was typically 70% of the control value at 2.1-2.2 μm, while it was about 80% at the extremes of the range studied. The author noted, however, that the depressant effect varied more between different fibres than at different lengths. Possibly, therefore, the volume independence of the depressant effect found in the present study on total guinea pig bladder can be explained by assuming that the length dependence of the individual fibres is obscured by a considerable fibre to fibre variability.

Shortening induced deactivation has also been demonstrated in situ.[10,11] The conclusion that it is found in the in vitro guinea pig bladder can therefore probably be extrapolated to the human bladder. In a group of 63 men with prostatism, Sullivan et al. compared the isovolumetric pressure after balloon occlusion of the bladder neck during voiding with the isovolumetric pressure that was generated when the patient tried to void against a continuously obstructed urethra.[12] Contrary to what would be expected, no significant difference was found. Perhaps the ability to generate maximal isovolumetric pressure was regained in the course of contraction in these experiments.

It is concluded that shortening induced deactivation has been demonstrated in the in

vitro guinea pig detrusor muscle. The magnitude of this effect was 8% in excess of the effect that was measured after passive shortening and was independent of the bladder volume for the values studied. The fact that deactivation is shown both by striated and smooth muscle preparations is in line with the assumption that it is caused by reduced actin-myosin interaction. The hypothesis that (in striated muscle) the latter is effected by a decrease in troponin-calcium binding, however, needs reconsideration. Shortening induced deactivation does not seem to invalidate clinical tests used in urology.

References

1. *Jewell, B.R. and Wilkie, D.R.: The mechanical properties of relaxing muscle. J.Physiol. 152: 30-47, 1960.*
2. *Edman, K.A.P.: Depression of mechanical performance by active shortening during twitch and tetanus of vertebrate muscle fibers. Acta Physiol.Scand. 109: 15-26, 1980.*
3. *Ekelund, M.C. and Edman, K.A.P.: Shortening induced deactivation of skinned fibers of frog and mouse striated muscle. Acta Physiol.Scand. 116: 189-199, 1982.*
4. *Boels, P.J., Claes, V.A. and Brutsaert, D.L.: Mechanics of K^+-induced isotonic and isometric contractions in isolated canine coronary microarteries. Am.J.Physiol. 258 (Cell Physiol. 27): C512-C523, 1990.*
5. *Uvelius, B.: Isometric and isotonic length-tension relations and variations in cell length in longitudinal smooth muscle from rabbit urinary bladder. Acta Physiol. Scand. 97: 1-12, 1976.*
6. *Groen, J., van Mastrigt, R. and Bosch, R.: Neurogenic modulation of micturition: the relation between stimulation intensity and the maximum shortening velocity of the guinea pig detrusor muscle. J.Urol. 153: 228-232, 1995.*
7. *Groen, J., van Mastrigt, R. and Bosch, R.: Contractility of the guinea pig bladder measured in situ and in vitro. Neurourol.Urodyn. 13: 587-595, 1994.*
8. *Sibley, G.N.A.: A comparison of spontaneous and nerve-mediated activity in bladder muscle from man, pig and rabbit. J.Physiol. 354, 431-443, 1984.*
9. *Griffiths, D.J., van Mastrigt, R., van Duyl, W.A. and Coolsaet, B.L.R.A.: Active mechanical properties of the smooth muscle of the urinary bladder. Med.Biol.Eng. Comput. 17: 281-290, 1979.*
10. *Joyce, G.C., Rack, P.M.H. and Westbury, D.R.: The mechanical properties of cat soleus muscle during controlled lengthening and shortening movements. J.Physiol. 204: 461-474, 1969.*
11. *Joyce, G.C. and Rack, P.M.H.: Isotonic lengthening and shortening movements of cat soleus muscle. J.Physiol. 204: 475-491, 1969.*
12. *Sullivan, M.P., DuBeau, C.E., Resnick, N. and Yalla, S.V.: Continuous outlet occlusion test: a simple, clinical urodynamic technique to assess detrusor contractile performance. Neurourol.Urodyn. 13: 421-422, 1994.*

Chapter 7

The influence of extracellular Ca^{2+} on the time course of isovolumetric pressure development in the guinea pig urinary bladder

J. Groen, R. van Mastrigt, R. Bosch

Acta Physiologica Scandinavica, in press

Abstract

Isovolumetric contractions were induced electrically in 5 guinea pig bladders in a 1.8 mM Ca^{2+} buffer solution at two different volumes. The rate of pressure development was studied by analysis of phase plots, which are plots of the rate of change of intravesical pressure as a function of pressure. At relatively large volumes the rate of pressure development showed a biphasic nature, suggesting that two different processes were involved in different phases of contraction. Extracellular Ca^{2+} was manipulated to identify these processes.

Removal of extracellular Ca^{2+} led to an 85% reduction of the maximum isovolumetric pressure in subsequent stimulations, but pressure developed faster. The biphasic nature of pressure development at large volumes tended to disappear.

It was concluded that at large bladder volumes pressure development in this type of smooth muscle is determined by the release of intracellularly stored Ca^{2+} in the early phase of contraction. In the final phase the rate of pressure development is limited by the extracellular Ca^{2+} influx. At small bladder volumes the Ca^{2+} influx takes place at a higher rate, so that the contribution of intracellular Ca^{2+} release to pressure development could not be identified at these volumes.

Introduction

Contraction of smooth muscle results from a complex series of events, in which Ca^{2+} plays a central role. It has been shown that force development is preceded by a rise in the cytoplasmic Ca^{2+} concentration $[Ca^{2+}]_i$. This Ca^{2+} originates from the extracellular space and intracellular stores, mainly the sarcoplasmic reticulum (SR). Binding of Ca^{2+} to calmodulin and of the $(Ca^{2+})_4$-calmodulin complex to myosin light chain kinase leads to phosphorylation of the myosin light chains. This permits the activation of myosin ATPase by actin.[1]

Muscle contraction can be triggered via a number of different stimulatory pathways, which results in different time courses for isometric force or isovolumetric pressure development. This time course can be investigated by phase plot analysis.[2] A phase plot is a plot of the rate of change of a variable as a function of that variable, which in the present study was intravesical pressure (Fig.1A). Phase plots of isovolumetric smooth muscle contractions can partly be characterized by a straight line (Fig.1B):

$$dp/dt = [P_{isv}-p]/C$$

where p is the measured active intravesical pressure, i.e. the pressure exceeding the (passive) pressure resulting from the viscoelastic properties of the bladder wall, P_{isv} is the maximum extrapolated active pressure, t is time and C is the time constant for isovolumetric pressure development. A linear relationship between dp/dt and p for a part of a contraction implies that pressure increased exponentially with time in that part.

It has been shown that in strips of pig detrusor muscle the time course of isometric force development, characterized by the time constant C as derived from phase plot analysis, is limited by the rate of influx of extracellular Ca^{2+}.[2] In contractions of complete guinea pig bladders in vitro it seemed that, depending on the bladder volume, two time constants instead of one were involved in isovolumetric pressure development.[3] It is tempting to hypothesize that these might be ascribed to extracellular Ca^{2+} influx and intracellular Ca^{2+} release. It was the aim of this study to test this hypothesis. Isovolumetric contractions of in vitro guinea pig bladders were induced electrically at two values of the extracellular Ca^{2+} concentration and at two different bladder volumes.

Materials and Methods

Five male guinea pigs weighing 905 to 975 g were urethane-anaesthetized (1.2 g/kg i.p.). The bladder was exposed via a midline incision in the abdominal wall. After ligation of both ureters it was excised and kept at room temperature in a buffer solution of the following composition: NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.0; $NaHCO_3$ 8.0; N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES) 10.5 and glucose 5.1 mmol/l; NaOH was added to adjust the pH to 7.4.

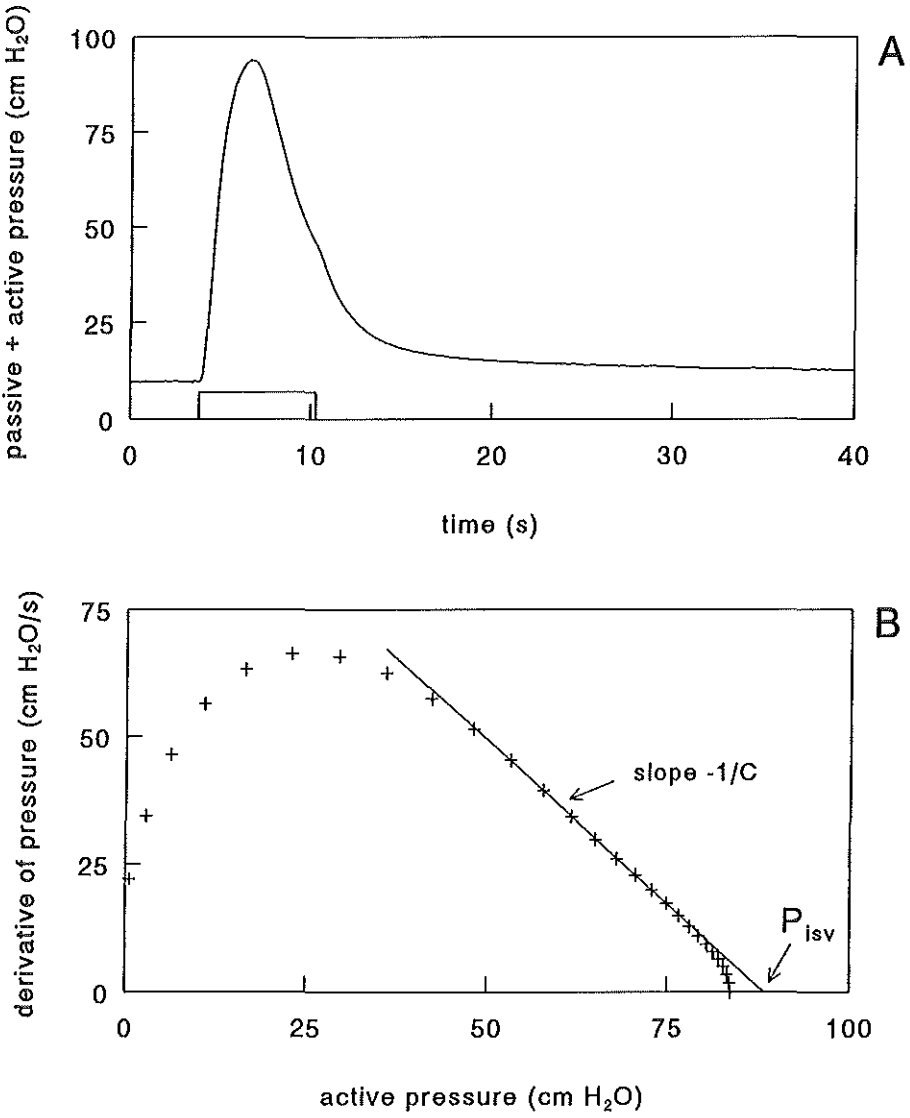


Figure 1. A: Pressure development as a function of time during an electrically induced isovolumetric contraction of a guinea pig bladder at a volume of 1.6 ml. The bladder was stimulated during the interval indicated by the box at the time axis. B: A phase plot of the contraction shown in A. The rate of pressure development is plotted as a function of the pressure. Part of the plot can be described by a straight line with parameters P_{isv} and C.

Two 5 F catheters were inserted via the remaining part of the urethra and connected to a disposable pressure transducer and to an infusion pump. The preparation was suspended in an organ bath containing 85 ml of the above buffer solution, which was called "1.8 mM Ca^{2+} buffer" and which was equilibrated with 95% O_2 - 5% CO_2 at a temperature of 37°C. The pH was in the range 7.2-7.4 and the osmolality was close to 300 mOsm. The solution could be refreshed within a few seconds from two heated and aerated storage containers; this was done repeatedly as described below. The bladder was filled with room temperature saline to a volume of 5 ml and allowed to accommodate for one hour before the start of the experiments.

Next, electrical stimulation was applied for 6.5 seconds at intervals of 5 minutes, using two parallel platinum electrodes (20 x 30 mm), one on each side of the bladder. The applied voltage, the pulse frequency and the pulse width were 10 V, 200 Hz and 2.5 ms (duty cycle 50%). Rectangular pulses, generated by a Dytech Corporation (Santa Clara, California) model 801 pulse generator and amplified by a Kepco (Flushing, New York) model BOP 72-5 (M) bipolar operational power amplifier, were used. A specially developed device inverting alternate pulses was used in order to minimize electrolysis of the buffer solution.

To examine the influence of extracellular Ca^{2+} on the time course of isovolumetric pressure development, the following scheme was applied: First the bladder was stimulated twice. Then the buffer solution was replaced by a buffer solution of the same composition but with the Ca^{2+} omitted. This solution was called the "low Ca^{2+} buffer"; the glass storage container containing this solution was cleaned with a 2 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) solution prior to the experiments. The bladder was stimulated 6 times, the first time 15 seconds after the buffer replacement. Next, the low Ca^{2+} buffer was replaced by the 1.8 mM Ca^{2+} buffer again and the bladder was subsequently stimulated 3 times, again the first time 15 seconds after the buffer replacement. To examine the bladder volume dependence of the time course of isovolumetric pressure development, the experiments were performed at volumes of 1.8 ± 0.1 (SEM) and 4.9 ± 0.2 ml. Previous work showed that isovolumetric pressure at these volumes is about 90 and 60% of the maximum, which is found at a volume of 1 ml.[3] All isovolumetric contractions were sampled at a rate of 10 Hz with a personal computer, using a Statham/Gould model SP1400 pressure monitor (Gould Inc., Statham Instruments Division, Oxnard, California).

Using the software package MATLAB the measured pressure signal in each contraction was filtered with a 1 Hz low pass filter. The derivative of the filtered pressure signal was calculated and plotted as a function of pressure to obtain a phase plot. A straight line was fitted to the part of the plot where the active pressure was between 78 and 90% of P_{max}, the maximum active isovolumetric pressure (Fig.2). The result of the fitting procedure was extrapolated to the maximum in the phase plot. In order to quantify objectively the degree to which the descending part of the phase plot could be described using one straight line (or time constant) only, two measures were

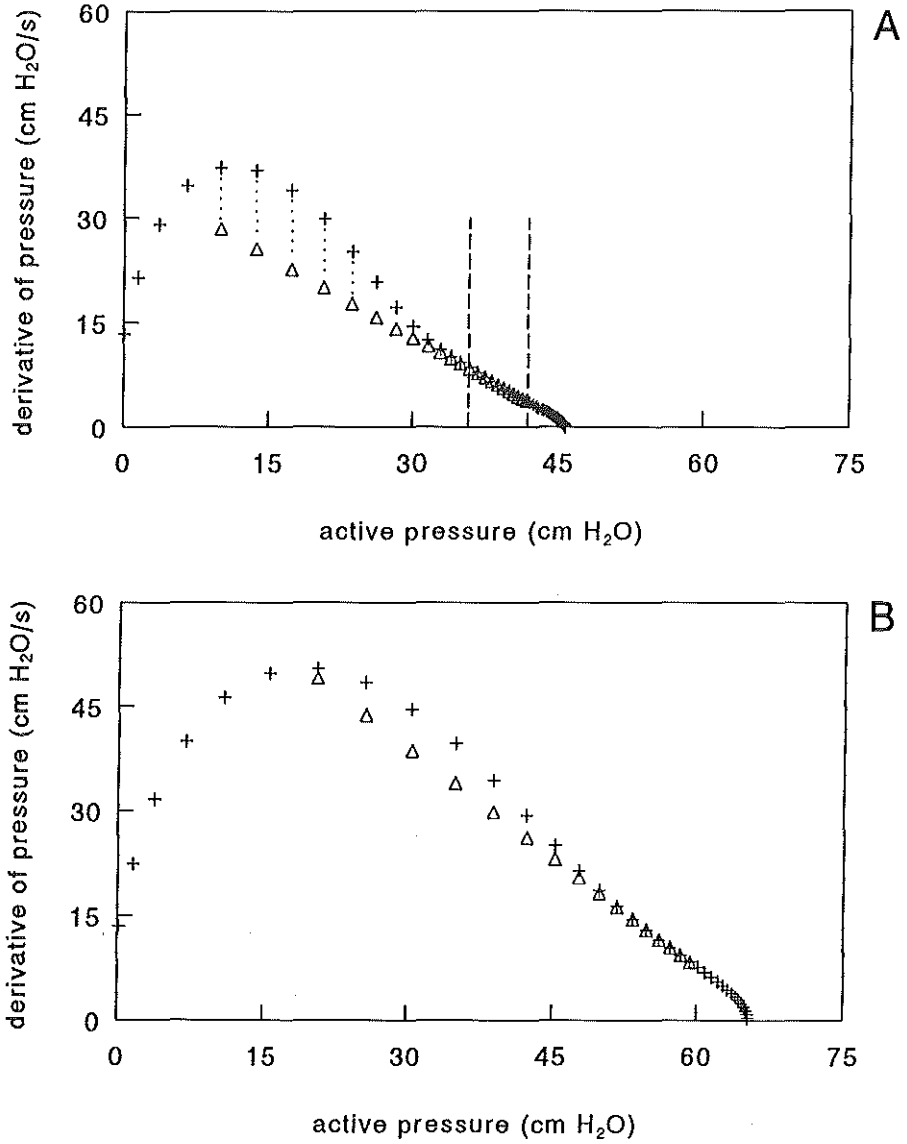


Figure 2. Phase plots of isovolumetric contractions of the same guinea pig bladder at a volume of 4.9 ml (A) and 1.8 ml (B) in the 1.8 mM Ca^{2+} buffer. Plus signs represent the measured data. To the part of the plot where active pressure was between 78 and 90% of maximum, as indicated by the dashed lines in A, a straight line was fitted. The triangles represent this line. The 5 dotted lines in A illustrate the definition of nssd. The dotted line at the lowest active pressure value illustrates the definition of δ_1; see text.

used. The first one was the normalized difference δ_1 between the maximum $(dp/dt)_{max}$ in the phase plot and the corresponding value $(dp/dt)_{fit,max}$ which resulted from the extrapolated fitted straight line, i.e.

$$\delta_1 = \frac{(\frac{dp}{dt})_{max} - (\frac{dp}{dt})_{fit,max}}{(\frac{dp}{dt})_{fit,max}}$$

This measure is indicated with the first dotted line in Fig.2A; see legend. The second measure was the normalized sum of squared deviations *nssd* between the actual and fitted phase plot of the first five samples starting from the maximum:

$$nssd = \sum_{i=1}^{5} \frac{[(\frac{dp}{dt})_i - (\frac{dp}{dt})_{fit,i}]^2}{[(\frac{dp}{dt})_{fit,ave}]^2}$$

where $(dp/dt)_i$ are the observed values of dp/dt, $(dp/dt)_{fit,i}$ are the values of dp/dt calculated from the extrapolated straight line and $(dp/dt)_{fit,ave}$ is the average of the 5 $(dp/dt)_{fit,i}$ values.

The averaged results for P_{max}, C, δ_1 and nssd of the first two contractions at each bladder volume were taken as baseline values. Analysis of variance was applied to compare the similarity of the δ_1 and nssd values at both volumes in the 1.8 mM Ca^{2+} buffer and the low Ca^{2+} buffer. The results obtained from the first contraction after the removal of extracellular Ca^{2+} were pooled with the 1.8 mM Ca^{2+} buffer because of the large similarity of the P_{max}, C, δ_1 and nssd values in this contraction to the baseline values (see Results).

Results

Examples of phase plots at volumes of 4.9 and 1.8 ml measured in the same guinea pig bladder are shown in Fig.2. The descending parts of the plots seem to consist of two phases. A straight line was fitted to the part where the active pressure was between 78 and 90% of the maximum pressure. This part was chosen to exclude the inflection in the plot and the curvature near maximum pressure from the fitting procedure. In the calculation of nssd 5 samples starting from the maximum of dp/dt were chosen to ensure that the samples near the inflection were not included in this measure.

Baseline values of the (passive) pressure preceding bladder contraction and of the parameters P_{max}, C, δ_1 and nssd are presented in Table 1. Fig.3A shows the relative decrease of the generated isovolumetric pressure after removal of the extracellular Ca^{2+}

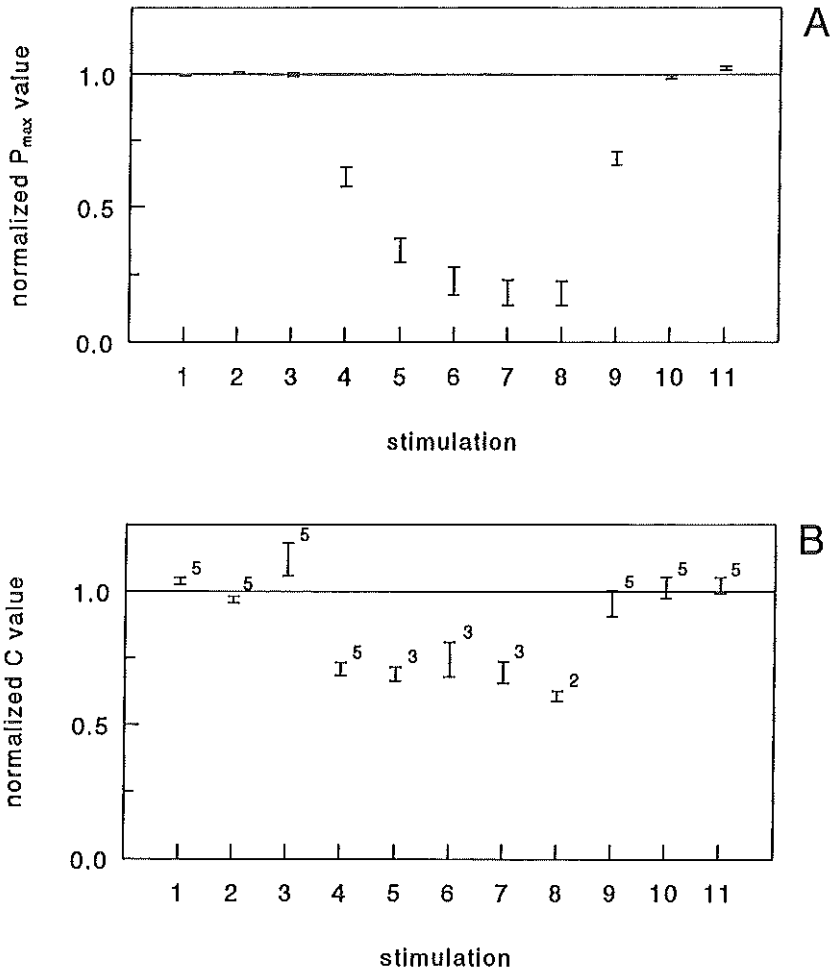


Figure 3. Mean values ± SEM of the measured maximum active pressure P_{max} (A) and of the time constant C (B) in the consecutive bladder contractions, normalized to the baseline values, at a volume of 4.9±0.2 ml. The numbers in B indicate the number of cases in which a straight line could be observed in the phase plot. Only in those cases were the C values used. A similar trend was found when the observations in B were confined to those from the two bladders in which a straight line could be observed in the phase plot of all contractions. The extracellular Ca^{2+} was removed and added again 15 seconds before the stimulations numbered 3 and 9. The time interval between the stimulations was 5 minutes.

Table 1. Baseline values for the parameters measured in the 5 guinea pig bladders at volumes of 4.9±0.2 and 1.8±0.1 ml.

	4.9 ml	1.8 ml	p
Passive pressure (cm H_2O)	9.9 ± 0.6	6.9 ± 0.9	0.101
P_{max} (cm H_2O)	46.8 ± 0.7	70.0 ± 2.5	0.001
C (s)	1.43 ± 0.08	1.11 ± 0.05	0.023
δ_1	0.37 ± 0.04	0.09 ± 0.04	0.001
nssd	1.08 ± 0.18	0.17 ± 0.07	0.007

Values shown are means and standard errors of the means. The p values resulted from the paired t test.

and the subsequent increase after its re-addition at a bladder volume of 4.9 ml. The proportional change in maximum pressure at 1.8 ml did not significantly differ from the one at 4.9 ml. In contrast to the active pressure, passive pressure did not change after exchange of the buffer solutions.

Comparing Fig.4 with Fig.2B illustrates the change in the shape of the phase plots

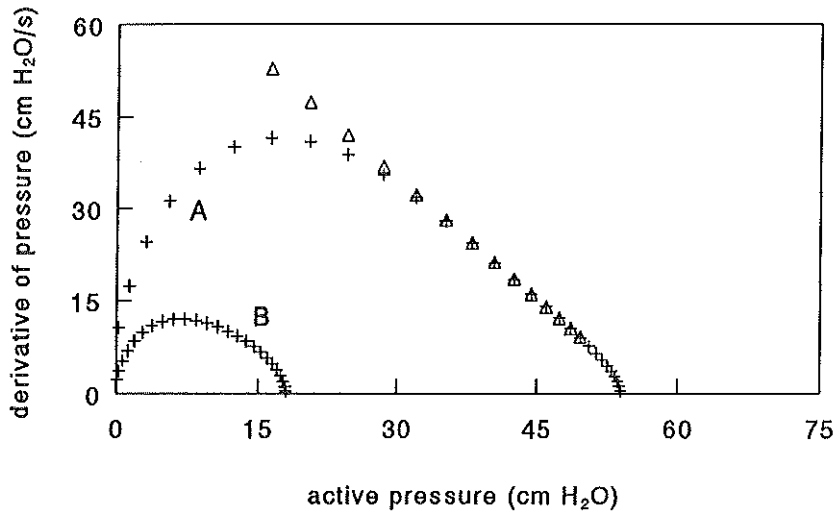


Figure 4. Phase plots of the fourth (A) and the seventh (B) isovolumetric bladder contraction at a volume of 1.8 ml. Just before the third contraction the extracellular Ca^{2+} was removed. Measurements were made on the same bladder as in Fig.2. Plus signs and triangles as in Fig.2. Note the absence of a straight line part in B.

after removal of the extracellular Ca^{2+}. When the plots clearly showed a convex aspect, as curve B in Fig.4, the calculated time constants were not used, because pressure development was obviously not monoexponential. The values of δ_i and nssd, however, were used, because they provide some information on the shape of the phase plots. Fig.3B shows the number of measurements where the time constant C could be reliably determined, and its decrease after removal of the extracellular Ca^{2+} at a volume of 4.9 ml. The time constant also decreased at 1.8 ml. It can be seen from Fig.5 that in the 1.8 mM Ca^{2+} buffer δ_i and nssd differed largely at 4.9 and 1.8 ml, while in the low Ca^{2+} buffer the values of these measures were more or less similar at both volumes. Analysis of variance confirmed this observation: in the 1.8 mM Ca^{2+} buffer the difference between δ_i and nssd at 4.9 and 1.8 ml was highly significant ($p < 0.0005$ for both measures), while in the low Ca^{2+} buffer the difference between the δ_i and nssd values at 4.9 and 1.8 ml resulted in p values of 0.021 and 0.084.

Discussion

The time course of isovolumetric pressure development was studied in guinea pig bladders in vitro. Phase plots were made of contractions measured at two values of the bladder volume. The shape of these plots depended on the volume. At a large volume three phases in pressure development could be discriminated, as illustrated in Fig.6. At both high and low bladder volumes the final phase of contraction, phase 3, could be characterized by a straight line in the phase plot. At high volumes the phase plots deviated significantly from the extrapolated straight line in phase 2. At small volumes there was no difference between the plotted data and this line. Phase 1 corresponds to the early onset of contraction and was of short duration. This phase was not further examined.

The biphasic nature of the descending part of the phase plots at large volumes suggests that two separate processes limit the rate of isovolumetric pressure development. Because Ca^{2+} is the primary regulator of contraction,[4] a close correlation between pressure development and changes in $[Ca^{2+}]_i$ may be expected. Ganitkevich and Isenberg studied $[Ca^{2+}]_i$ and its transients in muscle cells from the urinary bladder of the guinea pig after membrane depolarization.[5] They observed that the $[Ca^{2+}]_i$ transients consisted of two components. The first, phasic component was mainly caused by Ca^{2+} induced Ca^{2+} release (CICR): the release of Ca^{2+} from intracellular stores, induced by the influx of extracellular Ca^{2+}. The second, tonic component mainly originated from the ongoing extracellular Ca^{2+} influx. It was the aim of this study to test the hypothesis that intracellular Ca^{2+} release and extracellular Ca^{2+} influx correspond to the phases 2 and 3 in the phase plots. To this end the extracellular Ca^{2+} in the buffer solution was manipulated.

Maximum developed pressure fell to about 15% of the baseline value when the 1.8

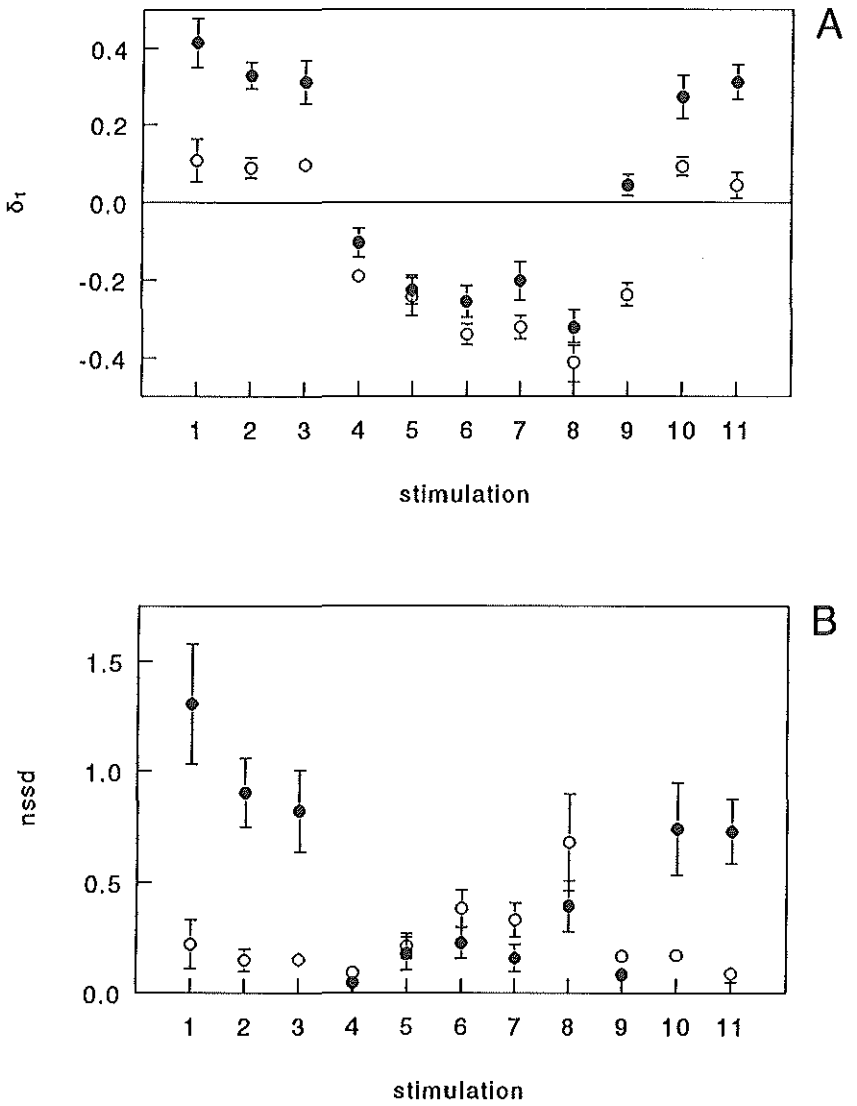


Figure 5. Mean values ± SEM of the measures δ_1 (A) and nssd (B) for the shape of the phase plot in consecutive bladder contractions at volumes of 4.9±0.2 ml (filled symbol) and 1.8±0.1 ml (open symbol). The extracellular Ca^{2+} was removed and added again 15 seconds before the stimulations numbered 3 and 9. The time interval between the stimulations was 5 minutes.

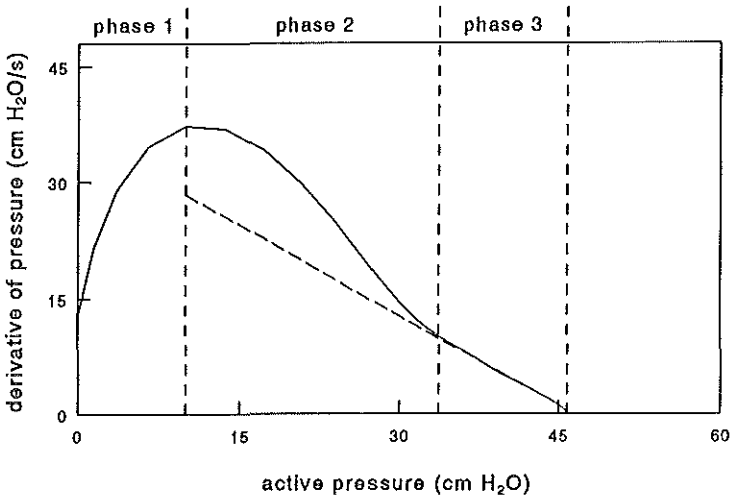


Figure 6. The same phase plot as in Fig.2A, schematically divided into three phases.

mM Ca^{2+} buffer was replaced by a low Ca^{2+} buffer (Fig.3A). This in itself does not necessarily prove that extracellular Ca^{2+} is the major source of activator Ca^{2+} in smooth muscle contraction, for it is known that removal of extracellular Ca^{2+} leads to depletion of SR Ca^{2+} stores.[4,6] In our study the decrease of the SR Ca^{2+} content was reflected by the fact that in the first bladder contraction after re-addition of extracellular Ca^{2+} P_{max} was about 68% and not 100% of the baseline value (Fig.3A): if the SR Ca^{2+} content had remained unchanged, an immediate return of P_{max} to baseline levels after restoration of the extracellular Ca^{2+} concentration $[Ca^{2+}]_o$ would have been expected.

In the first contraction after the removal of Ca^{2+}, i.e. in stimulation number 3 in Figs.3 and 5, maximum pressure was not significantly different from the baseline value, while the time constant for pressure development increased slightly, i.e. pressure developed a little slower. If phase 3 in the phase plot reflects the extracellular Ca^{2+} influx, these observations can be explained by assuming that sufficient Ca^{2+} had stuck to the surface of the bladder to generate a maximal contraction. An alternative explanation might, however, be that the intracellular Ca^{2+} stores were not depleted.

The low $[Ca^{2+}]_o$ led to a smaller, but faster pressure development in the next contractions. This is in line with the view that phase 3 reflects the extracellular Ca^{2+} influx. In the low Ca^{2+} buffer this phase would then tend to disappear, leaving only phase 2, in which pressure develops faster. Consequently, intracellular Ca^{2+} release must play a role in this phase. If phase 3 is caused by Ca^{2+} release, it is hard to imagine how pressure can develop faster when the Ca^{2+} stores become depleted. Phase 3 could not reliably be characterized by a straight line in all contractions in the low Ca^{2+} buffer. The tendency to show a more convex shape in the subsequent contractions is illustrated by the

increasingly negative values of δ_1 and the increasing values of nssd (Fig.5).

In the first contraction after restoration of $[Ca^{2+}]_o$, the straight line in phase 3 immediately reappeared. The time constant for pressure development in that contraction was approximately equal to the baseline value (Fig.3B), while maximum pressure was only 68% of baseline levels. Because the latter probably represents a depletion of the SR Ca^{2+} content, as argued above, these data are further evidence in favour of the hypothesis that phase 3 in the phase plots is related to extracellular Ca^{2+} influx. Because phase 3 is also the phase in which pressure develops at the lowest rate, this conclusion is in accordance with the results of previous work, performed in our laboratory, from which it was concluded that the influx of extracellular Ca^{2+} is the rate limiting factor in isometric force development in smooth muscle.[2] Most likely, the deviation from the straight line in phase 2 of the phase plots, as quantified by the objective parameters δ_1 and nssd, then originates from the release of intracellularly stored Ca^{2+}.

The mechanisms regulating smooth muscle contraction are complex. Intravesical pressure rises can therefore not be considered to be proportional to $[Ca^{2+}]_i$ transients. However, it is known that rates of force (or pressure) development are largely dominated by myosin light chain phosphorylation levels[7] and that the activation of myosin light chain kinase is Ca^{2+} dependent.[8] This, and the close temporal relationship between $[Ca^{2+}]_i$ and force,[1] stresses the central role of Ca^{2+} in pressure development and supplies arguments for the discussed interpretation of the shape of the phase plots in terms of $[Ca^{2+}]_i$ transients. It cannot, however, be excluded that membrane bound signal transduction mechanisms have been affected by the changes of $[Ca^{2+}]_o$ in our experiment. In vascular smooth muscle, a dual role of extracellular Ca^{2+} has been shown.[9] It not only serves as the activator of contraction, but also acts as a membrane stabilizer: the contractile response of rabbit aorta to epinephrine decreased when $[Ca^{2+}]_o$ increased. Therefore, membrane properties might depend on $[Ca^{2+}]_o$ in our experiment as well. In our view, however, an explanation for the biphasic nature of pressure development in terms of the $[Ca^{2+}]_o$ dependence of signal transduction mechanisms would be less convincing, especially when considering the biphasic nature of $[Ca^{2+}]_i$ transients demonstrated by Ganitkevich and Isenberg.[5]

The time constant for pressure development in phase 3 of contraction was significantly different at both volumes studied. At large volumes pressure developed much more slowly in this phase than in phase 2 (Fig.2A). This caused large deviations from the extrapolated straight line in phase 2. At a smaller volume the Ca^{2+} influx (or the processes following it) took place at a higher rate, so that the contribution of the intracellular Ca^{2+} release to pressure development could not be identified in the phase plot. Ganitkevich and Isenberg showed that the extracellular Ca^{2+} influx into guinea pig detrusor muscle cells almost entirely takes place through so-called L-type Ca^{2+} channels.[10] These are voltage-dependent channels of which the permeability is regulated by an additional Ca^{2+}-mediated negative feedback mechanism, i.e. Ca^{2+} influx is inhibited by an elevated $[Ca^{2+}]_i$. Possibly the permeability of these channels is configuration dependent.

Another explanation for the volume dependence of the time course of pressure development is a configuration dependence of one of the other factors by which this development is influenced, for instance, the Ca^{2+} sensitivity of myosin light chain phosphorylation, at high $[Ca^{2+}]_i$ values.

It is concluded that at large bladder volumes the rate of pressure development in an isovolumetric contraction has a biphasic nature. In the early phase of contraction pressure development is determined by the release of intracellularly stored Ca^{2+}, while in the final phase it is limited by the extracellular Ca^{2+} influx. Although Ca^{2+} influx leads to a lower rate of pressure development than Ca^{2+} release, it contributes to maximum pressure to a considerable extent. At small volumes Ca^{2+} influx seems to take place at a higher rate. As a consequence, the contribution of intracellular Ca^{2+} release to pressure development cannot be identified at these volumes.

References

1. *Somlyo, A.P. and Himpens, B.: Cell calcium and its regulation in smooth muscle. FASEB J. 3: 2266-2276, 1989.*
2. *van Koeveringe, G.A. and van Mastrigt, R.: Excitatory pathways in smooth muscle investigated by phase-plot analysis of isometric force development. Am.J.Physiol. 261: R138-R144, 1991.*
3. *Groen, J., van Mastrigt, R. and Bosch, R.: Contractility of the guinea pig bladder measured in situ and in vitro. Neurourol.Urodyn. 13: 587-595, 1994.*
4. *Himpens, B. and Somlyo, A.P.: Free-calcium and force transients during depolarization and pharmacomechanical coupling in guinea-pig smooth muscle. J.Physiol. 395: 507-530, 1988.*
5. *Ganitkevich, V.Y. and Isenberg, G.: Contribution of Ca^{2+}-induced Ca^{2+} release to the $[Ca^{2+}]_i$ transients in myocytes from guinea-pig urinary bladder. J.Physiol. 458: 119-137, 1992.*
6. *Janssen, L.J. and Sims, S.M.: Emptying and refilling of Ca^{2+} store in tracheal myocytes as indicated by ACh-evoked currents and contraction. Am.J.Physiol. 265: C877-C886, 1993.*
7. *Dillon, P.F., Aksoy, M.O., Driska, S.P. and Murphy, R.A.: Myosin phosphorylation and the cross-bridge cycle in arterial smooth muscle. Science 211: 495-497, 1981.*
8. *Murphy, R.A.: Crossbridges and contraction in smooth muscle. Neurourol.Urodyn. 11: 265-268, 1992.*
9. *Bohr, D.F.: Vascular smooth muscle: dual effect of calcium. Science 139: 597-599, 1963.*
10. *Ganitkevich, V.Y. and Isenberg, G.: Depolarization-mediated intracellular calcium transients in isolated smooth muscle cells of guinea-pig urinary bladder. J.Physiol. 435: 187-205, 1991.*

Chapter 8

Neurogenic modulation of the urethral resistance in the guinea pig

J. Groen, E. van Asselt, R. van Mastrigt, M. Kranse, R. Bosch

The Journal of Urology 155: 1471-1476, 1996

Abstract

Purpose: The resistance offered to urinary flow by the urethra is one of the factors determining the course of micturition. It was the aim of the present work to study the dependence of urethral resistance on the degree of relaxation of the urethra.

Materials and Methods: Experiments were done in the guinea pig. Ten animals were used. In 5 animals saline was forced through the (unrelaxed) urethra at imposed flow rates in the range of 1.1 to 43.0 ml/min while the urethral pressure was measured. Second degree polynomials were fitted to the pressure/flow data. In the other 5 animals micturition contractions were evoked and pressure/flow plots were derived from the measured signals. A straight line was fitted to the lowest pressure values at each flow rate in these plots. These pressure values represent the most relaxed state of the urethra in these voidings.

Results: The pressures measured in the unrelaxed urethra were much higher than the pressures measured during voiding in the same flow rate range, but the intercepts of the mathematical equations fitted to the pressure/flow data on the pressure axis were not significantly different in the two groups.

Conclusions: The unrelaxed urethra has a much "steeper" pressure/flow characteristic than the relaxed urethra. However, the urethral closing pressure, that is, the intercept of the pressure/flow characteristic on the pressure axis, does not depend on the state of relaxation of the urethra.

Introduction

The course of micturition depends on the contraction strength of the bladder and the resistance offered to urinary flow by the urethra. These two properties are modulated by the nervous system. A complete understanding of the function of the lower urinary tract therefore requires knowledge of the modulation mechanism. In previous work the dependence of bladder contractility on stimulation intensity was studied in a guinea pig bladder model in vitro.[1,2] The aim of the present work was to study the modulation of urethral resistance to flow by neurogenic stimulation in the guinea pig in vivo.

The resistance offered to flow by the urethra is reflected by the pressure head needed to drive a given flow rate through the urethra. The relationship between the instantaneous flow rate and the associated pressure was called "urethral resistance relation" by Griffiths.[3] In the normal urethra the urethral resistance relation depends on the state of relaxation of the urethral musculature, especially the urethral sphincter. This means that the urethral resistance relation varies with the intensity of neurogenic stimulation. In this study urethral resistance was measured at two levels of neurogenic stimulation: during a micturition contraction, when the urethra was relaxed, and in the absence of such a contraction, when it was contracted. Micturition contractions were volume evoked. Pressure/flow plots were derived from the measured pressure and flow rate signals. The lowest pressure values at each flow rate in these plots, which occur during the most relaxed state of the urethra, were compared with the pressures measured when flow was forced through the unrelaxed urethra.

Materials and Methods

Ten male guinea pigs weighing 817 to 1280 g were used. Each animal was anaesthetized with urethane (1.2 g/kg intraperitoneally) and rested on a heating pad to maintain body temperature. In 5 of the animals the urethral resistance relation was determined in the unrelaxed urethra, as follows: The bladder and proximal urethra as well as the most superficial distal part of the urethra were exposed through an abdominal incision. A 24 G angiocatheter was inserted into the proximal urethra, just distal to the bladder neck. Proximally, the urethra was sealed with a clamp to prevent leakage to the bladder. The catheter was connected to a Harvard infusion pump and a disposable pressure transducer with polyethylene tubes (inner diameter 1.5 mm) and a Y connector. The distance between the catheter tip and the pressure transducer was 50 cm. The distal part of the urethra was dissected from the underlying tissue. A 3-mm diameter ultrasonic flow probe was placed around this part and connected to a T106 flowmeter (Transonic Systems Inc., Ithaca, New York). The flow probe of this flow rate measurement system contains two ultrasound transducers located at one side of the urethra, while a reflector is located at the other side. The two transducers pass ultrasonic signals back and forth,

alternately intersecting the urine flow in upstream and downstream directions. The flowmeter measures the time it takes for the waves to travel from one transducer to the other. The difference between the upstream and downstream integrated transit times is a measure of the flow rate. Room temperature saline was pumped through the urethra. Flow rates of 1.1, 2.2, 5.4, 10.8, 21.4 and 43.0 ml/min were applied in a semirandom order while pressure was measured. The pressure and flow rate signals were sampled with a personal computer at a rate of 100 Hz. Fig.1 illustrates that, following a transient, steady state readings were attained for both the pressure and flow rate signal. The measured pressure signal was averaged over a time interval of 2.0 to 20 seconds (depending on the flow rate) during this steady state. The measured pressure values were corrected by subtracting the pressure loss in the measuring system caused by the imposed flow rate. This pressure loss, which is due to the resistance to flow in the catheter and to the Bernoulli effect, was determined in separate measurements (see Results). Atmospheric pressure was used as the reference level. Using the software package SPSS® (SPSS Inc., Chicago, Illinois), version 5.0.2, second degree polynomials defined by the equation

$$p_{ura} = p_{ura.clos.est} + a_1.Q + a_2.Q^2 \qquad (1)$$

were fitted to the measured pressure/flow data. In this equation, p_{ura} is the (corrected) measured urethral pressure and Q is the imposed flow rate. The parameter $p_{ura.clos.est}$ is the intercept of the urethral resistance relation on the pressure axis and was considered an estimate for the urethral closing pressure; the parameters a_1 and a_2 describe the slope and curvature of this relation. Two subsets of the polynomials were applied: one without a linear term ($a_1=0$ in equation (1)) and one with a linear term ($a_1 \neq 0$).

In the other 5 animals conventional pressure/flow studies were done, that is, pressure/flow plots were derived from micturition contractions. The bladder and the distal part of the urethra of these animals were exposed through an abdominal incision. A 24 G angiocatheter was inserted into the bladder dome. The infusion pump was used to fill the bladder via the catheter with room temperature saline at a rate of 0.53 ml/min until a micturition contraction occurred. Pressure was measured via the same catheter with a disposable pressure transducer connected by a Y connector. The pressure loss in the measuring system at the filling rate used was about 0.7 cm H_2O. The flow probe was placed around the distal urethra as described earlier and connected to the flowmeter. The pressure and flow rate signals were sampled with a personal computer at a rate of 10 Hz. The filling time was used to calculate the filled volume. The voided volume was collected in a glass funnel and measured. Residual volume was removed from the bladder with a syringe attached to the catheter. The time interval between two fillings was 10 to 15 minutes. Test measurements with plastic tubes simulating the urethra showed that the amplitude of the uncalibrated flow rate signal depended on the apposition of tube and flow probe, namely, detachment and reattachment of the flow probe generally resulted in a different amplitude of the flow rate signal at the same imposed flow rate. The variation in the signal amplitude depended on the material and the size of the tube. For this

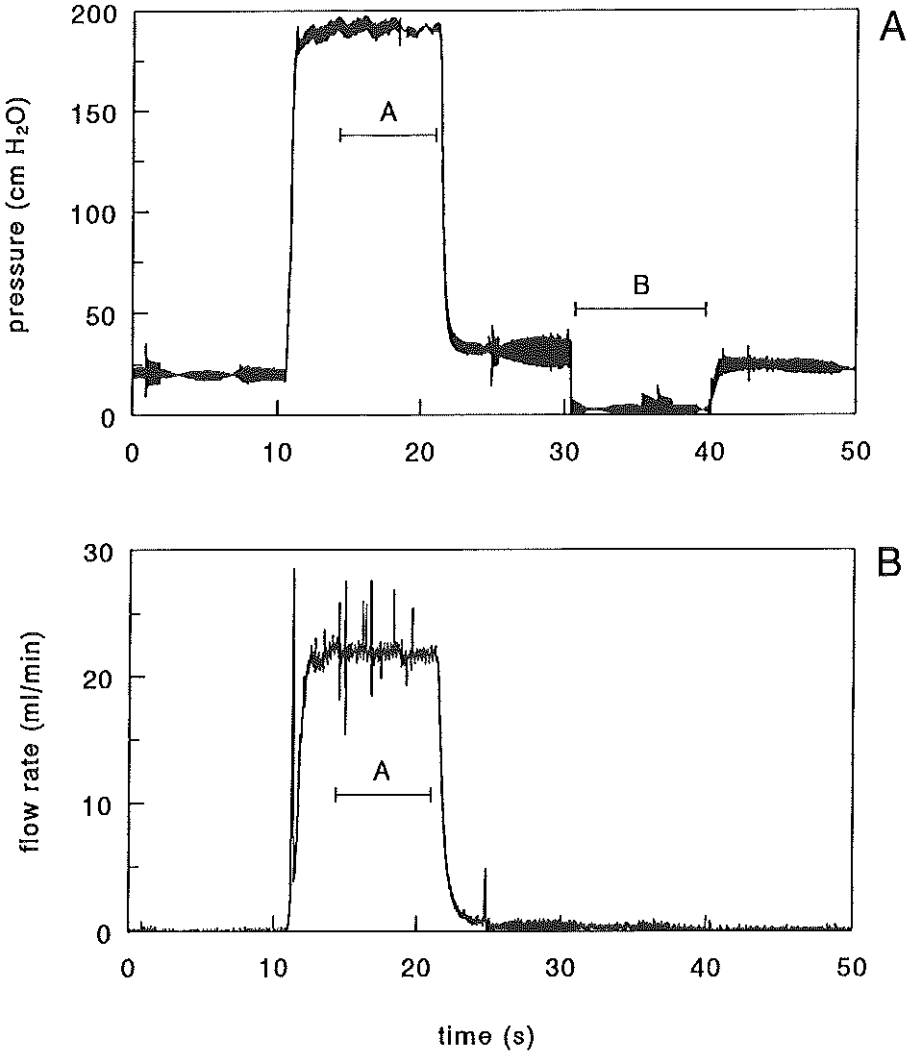


Figure 1. Typical example of pressure (A) and flow rate (B) measured in the unrelaxed urethra. At t=10.7 seconds, the infusion pump was switched on. A steady reading was attained in the time interval indicated with A. The signals were averaged over this interval. The pressure measured in time interval B is the atmospheric pressure, measured by opening the tubing system to air. In the absence of flow, the measured pressure does not reflect the pressure in the urethra. These parts of the pressure tracing should be ignored.

reason, the flow rate signals in all separate micturitions were multiplied by the quotient of the collected voided volume and the integrated flow rate signal as a calibration factor. The pressure and flow rate signals were filtered with a 1 Hz low pass filter and pressure/flow plots were made (Figs.2,3). No lag time correction between the pressure and flow rate signals was applied as both transducers were situated close together. The lowest part of each pressure/flow plot was selected according to the algorithm descibed by Kranse and van Mastrigt.[4] To exclude artifacts in the flow rate signal, only flow rate values exceeding 0.025 ml/s were used. A straight line was fitted to the selected data. The intercept $p_{ura.clos.est}$ on the pressure axis was considered an estimate of the urethral closing pressure. Frequently, the lowest part of the pressure/flow plot was characterized by a negative slope, as in Fig.3B. In those cases the minimum pressure $p_{ves.min.void}$ attained during voiding was read from the pressure/flow plot, while no value was assigned to $p_{ura.clos.est}$. Furthermore, the intravesical opening pressure $p_{ves.open}$, defined as the intravesical pressure recorded at the onset of measured flow,[5] was read from the pressure/flow plots (Figs.2,3).

Statistical significance was evaluated with the unpaired t test.

Results

Fig.1 shows an example of the measured urethral pressure and flow rate at an imposed flow rate of 21.4 ml/min. The pressure loss that was caused by filling of the bladder and pressure measurement through the same catheter is shown in Fig.4. A typical example of the urethral pressure, corrected for the pressure loss, as a function of the imposed flow rate is shown in Fig.5. A second degree polynomial with and without a linear term fitted to the data is also plotted in the figure. It is obvious that the linear term is necessary to adequately describe the urethral resistance relation. The (corrected) urethral pressure values measured at the imposed flow rates in the 5 animals are summarized in Table 1. The coefficients of equation (1) fitted to this data are shown in Table 2.

In the group of 5 animals in which conventional pressure/flow studies were done, a total of 31 voidings was analyzed. The mean bladder volume at the onset of micturition was 4.8 $\pm$ 0.3 (SEM) ml and the relative residue was 61 $\pm$ 3%. The calibration factor applied to the flow rate signal ranged from 0.009 to 0.049 in all animals as a result of its dependence on the apposition of urethra and flow probe. It varied as much as 40% within the same animal. The variation between two consecutive voidings of the same animal ranged from 0 to 20% and was 12 $\pm$ 2% on average. The shape of the pressure/flow plots was not uniform. Typical examples are shown in Figs.2B and 3B. Analysis was performed as follows: When the intercept of the straight line that was fitted to the lowest part of the pressure/flow plot on the pressure axis exceeded the minimum pressure $p_{ves.min.void}$, the latter was recorded. When the intercept did not exceed the minimum

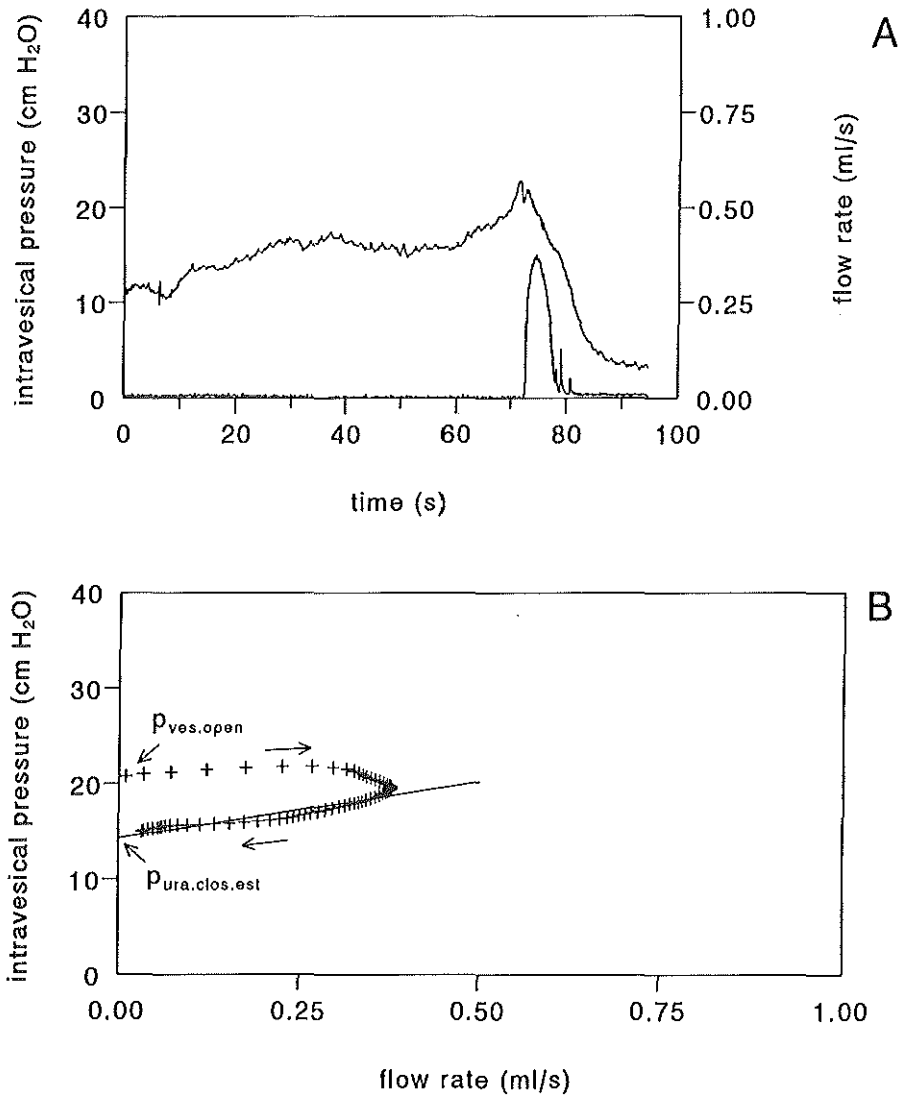


Figure 2. A: Typical example of an intravesical pressure and flow rate tracing in a pressure/flow study with positive slope of the pressure/flow plot. B: Pressure/flow plot derived from the data in A. The straight line was fitted to the lowest part of the plot as selected by the algorithm used. $p_{ura.clos.est}$, *the estimate for the urethral closing pressure, is the intercept of this line on the pressure axis.* $p_{ves.open}$, *the intravesical opening pressure, is the pressure at the onset of voiding. The arrows indicate the time course.*

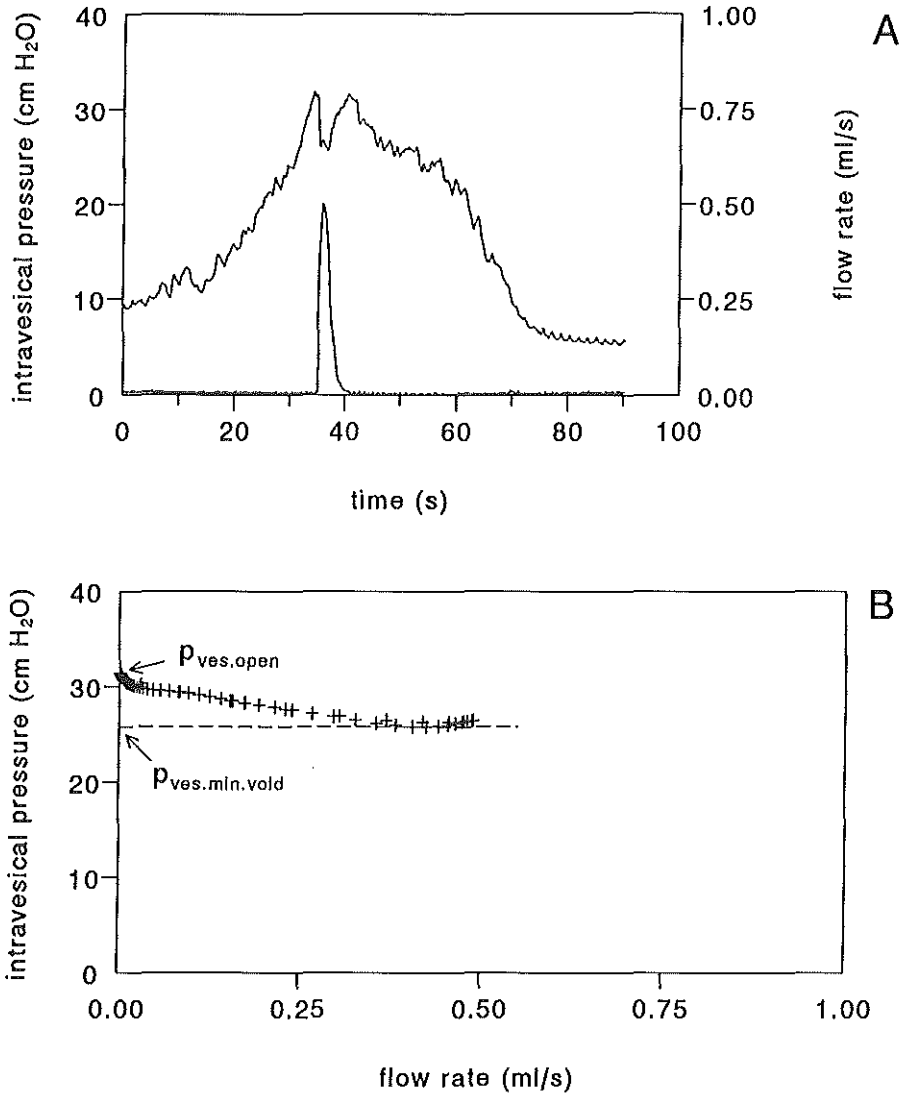


Figure 3. A: Typical example of an intravesical pressure and flow rate tracing in a pressure/flow study with negative slope of the pressure/flow plot. B: Pressure/flow plot derived from the data in A. $p_{ves,min,void}$ *is the minimum pressure attained during voiding.*

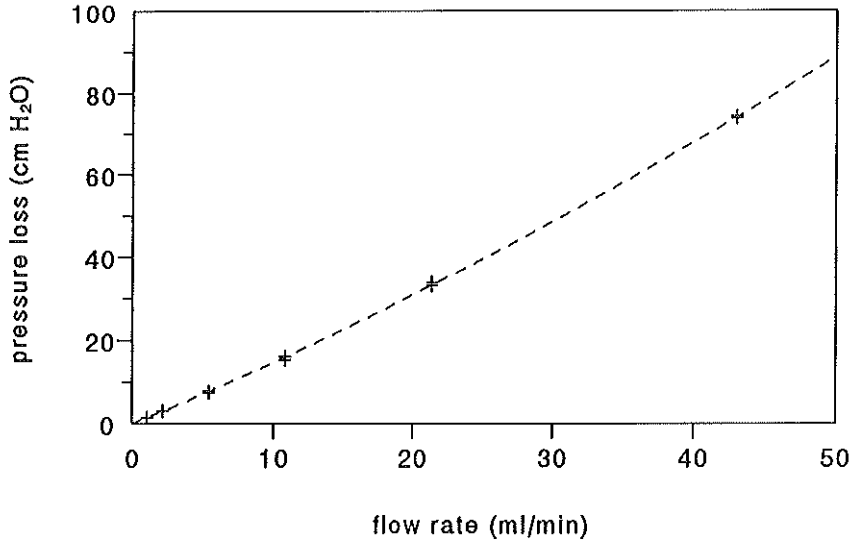


Figure 4. Pressure loss in the measuring system as a function of the imposed flow rate through it. The dotted line is the result of fitting a parabolic equation to the measured data and is merely meant as guide.

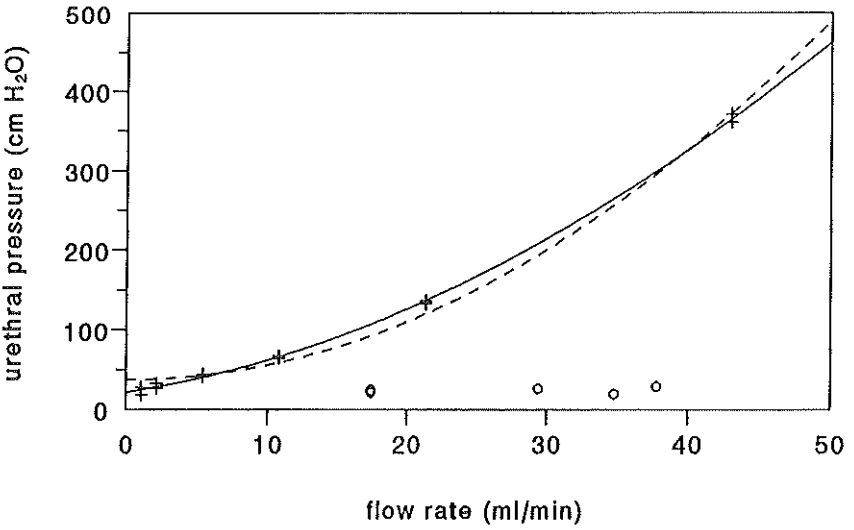


Figure 5. Urethral pressure as a function of the imposed flow rate as measured in one animal. The crosses indicate the measured data values. The solid and dashed curves represent the result of fitting a second degree polynomial with and without a linear term to the data. The circles represent the mean (Q_{max}, $p_{ves}(Q_{max})$) values obtained in the 5 animals in which conventional pressure/flow studies were done.

Table 1. Values of the urethral pressure measured at the imposed flow rates in the unrelaxed urethra of 5 guinea pigs.

flow rate *guinea pig*	1.1 ml/min	2.2 ml/min	5.4 ml/min	10.8 ml/min	21.4 ml/min	43.0 ml/min
1	23 ± 5	30 ± 3	43 ± 0.4	66 ± 1	135 ± 2	366 ± 5
2	24 ± 3	31 ± 9	47 ± 2	91 ± 15	153 ± 3	406 ± 6
3	25 ± 1	31 ± 2	42 ± 1	71 ± 5	161 ± 12	351 ± 6
4	28 ± 3	31 ± 4	48 ± 2	81 ± 3	155 ± 0.3	377 ± 6
5	17 ± 1	23 ± 1	42 ± 2	88 ± 3	196 ± 10	527 ± 19
mean	23 ± 2	29 ± 1	45 ± 1	79 ± 4	160 ± 9	406 ± 28

Values given for the individual animals are the means of 2 to 5 measurements at each flow rate and the corresponding standard deviations in cm H_2O. Values given in the last row are the mean values of these means ± the standard errors of the mean.

Table 2. Values of the parameters $p_{ura.clos.est}$, a_1 and a_2 obtained from fitting equation (1) to the pressure/flow data measured in the unrelaxed urethra of 5 guinea pigs.

guinea pig	$p_{ura.clos.est}$ (cm H_2O)	a_1 (cm H_2O/ml/min)	a_2 (cm H_2O/(ml/min)^2)
1	22.7 ± 2.0	2.64 ± 0.28	0.124 ± 0.006
2	22.2 ± 5.5	4.30 ± 0.84	0.107 ± 0.019
3	15.8 ± 4.9	5.39 ± 0.63	0.057 ± 0.014
4	22.1 ± 2.1	4.32 ± 0.32	0.091 ± 0.007
5	10.2 ± 4.7	5.44 ± 0.68	0.153 ± 0.015
mean	18.6 ± 2.2	4.42 ± 0.46	0.106 ± 0.014

Values given for the individual animals are the estimates and the corresponding standard errors as calculated by the software package SPSS®. Values given in the last row are the mean values of these estimates ± the standard errors of the mean.

pressure, it was considered an estimate for the urethral closing pressure $p_{ura.clos}$ and called $p_{ura.clos.est}$. Thirteen pressure/flow plots looked like Fig.2B and 18 were similar to Fig.3B. Both types of plots were found in 4 of the 5 animals. The $p_{ura.clos.est}$ and $p_{ves.min.void}$ values and the other parameters derived from the pressure/flow studies are listed in Table 3. The mean $p_{ves.min.void}$ values in each animal exceeded the mean $p_{ura.clos.est}$ values. Because the $p_{ves.min.void}$ values were read from the pressure/flow plots with a negative slope and the $p_{ura.clos.est}$ values were derived from those with a positive slope, this was as expected. The amount of residual urine relative to the bladder volume at the onset of voiding was similar in both types of micturition.

The $p_{ura.clos.est}$ values in Table 2, that is, in the unrelaxed urethra, were not significantly different from the $p_{ura.clos.est}$ values in Table 3 ($p=0.91$). The mean maximum flow rate (Q_{max}) values in the pressure/flow studies ranged from 0.29 to 0.63 ml/s, or from 17.4 to 37.8 ml/min, and occurred at a bladder volume of 4.1 $\pm$ 0.2 ml on average. The pressure values $p_{ves}(Q_{max})$ at these flow rates ranged from 19.4 to 28.8 cm H_2O. It can be seen from Fig.5 that these pressures are much lower than the pressures measured in the unrelaxed urethra in the same flow rate range. The pressure at which voiding started exceeded the urethral closing pressure by 9 cm H_2O on average.

Discussion

Micturition is initiated by relaxation of the external urethral sphincter.[6] The decrease in resistance of the urethra to urinary flow is caused by a change in neurogenic stimulation. It was the aim of this study to investigate this mechanism. The resistance to flow of the unrelaxed urethra in the guinea pig was measured by forcing saline through the urethra with an infusion pump. The results were compared with pressure/flow plots of voidings in these animals, where the urethra was (more) relaxed. Theoretically, in the former type of measurements (some) urethral relaxation may have been caused as a result of Barrington's (doubtful) fourth micturition reflex, which states that urethral flow induces sphincter relaxation.[7] Relaxation in the "unrelaxed urethra" could therefore not be excluded beforehand. Moreover, it could not be excluded that urethral tone was influenced by the anaesthetic used. Consequently, it cannot be stated that, in the two types of measurement, the urethra was fully contracted and fully relaxed. However, the pressures measured in both conditions were very different indeed, so that it is obvious that the measurements were performed at two very different levels of urethral relaxation. The dependence of the urethral resistance relation on the stimulation intensity could therefore be studied.

In the unrelaxed urethra the urethral resistance relation could adequately be described by a second degree polynomial with a non-zero linear term. It would have been most logical to fit second degree polynomials to the pressure/flow data in the relaxed urethra as well. However, this was not useful as a result of the variation in shape of the plots.

Table 3. Values of the parameters studied in the conventional pressure/flow studies in 5 guinea pigs.

guinea pig	$p_{ves.open}$ (cm H_2O)	Q_{max} (ml/min)	$p_{ves}(Q_{max})$ (cm H_2O)	$p_{ura.clos.est}$ (cm H_2O)	residue (%)	$p_{ves.min.void}$ (cm H_2O)	residue (%)
6	35.2 ± 0.9	37.8 ± 3.0	28.8 ± 2.5	23.6 ± 0.5 (2)	68 ± 11	24.3 ± 0.6 (2)	73±9
7	30.9 ± 1.6	29.4 ± 1.2	26.1 ± 1.9			24.4 ± 1.7 (7)	56±6
8	29.0 ± 1.6	17.4 ± 1.8	24.6 ± 1.4	19.7 (1)	80	23.4 ± 2.1 (4)	81±3
9	21.4 ± 0.2	34.8 ± 3.6	19.4 ± 0.2	15.9 ± 0.7 (4)	49 ± 3	17.4 ± 0.5 (3)	44±4
10	22.7 ± 1.1	17.4 ± 2.4	21.4 ± 1.2	16.7 ± 1.3 (6)	59 ± 4	20.8 ± 2.3 (2)	58±12
mean	27.8 ± 2.6	27.6 ± 4.2	24.1 ± 1.7	19.0 ± 1.7	64 ± 7	22.1 ± 1.3	62±7

Values given are mean values ± standard errors of the mean. The residue is given as a percentage of the bladder volume at the onset of micturition. The numbers in parentheses refer to the number of pressure/flow plots on which the mean $p_{ura.clos.est}$ and $p_{ves.min.void}$ values and the corresponding residues are based. The mean values of the other parameters are based on all pressure/flow studies in each animal.

Studies in humans also indicated that pressure/flow plots show a wide variety of shapes such that many different classes of mathematical functions have been proposed to describe them.[8-10] In most cases a straight line suffices to quantify the degree of urethral resistance during voiding.[11] Moreover, most often a simple straight line is the most complicated mathematical function that can reliably be fitted to pressure/flow data. As a result of the considerable variability of such data the parameters of more complicated functions cannot reliably be determined.[12] The variety of shapes in pressure/flow plots can be explained by the fact that the course of micturition not only depends on urethral properties, but also on properties of the detrusor muscle and the intensity of neurogenic stimulation.
Notwithstanding the differences in measurement accuracy, the urethral resistance relation in the contracted and relaxed urethra could adequately be compared in the present study. It was found that the intercept on the pressure axis was the same in both conditions, but the steepness of the curve was largely different. It can thus be concluded that the urethral resistance relation rotates around the urethral closing pressure and simultaneously flattens when the urethral musculature relaxes, as illustrated in Fig.6. The independence of the urethral closing pressure on the state of relaxation seems in correspondence with Schäfer's qualitative description of the urethral resistance relation in case of a urethral stricture.[8] In this author's view such a stricture steepens the urethral resistance relation but leaves the intercept on the pressure axis unaltered. On the other hand, the low value that we found for the urethral closing pressure in the contracted state seems to contradict the results of static urethral pressure profile measurements in humans. Typically, maximum pressures of about 100 cm H_2O are measured in such studies.[13] It should be recognized, however, that these pressure values may overestimate the real urethral pressure because of the size of the measuring catheter.[14] Assuming that our results can be extrapolated to the human situation, it follows that the effect of the catheter on the measured pressure is very large indeed, as is suggested by Griffiths' illustration 5.6.[14]

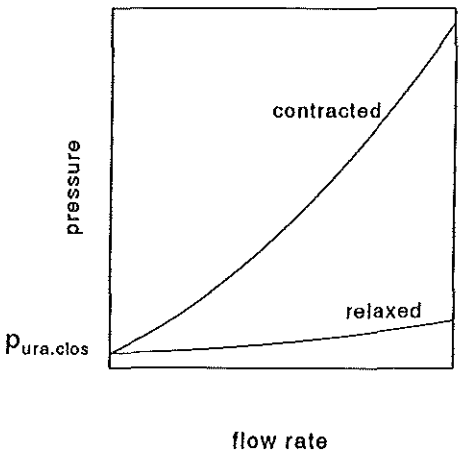


Figure 6. Schematic representation of the dependence of the urethral resistance relation on the degree of relaxation of the urethral musculature.

According to the simple view that, upon relaxation the urethral resistance relation rotates around the intercept on the pressure axis, it is to be expected that voiding starts when the intravesical pressure equals the urethral closing pressure. In our measurements this was not the case. Voiding started only when the intravesical pressure reached a

systematically higher pressure value (Table 3). The same is usually observed in humans.[15] Videoscopic studies in humans show that voiding is preceded by funnelling of the bladder neck, which is believed to be initiated by pelvic floor and urethral sphincter relaxation.[16,17] This renders it unlikely that the high intravesical pressure recorded at the onset of voiding, after the funnelling of the bladder neck, can be ascribed to this part of the tract. This view is supported by the general assumption that the bladder neck plays only a passive role in maintaining continence.[17] In a study on the pig ureter, Griffiths and Notschaele noted that the pressure needed to open the collapsed ureter was a few cm H_2O higher than the pressure needed to keep the ureter open.[18] As the walls of the collapsed ureter thus seem to stick together, the difference in the two pressures was called the unsticking pressure. The difference between the intravesical opening pressure and the urethral closing pressure might represent a similar unsticking pressure of the urethra. This explanation would be in line with experiments of Zinner et al., who showed that a higher pressure head was needed to cause leakage in a collapsible tube when the inner surface was coated with grease.[19] An alternative explanation is that the pressure difference must be ascribed to the viscoelastic properties of the urethra. Thind et al. studied the pressure response after inflation of a balloon located in the urethra.[20] It was found that, upon balloon inflation, the urethral pressure rose to a value higher than the eventual equilibrium value. The dynamic pressure excess increased with an increasing rate of balloon inflation.

A large amount of residual urine is frequently found in anaesthetized animals, as in this study.[21] Analysis of pressure/flow plots might contribute to understanding the cause of this. After the micturition corresponding to Fig.2 the residue was 58% and seems to be caused by a premature fading of the bladder contraction. After the micturition of Fig.3 the residue was 64%. In this case bladder contraction was sustained for about 30 seconds, but premature closing of the urethral sphincter, signified by a rise of pressure to its isovolumetric value, prevented further bladder emptying. It could be hypothesized that the course of micturition, as in Fig.2 or Fig.3, depends on the depth of anaesthesia. In our measurements no relation between the course of micturition and the duration of the experiment was found. It thus seems that the efficiency of voiding in urethane-anaesthetized guinea pigs may be reduced both by insufficiently sustained bladder contraction and by dyssynergic voiding.

References

1. *Groen, J., van Mastrigt, R. and Bosch, R.: Contractility of the guinea pig bladder measured in situ and in vitro. Neurourol.Urodyn. 13: 587-595, 1994.*
2. *Groen, J., van Mastrigt, R. and Bosch, R.: Neurogenic modulation of micturition: the relation between stimulation intensity and the maximum shortening velocity of the guinea pig detrusor muscle. J.Urol. 153: 228-232, 1995.*

3. *Griffiths, D.J.: The mechanics of the urethra and of micturition. Br.J.Urol. 45: 497-507, 1973.*
4. *Kranse, M. and van Mastrigt, R.: A computer algorithm to detect the lowest monotonically increasing part of a pressure flow plot. Neurourol.Urodyn. 10: 293-294, 1991.*
5. *International Continence Society: Standardisation of terminology of lower urinary tract function: pressure-flow studies of voiding, urethral resistance and urethral obstruction. Draft, 1995.*
6. *Tanagho, E.A.: The anatomy and physiology of micturition. Clin.Obstet.Gynaecol. 5: 3-26, 1978.*
7. *Morrison, J.F.B.: Reflex control of the lower urinary tract. In: The physiology of the lower urinary tract. Edited by M. Torrens and J.F.B. Morrison. London: Springer-Verlag, chapt.7, pp.207-208, 1987.*
8. *Schäfer, W.: The contribution of the bladder outlet to the relation between pressure and flow rate during micturition. In: Benign prostatic hypertrophy. Edited by F. Hinman, Jr. New York-Heidelberg-Berlin: Springer-Verlag, pp.470-498, 1983.*
9. *Griffiths, D., van Mastrigt, R. and Bosch, R.: Quantification of urethral resistance and bladder function during voiding, with special reference to the effects of prostate size reduction on urethral obstruction due to benign prostatic hyperplasia. Neurourol.Urodyn. 8: 17-27, 1989.*
10. *Spångberg, A., Teriö, H., Engberg, A. and Ask, P.: Quantification of urethral function based on Griffiths' model of flow through elastic tubes. Neurourol.Urodyn. 8: 29-52, 1989.*
11. *Schäfer, W.: Basic principles and clinical application of advanced analysis of bladder voiding function. Urol.Clin. North Am. 17: 553-566, 1990.*
12. *Kranse, M. and van Mastrigt, R.: Fitting orthogonal polynomials to the lowest part of a pressure flow plot. Neurourol.Urodyn. 10: 290-291, 1991.*
13. *Torrens, M.: Urodynamics. In: The physiology of the lower urinary tract. Edited by M. Torrens and J.F.B. Morrison. London: Springer-Verlag, chapt.9, pp.297-301, 1987.*
14. *Griffiths, D.J.: Hydrodynamics and mechanics of the bladder and urethra. In: Urodynamics: principles, practice and application, 2nd edition. Edited by A.R. Mundy, T.P. Stephenson and A.J. Wein. New York: Churchill Livingstone, chapt.5, pp.75-77, 1994.*
15. *Griffiths, D.J.: Urodynamics (Medical physics handbooks 4). Bristol: Adam Hilger Ltd, pp.130-132, 1980.*
16. *Tanagho, E.A. and Miller, E.R.: Initiation of voiding. Br.J.Urol. 42: 175-183, 1970.*
17. *Torrens, M.: Human physiology. In: The physiology of the lower urinary tract. Edited by M. Torrens and J.F.B. Morrison. London: Springer-Verlag, chapt.11, pp.343-344, 1987.*
18. *Griffiths, D.J. and Notschaele, C.: The mechanics of urine transport in the upper*

urinary tract: I. The dynamics of the isolated bolus. Neurourol.Urodyn. 2: 155-166, 1983.

19. *Zinner, N.R., Sterling, A.M. and Ritter, R.C.: Role of inner urethral softness in urinary continence. Urology 16: 115-117, 1980.*
20. *Thind, P., Lose, G. and Colstrup, H.: Resistance to forced opening of the resting urethra in healthy females assessed by experimental simulation of urine ingression. Neurourol.Urodyn. 11: 201-208, 1992.*
21. *van Asselt, E., Groen, J. and van Mastrigt, R.: A comparative study of voiding in the rat and the guinea pig: simultaneous measurement of flow rate and pressure. Am.J.Physiol. 269: R98-R103, 1995.*

Chapter 9

Computer simulation of micturition

Introduction

The two factors determining the course of micturition, bladder contractility and urethral resistance, are controlled by the nervous system. If the dependence of these factors on the bladder volume and on the intensity of neurogenic stimulation is known, it is possible to simulate the micturition process with a computer based on an assumed time course of the variation in nervous stimulation. A computer program for simulation of voiding was developed earlier in our laboratory by Hübener and van Mastrigt.[1] This was based on a set of assumptions for the above described relations. In the present chapter this model was refined and the assumptions were replaced by relations based on the results described in the previous chapters. The results of the simulation program were compared with clinical findings to check the validity of newly made assumptions. The program was developed in MATLAB on a personal computer.

The model

Neurogenic stimulation

It was assumed that the contraction of the detrusor muscle and the relaxation of the urethra are controlled by two different nerve signals. These two signals are illustrated in Fig. 1 and are described mathematically by

$$s_1(t) = 1 - e^{-t/\tau}$$

and

$$s_2(t) = 1 \qquad \text{for } t < \delta$$

$$s_2(t) = f + (1-f).(1+\omega(t-\delta)).e^{-\omega(t-\delta)} \qquad \text{for } t \geq \delta$$

In these equations, t is time and τ and ω represent time constants for the rate of development of bladder contractility and urethral relaxation; f is the final degree of "tone" in the urethra; δ is the delay time between the onset of stimulation of the bladder and the start of relaxation of the urethra and might be positive or negative. Unstable detrusor contractions measured in patients were used to estimate τ, the time constant for the development of bladder contractility. The time needed for the isovolumetric pressure to rise to 63% of the maximum was used as the value of τ. A value of 4 seconds appeared to be a good estimate.

Bladder contractility

Bladder contractility was described by the hyperbolic relation[2]

$$(p+p_0/4).(Q+Q_0/4) = 5.p_0.Q_0/16$$

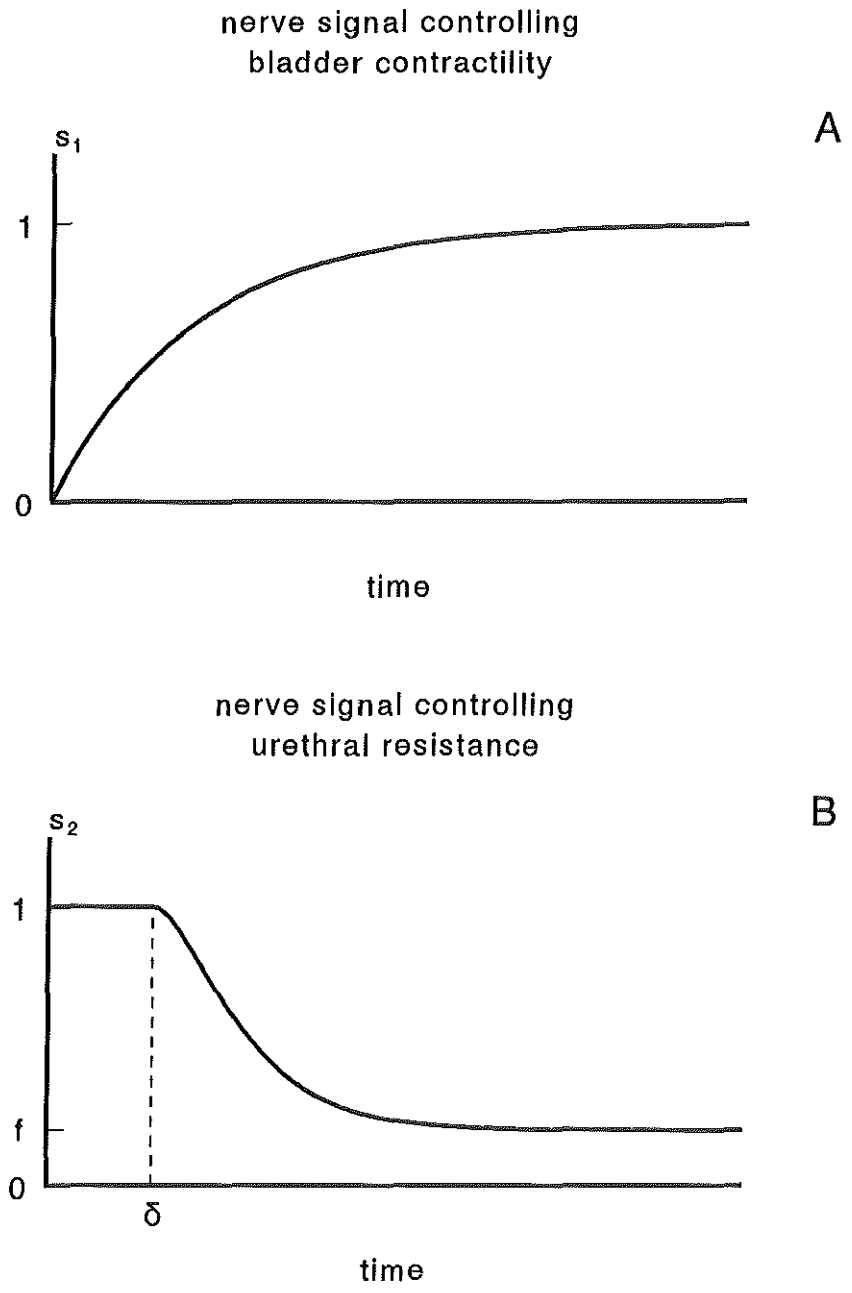


Figure 1. The assumed time course of the nerve signals s_1 and s_2 controlling the bladder and the urethra.

where:

p = (active) detrusor pressure
Q = flow rate
p_0 = isovolumetric pressure
Q_0 = flow rate at zero pressure

It was found in chapters 3 and 5 that in the guinea pig bladder the isovolumetric detrusor pressure and the maximum shortening velocity v_{max} of the detrusor muscle (which is proportional to Q_0 for a given bladder volume; see below) depended on the intensity of electrical stimulation (when measured in vitro). It was assumed in the model that the contractility parameters p_0 and Q_0 were both proportional to the nerve signal s_1. In other words, it was assumed that they depended on time according to

$$p_0(t) = p_{isv}.s_1(t)$$

$$Q_0(t) = Q_m.s_1(t)$$

where p_{isv} and Q_m denote the maximum bladder contractility values. In chapter 4 the isovolumetric detrusor pressure in guinea pigs in vivo was found to be independent of the bladder volume in the range of 0.5 to 7.8 ml. In the model it was assumed that the isovolumetric pressure is completely independent of the bladder volume. The value of p_{isv} was set at 100 cm H_2O.[3] Assuming a spherical bladder geometry and a zero detrusor muscle thickness, the flow rate Q and the bladder circumference (or detrusor) shortening velocity v are related as:[2]

$$Q = 2r^2v$$

where r is the bladder radius. As a special case,

$$Q_m = 2r^2v_{max}$$

In chapters 4 and 5 the maximum shortening velocity of the guinea pig detrusor muscle was found to be independent of the bladder volume in the range of 0.8 to 6.5 ml in vivo and in the range of 0.6 to 6.1 ml in vitro. It was therefore assumed in the model that the maximum shortening velocity of the human detrusor muscle was completely independent of the bladder volume. A value of 2.4 cm/s was chosen.[3] It was furthermore assumed that the detrusor muscle enclosed a volume of 10 ml of noncontracting tissue at the end of voiding.[3] The non-zero value of this amount of tissue implies that the bladder radius r, and consequently Q_m, do not equal zero when the bladder is empty.

Urethral resistance

In chapter 8 the urethral resistance relation, i.e. the relation between the flow rate Q through the urethra and the pressure head p needed for that flow rate, was studied at two levels of neurogenic stimulation in anaesthetized guinea pigs. It was found that the urethral resistance relation in the unrelaxed urethra - at steady state pressure and flow

rate values - could adequately be described by a second degree polynomial:

$$p = p_{ura.clos} + a_1.Q + a_2.Q^2$$

where $p_{ura.clos}$, the urethral closing pressure, is the intercept of this relation on the pressure axis and a_1 and a_2 describe its slope and curvature. The value of $p_{ura.clos}$ in the unrelaxed urethra was not significantly different from its value in the relaxed urethra. The latter was derived from analysis of pressure/flow plots, obtained from pressure and flow rate recordings during micturition. It was concluded that the urethral resistance relation rotates around its footpoint, the urethral closing pressure, when the urethra relaxes. In the model, the urethral resistance relation was therefore written as

$$p(t) = p_{ura.clos} + a_1(t).Q(t) + a_2(t).Q^2(t)$$

with

$$a_1(t) = A_1.s_2(t)$$

$$a_2(t) = A_2.s_2(t)$$

A_1 and A_2 are the values of $a_1(t)$ and $a_2(t)$ before urethral relaxation starts.

It was also found in chapter 8 that voiding did not start when the intravesical pressure equaled $p_{ura.clos}$, but only when it reached a systematically higher pressure value, the intravesical opening pressure $p_{ves.open}$. It was hypothesized that this phenomenon could be attributed to "unsticking" of the walls of the collapsed urethra or alternatively represented the viscoelastic properties of the urethra. For the equations used in the simulation model, and consequently for its results, the differences between these hypotheses are not relevant. For convenience, the first hypothesis will be maintained in the present chapter. The pressure difference $p_{ves.open}$-$p_{ura.clos}$ will accordingly be called the unsticking pressure p_{us}. It was assumed in the model that voiding starts when the intersection of the urethral resistance relation and the hyperbola describing bladder contractility exceeds the sum of $p_{ura.clos}$ and p_{us}, as illustrated in Fig.2. Based on clinical data, values of 20 and 10 cm H_2O were taken for $p_{ura.clos}$ and p_{us}, respectively.

To compare the results of the simulation model with clinical findings, the variable w, which is clinically used as a measure of bladder contractility, was also calculated:[3]

$$w = [(p+p_{isv}/4).(v+v_{max}/4)-p_{isv}.v_{max}/16]/2\pi$$

An initial bladder volume of 400 ml was chosen. The parameters in the model that could not be derived from clinical studies were varied until good results were obtained. All parameters and the final values used are listed in the Table. Calculations were made at increments of 0.1 second in the time variable, simulating a sample frequency of 10 Hz.

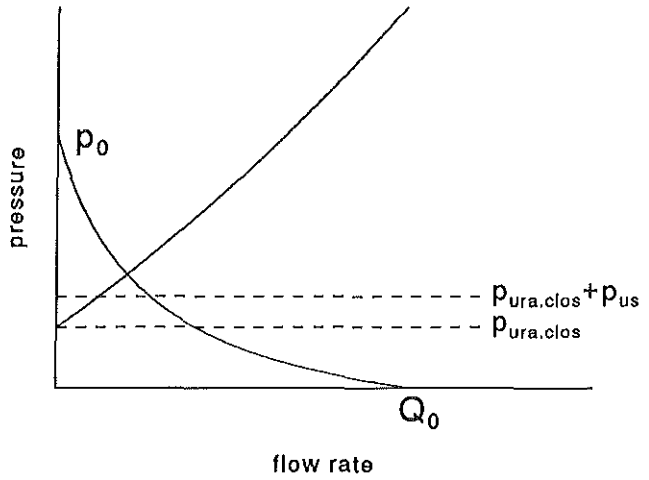


Figure 2. The hyperbolic pressure/flow relationship defining the bladder contractility, with intercepts p_0 and Q_0 on both axes, and the urethral resistance relation, with footpoint $p_{ura.clos}$ (urethral closing pressure). Flow starts when the intersection of both curves exceeds the sum of $p_{ura.clos}$ and p_{us}, the unsticking pressure.

Results

An example of the flow rate as a function of time during voiding as calculated from the simulation model is shown in Fig.3. Fig.4 shows the corresponding pressure/flow plot. Generally, both curves resemble clinical measurements. Two obvious deviations from such measurements, however, can be observed: at the start and at the end of voiding. At the start of voiding, the flow rate in the model suddenly jumped up, causing a sharp decrease in pressure, which is never seen clinically. This was found in spite of the fact that the delay time δ between both nerve signals was chosen positively. A negative value of δ, implying urethral relaxation preceded bladder contraction, resulted in an even larger discontinuity. The delayed urethral relaxation is reflected by the ascending part of the pressure/flow plot. Because normally a progressive drop in urethral pressure can be observed a few seconds before detrusor contraction,[4] a positive value of δ is not representative of normal voiding. At the end of voiding, i.e., when the bladder was empty, flow stopped abruptly in the model. Real flow rate curves usually approach the zero line smoothly. In parallel, in the model the contractility parameter w remained high at the end of voiding, as can be seen in Fig.5. Normally, this parameter is maximum at a relatively small volume, e.g. 50 ml. When the bladder empties further, it rapidly decreases. The results indicate that the model needed modification with respect to the start and the end of voiding.

Table. The parameters used in the first simulation model and the modified version.

organ	parameter		first model	modified model
nervous system	τ (s)		4.0	4.0
	f	*	0.02	0.02
	ω (s^{-1})	*	0.7	0.7
	δ (s)	*	3.0	-3.0
bladder	V_0 (ml)		400	400
	V_t (ml)		10	10
	p_{isv} (cm H_2O)		100	100
	v_{max} (cm/s)		2.4	-
	$v_{max,max}$ (cm/s)		-	2.4
	Φ (ml)	*	-	10
urethra	$p_{ura,clos}$ (cm H_2O)		20	20
	A_1 (cm H_2O/ml/min)	*	8.75	8.75
	A_2 (cm H_2O/(ml/min)^2)	*	0.45	0.45
	p_{os} (cm H_2O)		10	-
	P_{us} (cm H_2O)		-	10
	κ (s^{-1})	*	-	0.2

For symbols: see text. Additionally: V_0 is the bladder volume at the onset of micturition, V_t is the volume of noncontracting bladder tissue enclosed by the detrusor muscle. Whenever possible, parameter values were chosen based on clinical findings (see text). The remaining parameters, indicated with an asterisk, were varied until good results were obtained.

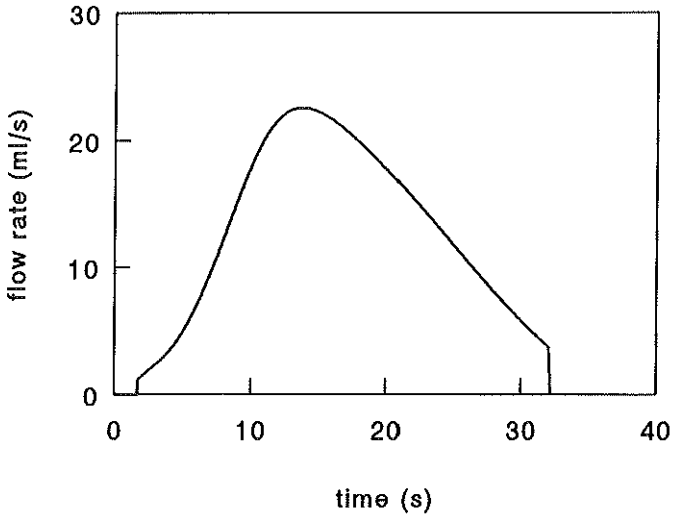


Figure 3. The flow rate as a function of time calculated from the simulation model.

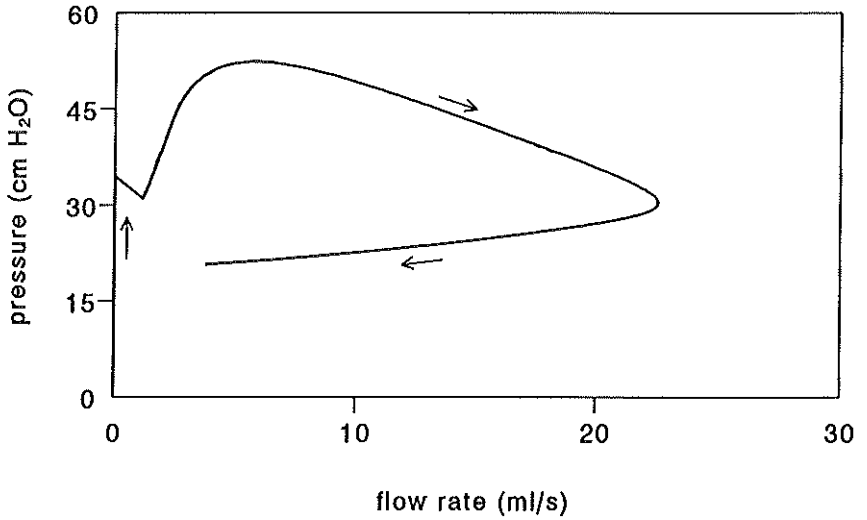


Figure 4. Pressure as a function of flow rate during voiding calculated from the simulation model. The arrows show the direction in which the curve was traced as a function of time.

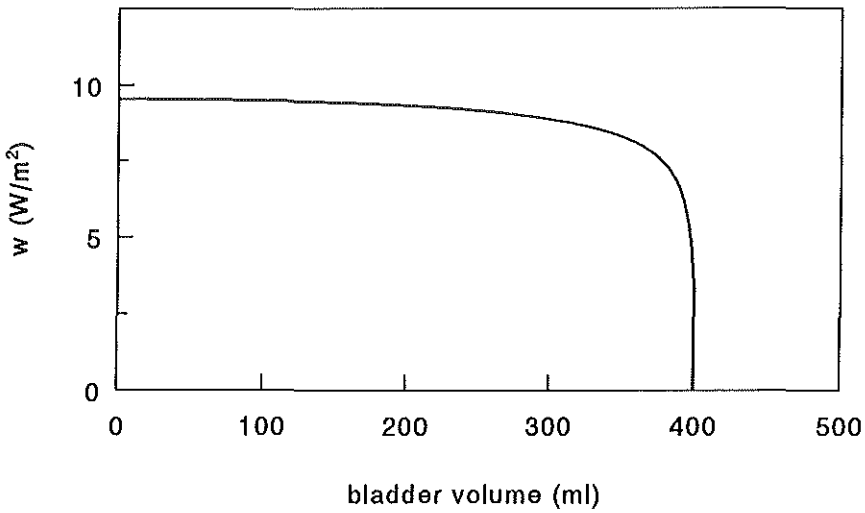


Figure 5. The calculated value of w, a measure of bladder contractility, as a function of the instantaneous bladder volume during simulated voiding.

Modification of the model

It was concluded in chapter 8 that the urethral resistance relation rotates around its footpoint, the urethral closing pressure, when the degree of relaxation is varied once flow has started. The simulation program showed that this conclusion cannot be extrapolated to the onset of micturition. In order to obtain a gradual start of voiding, it was additionally assumed that the footpoint of the urethral resistance relation is composed of two components: the urethral closing pressure $p_{ura.clos}$ (which can be estimated from a pressure/flow plot when the urethra is fully relaxed) and an unsticking pressure p_{us}, an extra pressure needed to separate the walls of the collapsed urethra. This means that the urethral resistance relation was rewritten as

$$p = p_{us} + p_{ura.clos} + a_1.Q + a_2.Q^2$$

It was furthermore assumed that p_{us} is a function of time once voiding has been started:

$$p_{us}(t) = P_{us} \qquad \text{for } t<T$$

$$p_{us}(t) = P_{us}.[1+\kappa(t-T)].e^{-\kappa(t-T)} \qquad \text{for } t\geq T$$

where P_{us} is the value of $p_{us}(t)$ before voiding starts, κ is a time constant describing the rate of unsticking and T is the moment in time when voiding started. The latter

corresponds to the first time the urethral resistance relation and the hyperbola describing bladder contractility intersect for a positive flow rate value. The time dependence of p_{us} is similar to that of the nerve signal s_2 in Fig.1, with $f=0$ and δ replaced by T. Implementing these equations in the model will effect that micturition starts when the detrusor pressure equals $p_{ura,clos}+P_{us}$, so that the intravesical opening pressure $p_{ves,open}$ is systematically higher than the urethral closing pressure $p_{ura,clos}$ as was found in chapter 8. Because $p_{us}=0$ for large values of t, the footpoint of the urethral resistance relation is still given by $p_{ura,clos}$ once the initial stage of voiding has elapsed.

In the original simulation model flow stopped abruptly when the bladder was empty. In clinical measurements, the flow rate curve usually ends smoothly. The high value of the flow rate at the end of voiding in the particular example of Fig.3 renders it unlikely that the discrepancy between the results of the model and the clinically observed pattern must be ascribed to the physical properties of the flowmeter, although this factor should be kept in mind when comparing the results of a mathematical model with real measurements. Several options for modification of the model were considered. In Hübener and van Mastrigt's model[1] pressure depended on the bladder volume in such a way that pressure was zero when the bladder was exactly empty. This resulted in a smooth ending of the flow rate curve. The assumed volume dependence of pressure, however, was derived from in vitro measurements. It was found in chapter 4 that the in vitro volume dependence of pressure cannot be extrapolated to the in vivo situation. The occurrence of (sometimes high) aftercontractions in patients after residual free voiding supplies arguments to maintain the assumption that the in vivo isovolumetric pressure is volume independent. For the same reason the possibility that the nerve signal s_1 decreases towards the end of voiding was rejected. Instead, the hypothesis that the maximum shortening velocity v_{max} of the detrusor muscle is independent of the bladder volume was abandoned. In experiments on striated muscle fibres Edman found that v_{max} decreased at short sarcomere lengths.[5] He ascribed this to an increase of the passive resistance to shortening. It was, therefore, assumed that v_{max} depends on bladder volume in the following way:

$$v_{max}(V) = v_{max,max}.(1-e^{-V/\Phi})$$

where $v_{max,max}$ is the maximum value of v_{max}, V is the instantaneous bladder volume and Φ is a characteristic volume constant. This dependence is similar to the dependence of s_1 on t in Fig.1.

Results of the modified model

Apart from the modifications described in the previous section, the modified model was identical to the basic model (see Table of parameter values). The adaptations resulted in a flow rate curve as shown in Fig.6A. Fig.6B illustrates the good resemblance of the

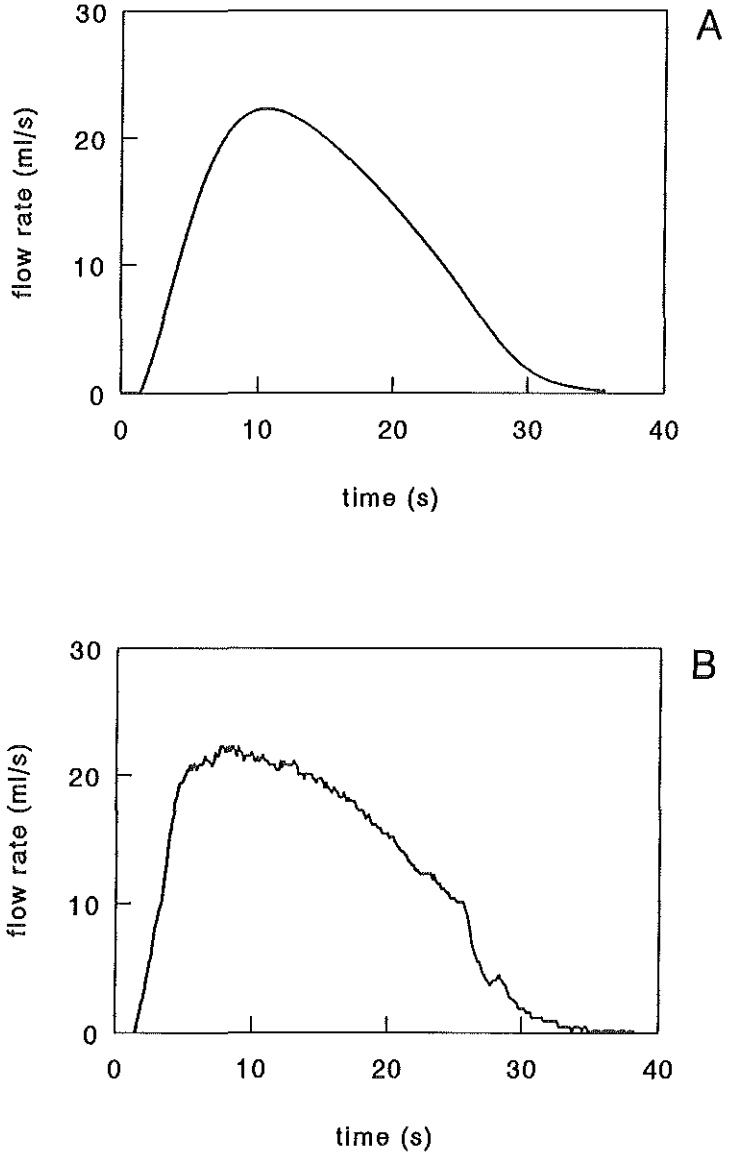


Figure 6. A: Flow rate as a function of time calculated from the modified simulation model. B: Flow rate curve measured in a patient, rescaled so that maximum and duration of the curve were identical to that in A.

newly obtained result to clinical measurements. The value of the delay time δ between the nerve signals s_1 and s_2 could now be chosen negatively, i.e., it could now be assumed that urethral relaxation preceded bladder contractility development, without obtaining an abrupt start of flow. The negative value of δ chosen, -3 seconds, resulted in a much less pronounced ascending part in the pressure/flow plot (Fig.7A vs Fig.4). The ascending part disappeared completely when δ was chosen even more negatively. The variability in the occurrence of a slightly ascending part in the pressure/flow plot in the early phase of the simulated micturition is in agreement with the variability that can be observed in real measurements, an example of which is shown in Fig.7B.

The assumed volume dependence of v_{max} caused flow to end gradually. However, an unrealistically prolonged flow rate curve, with infinitesimally small flow rate values, was now obtained: the flow rate curve approached the time axis asymptotically. For that reason the simulation was terminated when the bladder volume was less than 0.5 ml. The result could be improved mathematically by shifting the volume dependence of v_{max} to the left, so that v_{max} was non-zero at zero bladder volume. The bladder volume dependence of w (Fig.8A) now resembled clinical curves. An example of such a curve is shown in Fig.8B.

Discussion

With the modified simulation model, results were obtained that were in good comparison with clinical findings. It was assumed that the footpoint of the urethral resistance relation consists of two components: the urethral closing pressure and an unsticking pressure. The latter was assumed to be time dependent once flow has started, while the former was considered independent of the nerve signal controlling the urethra. The facts that most patients and volunteers can interrupt urinary flow by squeezing the pelvic floor, as is done in a so-called stop test, and that many patients can maintain continence despite high unstable detrusor contractions, show that one can make the footpoint of the urethral resistance relation rise above the instantaneous isovolumetric detrusor pressure. This implies that it is possible to increase the footpoint by neurogenic modulation. Consequently, an additional term in the urethral resistance relation is needed to adequately describe this relation when the pelvic floor is squeezed. In chapter 1 the role of the periurethral muscle of the levator ani in the maintenance of continence was described. It seems most logical to ascribe this extra term in the urethral resistance relation to increased activity of this muscle. In the absence of unstable detrusor contractions and during relaxed voiding the term is zero. In modelling micturition, therefore, it is irrelevant.

In the present model incomplete bladder emptying is impossible. This is caused by the fact that the isovolumetric pressure p_0 remains high towards the end of micturition and the maximum flow rate Q_m (at zero pressure) cannot equal zero when the bladder is

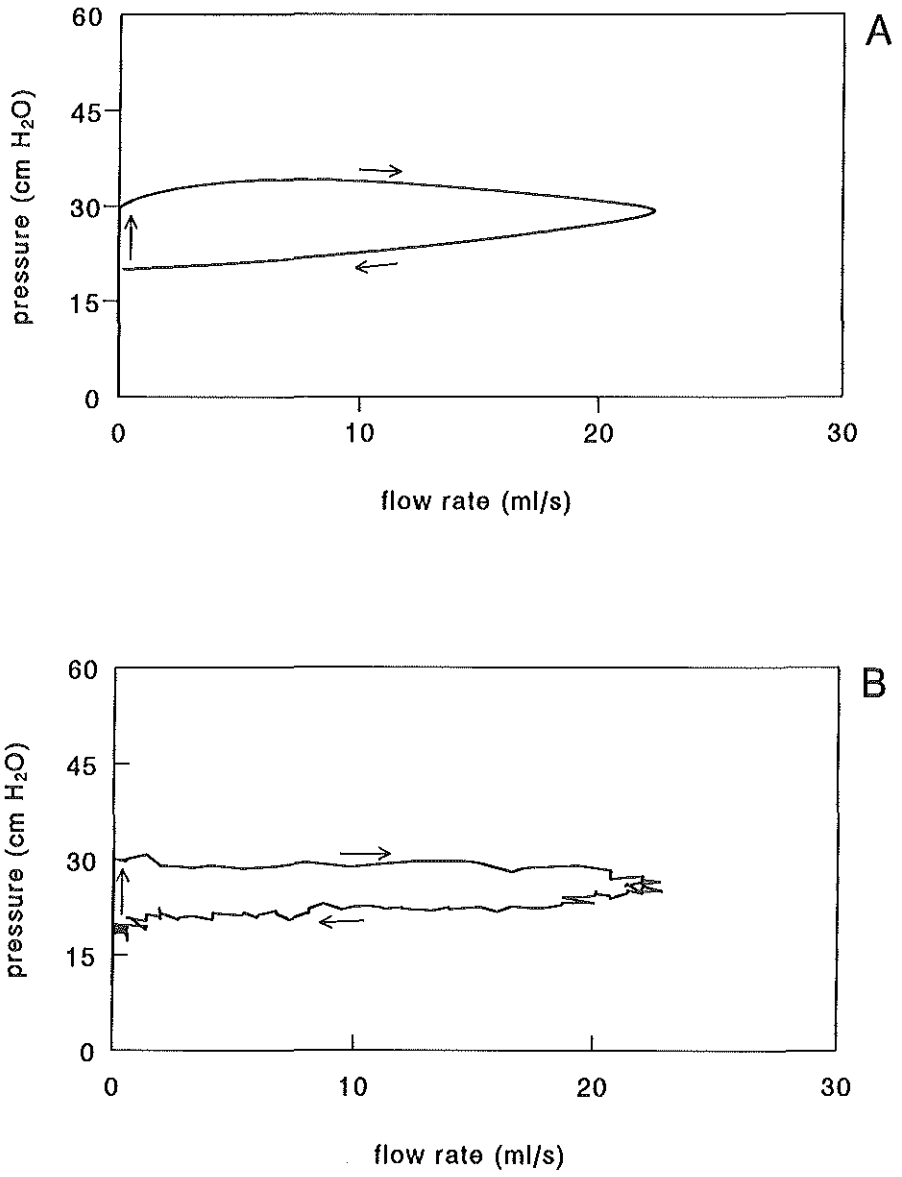


Figure 7. Pressure as a function of flow rate during voiding calculated from the modified simulation model (A) and, rescaled, as measured in a urodynamic examination (B).

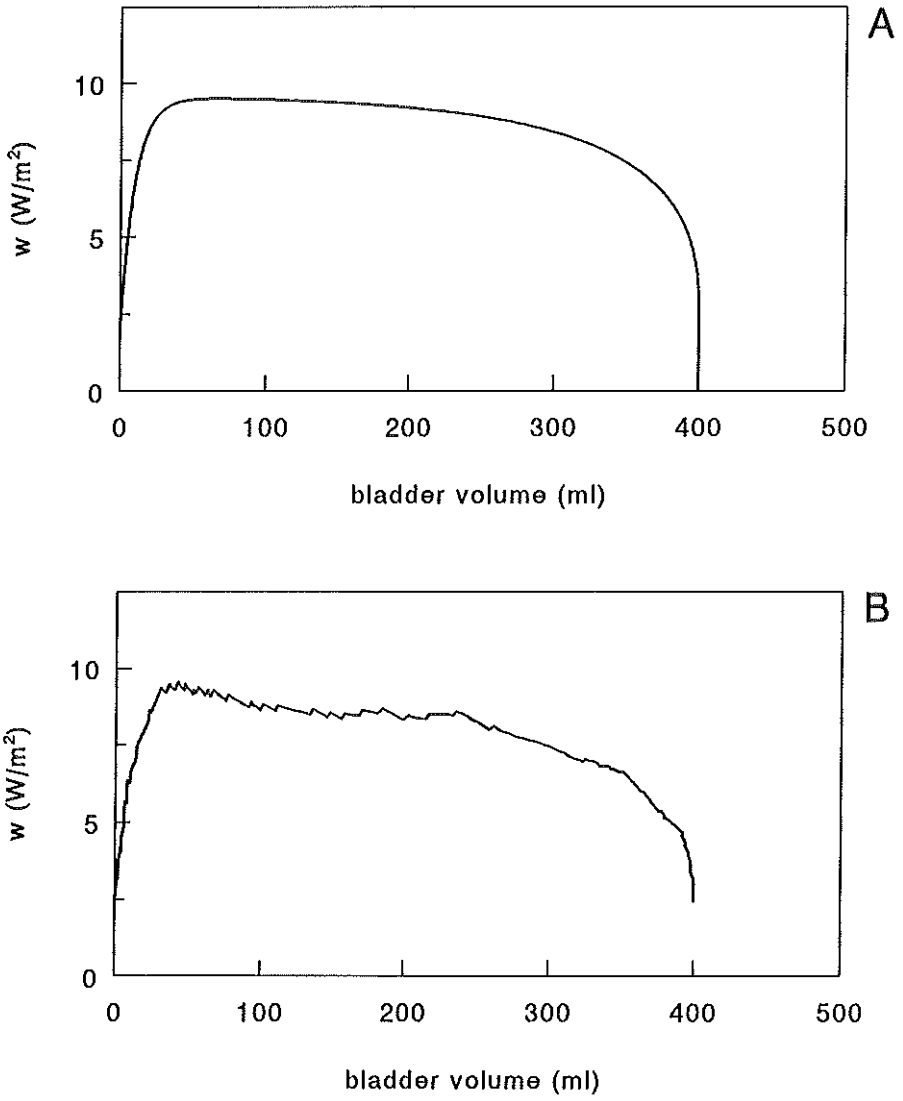


Figure 8. The value of the bladder contractility variable w as a function of the instantaneous bladder volume calculated from the modified simulation model (A) and, rescaled, as calculated from a urodynamic measurement (B). The first value of w in B is non-zero and relatively high due to the facts that the registration of flow rate only started when a threshold was surpassed and no correction for passive pressure takes place in the w concept.

not empty, so that the bladder contractility curve will always intersect the urethral resistance relation and flow rate will be positive. In patients residual urine may originate from a fading detrusor contraction, probably as a consequence of decreasing neurogenic stimulation or muscle fatigue. By letting the nerve signal s_1 decrease prematurely, so that p_0 gets smaller than the urethral closing pressure before the bladder empties completely, incomplete bladder emptying can be simulated. It may be hypothesized that in normal micturition the neurogenic stimulation of the bladder depends on the bladder volume via some feedback mechanism in such a way that p_0 decreases below $p_{ura,clos}$ when the bladder is empty.[6] In that case no truncation of the flow rate curve as in the first model (Fig.3) or a prolonged flow rate curve as in the modified model (Fig.6A) would be observed. Aftercontractions could then be explained by flaring up of the nervous stimulation of the bladder.

Conclusion

It was the aim of this thesis to investigate the dependence of the (mechanical) parameters determining the course of micturition on neurogenic stimulation. In the present chapter, the derived results were implemented in a simulation model of micturition. The assumed nerve signals controlling bladder contractility and urethral resistance were among the simplest possible. Deviations of the simulation model from real measurements probably mainly originate from the fact that the in situ nerve signals are more complicated than those used in the model. Neurophysiologic research is indicated to support this hypothesis. In spite of the simplicity of the nerve signals, the simulation model led to responses that closely resembled real measurements.

References

1. *Hübener, U. and van Mastrigt, R.: Computer simulation of micturition. Urodinamica 4: 81-90, 1994.*
2. *van Mastrigt, R. and Griffiths, D.J.: Contractility of the urinary bladder. Urol.Int. 34: 410-420, 1979.*
3. *Griffiths, D.J., Constantinou, C.E. and van Mastrigt, R.: Urinary bladder function and its control in healthy females. Am.J.Physiol. 251: R225-R230, 1986.*
4. *Tanagho, E.A. and Miller, E.R.: Initiation of voiding. Br.J.Urol. 42: 175-183, 1970.*
5. *Edman, K.A.P.: The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibers. J.Physiol. 291: 143-159, 1979.*
6. *Morrison, J.F.B.: Bladder control: role of higher levels of the central nervous system. In: The physiology of the lower urinary tract. Edited by M. Torrens and J.F.B. Morrison. London: Springer-Verlag, chapt.8, 1987.*

Chapter 10

Summary

It is the function of the lower urinary tract to temporarily store the urine that is produced in the kidneys and expel it under appropriate circumstances. The evacuation of the bladder normally results from a coordinated interaction of the bladder and the urethra. Conceptually, the course of micturition depends on three factors: the bladder contractility, the urethral resistance to urinary flow and the nervous system that modulates both these properties. It was the aim of this thesis to study this modulation and, more specifically, the dependence of bladder contractility and urethral resistance on neurogenic stimulation. Detailed knowledge of this dependence is necessary for the correct interpretation of cystometric data in patients, resulting in a correct diagnosis, and for the development of treatment modalities. The guinea pig was used as a model. The course of voiding in this animal is similar to that in humans. Measurements were done in situ and in vitro.

First, bladder contractility was studied. The concept of muscle contractility refers to the ability of a muscle to develop pressure (or force) and/or to shorten. It is known that a relation exists between the pressure developed by the detrusor muscle and the detrusor muscle (or bladder circumference) shortening velocity. This relation can be described by a hyperbolic equation. It is usually assumed that the parameter describing the "curvature" of the relation between pressure and shortening velocity is the same for all bladders. It then follows that the contractility of a given bladder is determined by two parameters: the isovolumetric detrusor pressure and the maximum detrusor shortening velocity (at zero pressure). Both these parameters may depend on the neurogenic stimulation. Both may also depend on the bladder volume. They were therefore studied at a wide range of volumes.

To estimate the degree of the neurogenic modulation of bladder contractility, the isovolumetric pressure in micturition contractions in situ was compared with the isovolumetric pressure in electrically induced contractions in vitro. It was concluded that the in situ bladder is not fully stimulated at a normal volume, i.e., contractility in situ was much smaller than in vitro. It was, however, noted that the bladder volume dependence of isovolumetric pressure was considerably different in situ and in vitro. After an initial maximum, isovolumetric pressure in vitro decreased as the volume increased. This dependence is caused by geometrical factors, as is explained in the sliding filament-cross bridge theory of contraction. In situ, no volume dependence of isovolumetric pressure was found. This suggests that in vivo neurogenic stimulation intensifies with increasing bladder volume.

To further study the importance of geometrical factors in bladder contractility, the contractility of strips of full bladder wall thickness was compared with that of whole organ preparations. The time course of contraction development was the same in both kinds of preparations, but in whole bladders the maximum was about twice as high as in strips. This difference could be explained in terms of the bladder wall structure. Taking this effect into account, the strip model constitutes a good alternative for the whole bladder in contractility studies.

The time course of the isometric contractions both in strips and in whole bladders

was studied with phase plot analysis. Phase plots in the present context are plots of the time derivative of pressure (or force in strips) as a function of the pressure itself. It seemed that the shape of these plots depended on the bladder volume (or the strip length). At relatively large volumes it showed a biphasic nature, suggesting that the rate of pressure development at these volumes was limited by two separate processes. It was hypothesized that these processes were the release of Ca^{2+} from intracellular stores and the influx of extracellular Ca^{2+}. The effect of the extracellular Ca^{2+} concentration on the maximum pressure and the shape of the phase plots was studied. Removal of extracellular Ca^{2+} led to a 85% reduction of the maximum isovolumetric pressure in subsequent stimulations, but pressure developed faster. The biphasic nature of pressure development at large volumes tended to disappear. It was concluded from the measurements that pressure development is determined by the release of intracellularly stored Ca^{2+} in the early phase of contraction and by the extracellular Ca^{2+} influx in the final phase. At small volumes Ca^{2+} influx takes place at a higher rate, so that the two processes cannot be identified in the phase plots.

Bladder contractility was not only studied in isovolumetric contractions, but also in nonisovolumetric contractions. This permitted the estimation of the maximum detrusor shortening velocity from the hyperbolic equation describing bladder contractility. To this end it was necessary to determine the instantaneous detrusor shortening velocity during voiding. As this velocity can be derived from the flow rate and the bladder volume, a method for measuring flow rates in guinea pigs was developed. It was found that the maximum shortening velocity of the guinea pig detrusor in situ did not depend on the bladder volume. This was as expected from the sliding filament-cross bridge theory: in this theory, the maximum shortening velocity of a muscle is not determined by the stretched length, i.e. by the number of interacting cross bridges, but by the cross bridge cycling rate.

The maximum shortening velocity of the detrusor muscle might not only depend on the bladder volume, but also on the intensity of stimulation. This was studied in vitro, using the stop-flow technique. With this technique, two pressure values were obtained at the same bladder volume: one at a known value of the bladder circumference shortening velocity, and one in the absence of shortening, i.e. the isovolumetric value. The maximum shortening velocity could then, as in the in vivo measurements, be derived from the hyperbolic equation describing bladder contractility. Its value depended on the intensity of stimulation. As in vivo, it did not depend on the bladder volume in the range studied.

It was noted in these stop-flow measurements that the isovolumetric pressure measured after active shortening of the detrusor muscle was significantly smaller than the isovolumetric pressure measured without preceding shortening. Active shortening in this context means that the bladder was stimulated during shortening. It was hypothesized that the observed phenomenon was caused by shortening induced deactivation, the depressant effect of active muscle shortening on the subsequently measured isometric force (or

pressure). To test this hypothesis, the effects of active and passive detrusor shortening on the subsequently measured isovolumetric pressure were compared. The isovolumetric pressures measured after five minute periods of recovery were taken as control values. The isovolumetric pressure measured after passive shortening was on average about 7% smaller than the control value. The difference was ascribed to viscoelastic relaxation during shortening. Active shortening had an additional 8% depressant effect on the isovolumetric pressure. The effects of active and passive shortening differed significantly. Shortening induced deactivation in the guinea pig bladder was therefore considered proven. This phenomenon is generally ascribed to a cross bridge cycling induced decrease of the calcium affinity of the contractile system. The hypothesis that it is specifically the calcium affinity of troponin that changes needs reconsideration, because this protein is not found in smooth muscle.

As explained before, the contractility of a given bladder is fully specified by the isovolumetric detrusor pressure and the maximum detrusor shortening velocity. Because generally these two parameters cannot be derived from an unmanipulated micturition, the contractility parameter W_{max} has been developed. This parameter was studied in isovolumetric and nonisovolumetric micturition contractions, using the average values found for the isovolumetric detrusor pressure and the maximum detrusor shortening velocity. It was found that W_{max} was not significantly different in these two kinds of contractions. In addition, W_{max} appeared to be suitable for detecting differences in the contractility of different bladders. Both results support the validity of the W_{max} concept as a measure of bladder contractility.

The course of micturition not only depends on the contractility of the bladder, but also on the resistance of the urethra to urinary flow. Urethral resistance is defined by the pressure head needed to drive a given flow rate through the urethra; the relation between these two variables, the flow rate and the needed pressure head, is called the urethral resistance relation. To study the dependence of the urethral resistance on neurogenic stimulation, the urethral resistance relation was examined at two levels of urethral relaxation: during a micturition contraction (when the urethra is relaxed) and in the absence of such a contraction (when the urethra is not relaxed). The intercept of the urethral resistance relation on the pressure axis was not significantly different in these two conditions, i.e., the urethral resistance relation rotates around this intercept when the degree of urethral relaxation changes. Yet it is generally observed in clinical urodynamics, as in the guinea pigs in the present study, that the detrusor pressure at the onset of voiding exceeds the pressure at the end of voiding. This pressure difference might be a manifestation of the viscoelastic properties of the urethra or represent the force necessary to "unstick" the sealed walls of the collapsed urethra.

To test the validity of the combined results, a computer program was developed to simulate human micturition. An exponential time course of the nerve signals controlling the bladder and the urethra was assumed, i.e., it was assumed that the neurogenic stimulation of bladder contractility increased exponentially at the onset of micturition until

the bladder was fully stimulated, and that the stimulation of the urethra decreased exponentially until it was fully relaxed. If possible, the values of the parameters in the model were estimated from clinical studies. Generally, the results of the simulation program resembled clinical measurements. Differences were observed, however, at the start and at the end of voiding. Modification of the model led to better, but not perfect results. The remaining discrepancies between the model and real measurements may have been caused by unjustified assumptions relating to the mechanical factors involved in the process of micturition, in particular to the bladder volume dependence of the isovolumetric detrusor pressure and the maximum detrusor shortening velocity at small volumes. The simplicity of the assumed nerve signals, however, constitutes a more likely cause for these discrepancies. In the model, the neurogenic stimulation of the bladder and the urethra did not depend on the bladder volume. Mechanisms causing such a dependence could be hypothesized. More neurophysiologic research is indicated to study the relation between the bladder volume and the stimulation of the lower urinary tract during a micturition contraction. In spite of the need for further research, the simulation program demonstrated that the course of normal voiding can generally be explained by the interaction between neurogenic and mechanical factors as described in this thesis.

Samenvatting

De lagere urinewegen, bestaande uit de blaas en de plasbuis, slaan de in de nieren geproduceerde urine tijdelijk op en lozen deze onder geschikte omstandigheden. De ontlediging van de blaas is normaliter het gevolg van een gecoördineerde wisselwerking van de blaas en de plasbuis. Het verloop van de urinelozing wordt daarom bepaald door drie factoren: het vermogen van de blaas om samen te trekken (de zogenaamde contractiliteit van de blaas), de weerstand die de plasbuis (urethra) aan de urinestroom biedt (de urethrale weerstand) en het zenuwstelsel, dat de lozing coördineert door deze beide eigenschappen te regelen. Het doel van dit proefschrift was deze regulatie en, specifieker, de afhankelijkheid van de blaascontractiliteit en de urethrale weerstand van de zenuwstimulatie te bestuderen. Gedetailleerde kennis van deze afhankelijkheid is onontbeerlijk voor de juiste interpretatie van de resultaten van blaasfunctie-onderzoek bij patiënten, en daarmee voor een juiste diagnose, en voor de ontwikkeling van nieuwe behandelingsmethoden. De cavia werd in dit onderzoek als proefdier gebruikt. Het verloop van de urinelozing komt bij dit dier overeen met dat bij de mens. Metingen werden gedaan in situ, d.w.z. in het levende, onder narcose gebrachte dier, en in vitro, d.w.z. dat de blaas uit het dier werd verwijderd alvorens eraan te meten.

In eerste instantie werd aandacht besteed aan de contractiliteit van de blaas. Het begrip "spiercontractiliteit" heeft betrekking op het vermogen van een spier om druk (of kracht) te ontwikkelen en/of te verkorten. Het is bekend dat er een verband bestaat tussen de door de blaasspier ontwikkelde druk en de snelheid waarmee deze spier (en dus de blaasomtrek) zich verkort. Dit verband kan wiskundig worden beschreven door een hyperbolische vergelijking. Doorgaans wordt aangenomen dat de "kromming" van het hyperbolische verband tussen druk en verkortingssnelheid voor alle blazen hetzelfde is. In dat geval wordt de contractiliteit van een bepaalde blaas volledig bepaald door slechts twee kentallen: de isovolumetrische blaasspierdruk (d.w.z. de druk die door de blaasspier kan worden ontwikkeld als de blaasinhoud niet verandert) en de maximale verkortingssnelheid. Beide kentallen hangen mogelijk af van de zenuwstimulatie. Beide zouden ook kunnen afhangen van het blaasvolume. Ze werden daarom bestudeerd over een groot volume-traject.

Om een indruk te krijgen van de mate van zenuwregulatie van de blaascontractiliteit werd de isovolumetrische druk tijdens een blaascontractie in situ, d.w.z. de maximale druk tijdens een poging tot plassen terwijl de plasbuis van de cavia werd dichtgehouden, vergeleken met de isovolumetrische druk in elektrisch opgewekte blaascontracties in vitro. Het bleek dat de blaas in situ bij een normale blaasinhoud niet volledig gestimuleerd wordt, d.w.z. de contractiliteit was in situ veel kleiner dan in vitro. Het viel echter op dat de volume-afhankelijkheid van de isovolumetrische druk in situ en in vitro nogal verschilde. Na een aanvankelijk maximum nam de isovolumetrische druk in vitro af als het volume toenam. Deze afhankelijkheid wordt veroorzaakt door geometrische factoren, zoals wordt uitgelegd in de zogenaamde glijdende-filamententheorie. In situ werd geen volume-afhankelijkheid van de isovolumetrische druk gevonden. Dit doet vermoeden dat de intensiteit van de zenuwstimulatie toeneemt met toenemend blaasvolume.

Om het belang van geometrische factoren met betrekking tot de blaascontractiliteit nader te bestuderen werd de contractiliteit van reepjes (strips) van de blaas vergeleken met die van gehele blazen. Het tijdsverloop van de ontwikkeling van de contractie was in beide soorten preparaten hetzelfde, maar het maximum was in gehele blazen ongeveer twee keer zo groot als in strips. Dit verschil kon worden verklaard vanuit de structuur van de blaaswand. Dit effect in aanmerking genomen vormen strips bij contractiliteits-onderzoek een goed alternatief voor gehele blazen.

Het tijdsverloop van de isometrische contracties werd zowel in strips als in gehele blazen bestudeerd met behulp van faseplot-analyse. Faseplots zijn in dit verband grafieken van de snelheid van verandering van de druk (of van de kracht als het gaat om strips) uitgezet tegen de druk zelf. De indruk ontstond dat de vorm van deze plots afhing van het blaasvolume (of de striplengte). Bij betrekkelijk grote volumina konden twee fasen worden onderscheiden, wat deed vermoeden dat de snelheid van de drukontwikkeling bij deze volumina wordt bepaald door twee afzonderlijke processen. Verondersteld werd dat deze processen het vrijkomen van Ca^{2+} uit intracellulaire (in de spiercel gelegen) opslag-plaatsen en de instroming van extracellulair (buiten de spiercel aanwezig) Ca^{2+} waren. Daarom werd het effect van de extracellulaire Ca^{2+}-concentratie op de maximale druk en de vorm van de faseplots bestudeerd. Verwijdering van het extracellulaire Ca^{2+} leidde tot een afname van 85% van de maximale isovolumetrische druk in de daarop volgende contracties, maar de druk ontwikkelde zich sneller. Het tweefasische patroon van de druk-ontwikkeling bij grotere volumina verdween geleidelijk. Uit de metingen werd geconclu-deerd dat de drukontwikkeling in de eerste fase van de contractie wordt bepaald door het vrijkomen van intracellulair opgeslagen Ca^{2+} en in de latere fase door de instroming van extracellulair Ca^{2+}. Bij kleine volumina vindt deze instroming met een hogere snelheid plaats, waardoor de twee processen niet te onderscheiden zijn in de faseplots.

De blaascontractiliteit werd niet alleen bestudeerd in isovolumetrische, maar ook in niet-isovolumetrische contracties. Dit maakte het mogelijk de maximale verkortings-snelheid van de blaasspier in de hyperbolische vergelijking die de blaascontractiliteit beschrijft te bepalen. Hiertoe was het noodzakelijk op ieder moment tijdens de urine-lozing de verkortingssnelheid van de blaasspier te kennen. Omdat deze snelheid kan worden berekend uit de urinestroomsnelheid en de blaasinhoud werd een methode ont-wikkeld om de urinestroomsnelheid in cavia's te meten. De maximale verkortingssnelheid van de blaasspier bleek niet van het blaasvolume af te hangen. Dit werd ook verwacht op grond van de eerder genoemde glijdende-filamententheorie.

Behalve van het blaasvolume zou de maximale verkortingssnelheid van de blaasspier ook kunnen afhangen van de intensiteit van de stimulatie. Dit werd in vitro onderzocht met behulp van de zogenaamde stop-flowtechniek. Met behulp van deze techniek werden twee drukwaarden verkregen bij hetzelfde blaasvolume: een waarde bij een bekende verkortingssnelheid van de blaasspier en een waarde in de afwezigheid van verkorting, d.w.z. de isovolumetrische waarde. De maximale verkortingssnelheid kon daarna, net zoals bij de metingen in de cavia, worden uitgerekend met behulp van de hyperbolische

vergelijking die de blaascontractiliteit beschrijft. De waarde hing af van de intensiteit van de stimulatie, maar niet van het blaasvolume.

In de experimenten met de stop-flowtechniek viel het op dat de isovolumetrische druk na actieve verkorting van de blaasspier significant kleiner was dan de isovolumetrische druk zonder voorafgaande verkorting. Met actieve verkorting wordt in dit verband bedoeld dat de blaas tijdens het verkorten werd gestimuleerd. Verondersteld werd dat het waargenomen verschijnsel berustte op "shortening induced deactivation", het verlagende effect van actieve spierverkorting op de vervolgens gemeten isometrische kracht (of druk). Om deze veronderstelling te toetsen werden de effecten van actieve en passieve verkorting van de blaasspier op de daarna gemeten isovolumetrische druk vergeleken. De isovolumetrische drukken die na een herstelperiode van vijf minuten werden gemeten werden als controlewaarden gebruikt. De na passieve verkorting gemeten isovolumetrische druk was gemiddeld ongeveer 7% kleiner dan de controlewaarde. Het verschil werd toegeschreven aan visco-elastische relaxatie tijdens verkorting. Actieve verkorting had een extra verlagend effect van 8% op de isovolumetrische druk. De effecten van actieve en passieve verkorting verschilden significant. "Shortening induced deactivation" in de cavia-blaas werd dan ook bewezen geacht. Dit verschijnsel wordt algemeen toegeschreven aan een afname van de gevoeligheid van het contractiele systeem van spierweefsel voor calcium. De veronderstelling dat het in het bijzonder gaat om de calcium-gevoeligheid van troponine moet echter herzien worden, omdat dit eiwit niet voorkomt in glad spierweefsel, zoals dat van de blaas.

Zoals eerder werd uitgelegd wordt de contractiliteit van een bepaalde blaas volledig beschreven door de isovolumetrische blaasspierdruk en de maximale verkortingssnelheid van de blaasspier. Omdat in het algemeen de waarde van deze twee kentallen niet kan worden afgeleid uit een normale urinelozing is in een eerder onderzoek het gecombineerde kental W_{max} ontwikkeld als maat voor blaascontractiliteit. W_{max} werd bestudeerd in isovolumetrische en niet-isovolumetrische blaascontracties. De waarde van W_{max} bleek in deze twee soorten contracties niet significant te verschillen. Verder bleek W_{max} in staat te zijn om de verschillen in de contractiliteit tussen verschillende blazen weer te geven. Beide resultaten laten zien dat W_{max} geschikt is om als maat voor blaascontractiliteit te dienen.

Het verloop van de urinelozing hangt niet alleen af van de contractiliteit van de blaas, maar ook van de weerstand die de plasbuis aan de urinestroom biedt: de urethrale weerstand. Hieronder wordt verstaan de druk die nodig is om de urine met een gegeven stroomsnelheid, de zogenaamde flow rate, door de plasbuis voort te stuwen. Het verband tussen deze twee variabelen, de flow rate en de benodigde druk, wordt de urethrale-weerstandsrelatie genoemd. Om de afhankelijkheid van de urethrale weerstand van de zenuwstimulatie te bestuderen werd de urethrale-weerstandsrelatie onderzocht in twee toestanden van de plasbuis: tijdens een urinelozing (wanneer de plasbuis ontspannen is) en in rust (wanneer de plasbuis gesloten is). Het snijpunt van de urethrale-weerstandsrelatie met de druk-as was in deze twee toestanden niet significant verschillend, d.w.z. dat de

urethrale-weerstandsrelatie om dit snijpunt draait als de mate van ontspanning van de plasbuis verandert. Toch wordt bij blaasfunctie-onderzoek bij patiënten, net als bij de cavia's in dit proefschrift, doorgaans waargenomen dat de blaasspierdruk bij het begin van de urinelozing groter is dan de druk aan het eind van de lozing. Dit drukverschil zou verband kunnen houden met de visco-elastische eigenschappen van de plasbuis of een uitdrukking kunnen zijn van de kracht die nodig is om de aan elkaar klevende wanden van de gesloten plasbuis los te maken.

Om de geldigheid van de experimentele resultaten in dit proefschrift te toetsen werd een computerprogramma ontwikkeld om de menselijke urinelozing na te bootsen. Aangenomen werd dat de zenuwstimulatie van de blaascontractiliteit in het begin van de urinelozing toenam tot de blaas volledig werd gestimuleerd en dat de stimulatie van de plasbuis afnam tot hij volledig ontspannen was. Voor zover mogelijk werden voor de kentallen in het model waarden gebruikt die konden worden afgeleid uit onderzoek bij patiënten. In het algemeen kwamen de resultaten van het computerprogramma goed overeen met wat bij patiënten gevonden wordt. Aan het begin en het einde van de urinelozing werden echter verschillen waargenomen. Aanpassing van het model leidde tot betere, maar nog steeds niet perfecte resultaten. De resterende verschillen tussen het model en echte metingen zouden het gevolg kunnen zijn van onterechte aannamen over de mechanische factoren die een rol spelen bij de urinelozing, in het bijzonder over de blaasvolume-afhankelijkheid van de isovolumetrische blaasspierdruk en de maximale blaasspierverkortingssnelheid bij kleine volumina. De eenvoud van de aangenomen zenuwsignalen vormt echter een waarschijnlijker oorzaak van deze verschillen. In het model hing de zenuwstimulatie van de blaas en de plasbuis niet af van de blaasinhoud. Mechanismen die voor een dergelijke afhankelijkheid zorgen zijn echter denkbaar. Meer neurofysiologisch onderzoek is nodig om het verband tussen de blaasinhoud en de stimulatie van de lagere urinewegen tijdens een blaascontractie te bestuderen. Ondanks deze behoefte aan verder onderzoek liet het computerprogramma zien dat het verloop van de normale urinelozing in het algemeen verklaard kan worden met de in dit proefschrift beschreven wisselwerking tussen het zenuwstelsel enerzijds en de blaas en de plasbuis anderzijds.

Dankwoord

Bij een proefschrift past een woord van dank aan degenen die aan de totstandkoming ervan hebben bijgedragen. Zonder daarbij verder enige volgorde van belangrijkheid te willen hanteren verdient mijn co-promotor, Ron van Mastrigt, het om als eerste genoemd te worden. Hij was de initiator van het beschreven onderzoek en heeft me intensief begeleid op de weg van experiment naar publicatie. Ruud Bosch bedank ik vooral voor de voortreffelijke wijze waarop hij mij heeft ingewijd in de klinische urodynamica, het onderdeel van de urologie ten behoeve waarvan het onderzoek werd verricht. Behalve de twee genoemden kennen de artikelen waarop dit proefschrift is gebaseerd nog drie co-auteurs: Els van Asselt, Gommert van Koeveringe en Ries Kranse. Ieder van hen had zo z'n eigen specifieke bijdrage. Gommert heeft mij leren werken met cavia's en bedacht oplossingen voor enkele kleine, maar belangrijke praktische problemen. Samen met Els deed ik een deel van de experimenten en Ries heeft mij geholpen bij het verwerken van de resultaten. Een aantal malen deed ik een beroep op enkele diensten van de faculteit: het Erasmus Dierexperimenteel Centrum en de glasinstrumentmakerij, de mechanische werkplaats en de elektronische afdeling van de Centrale Instrumentele Dienst. Steeds werd ik snel en op adequate wijze geholpen.

Natuurlijk ben ik ook dank verschuldigd aan professor Schröder, mijn promotor, die me in de gelegenheid stelde dit proefschrift te bewerken, en aan de leden van de promotiecommissie, die het verdedigbaar achtten. Jannie heeft mijn werk letterlijk en figuurlijk op afstand gevolgd, maar was altijd op de hoogte wanneer ik weer een cavia had 'gedaan'. Tenslotte bedank ik mijn ouders voor de mogelijkheden die ze mij steeds hebben geboden.

Curriculum vitae

De schrijver van dit proefschrift werd geboren op 16 juni 1958 te Zuidveen. Hij volgde het middelbaar onderwijs aan het Nassau-College in Heerenveen en behaalde het diploma Atheneum-B in 1976. Aansluitend ging hij natuurkunde studeren aan de Rijksuniversiteit Groningen. Het doctoraal examen werd afgelegd op 31 augustus 1983. Na het vervullen van de militaire dienstplicht begon hij in 1984, wederom aan de Rijksuniversiteit Groningen, met de studie geneeskunde, die werd afgesloten met het artsexamen op 31 januari 1991. Sinds februari van datzelfde jaar werkt hij als artsonderzoeker bij de afdeling Urologie van het Academisch Ziekenhuis Rotterdam, de eerste jaren in het kader van het ontwikkelingsgeneeskunde-project "Neuromodulatie". De in dit proefschrift beschreven experimenten werden uitgevoerd bij de vakgroep Urodynamica van de Erasmus Universiteit Rotterdam.