# Regulation of the contractility of the urinary bladder under healthy and diseased condition

Ph.D. Thesis

2006

Enikő Á Széll M.D.

University of Debrecen, Medical-, Health- and Science Centre

Department of Pediatrics

## Introduction

During aging, the human bladder undergoes significant plasticity that contributes to the development of detrusor overactivity. For example, the originally flawless storage and emptying function of the bladder becomes altered over time as more and more pathological features begin operating that are partly the result of the changes in bladder smooth muscle and connective tissue morphology in addition to changes in afferent and efferent innervation. In the elderly, a common symptom in many patients is the development of urinary frequency, urgency and/or urge incontinence that is thought to result from bladder contractions during the bladder filling.

### α adrenergic receptor subtypes in the rat urinary bladder

Three subtypes of $\alpha_1$ adrenoceptors are present in peripheral tissues: $\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$. These subtypes can be identified pharmacologically with antagonists that are selective for $\alpha_{1A}$ (5 methyl-urapidil;5-MU) for $\alpha_{1D}$ (BMY 7378) or more effective for $\alpha_{1B}$ and $\alpha_{1D}$ adrenoceptors (chloroethyl-clonidine; CEC). In the rat bladder all three subtypes of $\alpha_1$ adrenoceptors have been detected in the smooth muscle. In addition $\alpha_1$ adrenoceptors are located prejunctionally on cholinergic terminals in the rat urinary bladder. Activation of prejunctional $\alpha_1$ adrenoceptors facilitates acetylcholine (ACh) release and enhances neurogenic contractions, whereas, activation of $\alpha_1$ adrenoceptors in the smooth muscle increases basal tone. The non-selective $\alpha_1$ adrenoceptor antagonist, terazosin, inhibited phenylephrine (PE)-induced facilitation of the neurally evoked contractions and facilitation of ACh release as well as the PE-evoked increase in the basal tone. The types of $\alpha_1$ adrenoceptors mediating the pre-and postjunctional effects of PE in the urinary bladder are not known.

**Developmental modifications of the spontaneous bladder activity in neonatal rats**

In neonatal animals during the early postnatal period, micturition is mediated by a somatobladder spinal reflex pathway, which is activated when the mother licks the perineum of the neonate. During postnatal development this primitive reflex is replaced by supraspinal mechanisms, which underlie the mature bladder-to-bladder reflex and voluntary voiding. In the rat, this developmental change in the central neural control of voiding occurs in concert with changes in peripheral neurotransmission and intrinsic properties of the bladder smooth muscle. The latter have been demonstrated in whole bladder preparations in vitro and in bladder strips of the neonatal rat. Whole bladders removed from pups during the first postnatal week exhibit low-amplitude spontaneous contractions. These contractions increase in amplitude during the second and third postnatal week. In bladder strips, spontaneous activity was absent during the first week but was detectable during the second postnatal week.

Excitatory and inhibitory neural mechanisms also change during development. For example, neurally evoked bladder contractions were mediated entirely by cholinergic mechanisms in the bladder strips from 1-wk-old rats but became primarily purinergic in strips obtained from 2-wk-old animals. Inhibitory neural mechanisms driven by tonic out-flow from the spinal cord have been detected in neonatal rat spinal cord-bladder preparations in vitro. Inhibitory responses were also elicited by electrical field stimulation in bethanechol-contracted in vitro fetal bovine bladders and bladder strips. These inhibitory responses were not detectable in strips from postnatal and adult animals.

The neonatal rat bladder also exhibits prominent changes in activity in response to alterations in temperature. In bladders from 1- to 2-wk-old animals the amplitude

of spontaneous contractions is maximal at body temperature and decreases as the temperature is reduced. On the other hand, in bladders from neonatal animals older than 3 wk of age and from adult animals, spontaneous contractions are of low amplitude at body temperature and increase in amplitude at lower temperatures. This dramatic change in temperature sensitivity occurs during the developmental period when central micturition pathways are maturing. Thus under physiological conditions the neonatal bladder is capable of generating large-amplitude intrinsic contractions, which presumably reflect pacemaker activity and efficient mechanisms for conducting this activity throughout the bladder. This activity may be necessary to promote voiding when the neural control of the bladder is immature. Conversely, the bladder of mature animals exhibits minimal intrinsic activity, which improves the urine storage capabilities but makes voiding entirely dependent on neural mechanisms.

**Effect of the cryoinjury on the contractile responses of rat urinary bladders**

Bladder smooth muscle undergoes marked changes during aging or neurological diseases that may result in impaired detrusor contractility. However, no adequate experimental model exists to investigate impaired contractility of the bladder. The deficiency of a precise and reproducible model of impaired bladder contractility makes it difficult to examine potential therapeutic solutions. Recently an acute invasive bladder injury model was elaborated in our lab by short intense cooling of the bladder muscle. Under our experimental conditions, the cryoinjured bladder smooth muscle exhibits reproducibly impaired contractile parameters such that it may be applied as a model of insufficiently functioning smooth muscle. For example, cryoinjury may be applied for testing of therapies to improve detrusor contractility such as tissue engineering.

Similar cryoinjury models have been used to model myocardial necrosis and to test myoblast transplantation in injured heart muscles. The cryoinjured heart muscle develops scar tissue and its contractile capability is greatly impaired. Striated muscles have also been subjected to cryoinjury to evoke degraded muscle function.

**List of the objectives**

1. In the present study we used subtype selective antagonists to examine the $\alpha_1$ adrenoceptor subtypes located pre- and postjunctionally in the rat bladder.
2. The present study was undertaken to examine the changes in spontaneous activity in the dome and base of the neonatal rat bladder during postnatal development and to determine the influence of cholinergic mechanisms on this activity.
3. The present investigation deals with the altered contractile function of bladder tissue after cryoinjury. The contractile response was evoked either by electrical stimulation or by activating postjunctional purinergic receptors with $\alpha,\beta$ methylene-ATP ($\alpha,\beta$ m-ATP).

## Methods

### Animals

Adult female Sprague-Dawley rats (at least 7 months old; 350–450 g) were used for the adrenergic receptor subtypes experiments, neonatal rats (from 3- to 35-day-old) were used for the spontaneous contractility measurements, and Sprague–Dawley rats (250–300 g) were used for cryoinjury.

### Preparation of urinary bladder.

Urinary bladders were removed rats that were killed by inhalation of 100% $CO_2$. After opening the abdomen, the urinary bladder was rapidly excised and held in Krebs solution containing (in mmol/l) 113 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 25 $NaHCO_3$, 1.2 $KH_2PO_4$, and 11.5 glucose. The bladder was cut transversally into two equal parts, base and dome, by sectioning the bladder just rostral to the vesicoureteral junction for spontaneous contractility measurements. In other cases the bladder was removed from the abdomen following decapitation and two to four circular slices were cut from the bladder body. These two ring-shaped pieces of bladder were then cut open and suspended in double-jacketed organ baths of 5 ml in Krebs solution and bubbled with a mixture of 95% $O_2$-5% $CO_2$ at 37°C.

### Cryoinjury

Rats were anesthetized with 2% halothane in pure oxygen and a low midline incision was made to expose the bladder and urethra. Tissue damage was performed with an aluminum rod of 8 mm dia. chilled on dry ice (–40°C) (cryoprobe). The chilled probe was placed against the serosal surface of the bladder wall for 30 s, while the bladder was filled with 1 ml saline.

**Contractile experiments**

The initial tension was set at 10 mN and isometric contractions were measured with strain-gauge transducers and recorded with a computerized data acquisition program (Windaq, DATAQ Instruments Inc, Akron, OH, U.S.A). Electrical field stimulation with a Grass 88 stimulator (Grass, ASTROMED, RI, U.S.A.) was delivered through platinum electrodes inserted from the top and bottom of the organ bath and separated by 4 cm. A stimulus intensity-response curve was constructed at the beginning of each experiment and unless otherwise stated the preparations were stimulated (20 Hz with 0.25 ms pulse duration) at a voltage producing 50% of the maximal response. Long (100 shocks) and short (10 shocks) duration trains of stimuli were used. The amplitudes and areas of the stimulation-evoked contractions were computed by the WindaqEx program (DATAQ). When the spontaneous activity was studied we have to wait until the contraction stabilized, then carbachol was applied (30-40 min) and then washed out. When the regular spontaneous activity returned (30-50 min), either TTX or atropine was applied..

**ACh release**

Tissue slices were placed into an incubation bath with 1 ml of Krebs solution containing 5 uCi $ml^{-1}$ $^{3}$H-choline (80 Ci $mmol^{-1}$), and constantly bubbled with a mixture of 95% $O_2$ and 5% $CO_2$ for 30 min at 37°C. Each slice was suspended in a separate bath after incubation and superfused at a rate of 0.3 ml $min^{-1}$ with oxygenated Krebs solution. After 60 min washing (precollection period), 3 min effluents were collected with a fraction collector. All drugs except CEC were added to the perfusion solution 15 min before the stimulation and were kept in the solution until the end of the experiments. In experiments with CEC, the drug was added to the solution for 30 min, then it was washed out 15 min before the

stimulation. When PE was applied to CEC treated preparations it was added to the perfusion solution after CEC was washed out. Electrical field stimulation (10 Hz, 100 shocks, 100 V, 0.25 ms stimulus duration) was delivered by a Grass S-88 stimulator through platinum plate electrodes positioned at the top and the bottom of the perfusion bath The strips were stimulated either once (experiments with WB 4101) or twice (CEC experiments) with the above parameters. When two stimulation paradigm was used, the time interval between the two stimulations was 50 min. The radioactivity in the effluent was measured by using Optifluor scintillation reagent (Packard Industries) with a Beckman Spectrometer (Model 5801). The measured counts were corrected to absolute activity using an external standard technique. To determine the total uptake of radioactivity, the acid soluble radioactive content of the tissue slices was determined after the experiments by placing them into 1 ml of 0.1 N perchloric acid for 16 h and an aliquot of 0.1 ml was removed for counting. The released amount of radioactivity for each sample was expressed as per cent of the tissue radioactive content (fractional release). The net evoked release of ACh reflects the number of counts or fractional release contained in the efflux curve during stimulation minus baseline (non-stimulated) release. To compute net evoked release the mean of two baseline samples taken immediately before and then after the recovery from stimulation was subtracted from the total radioactivity released due to the. Drug effects were calculated either as change in the fractional release or when two stimulation paradigm was used as change in $S_2/S_1$ ratio.

**Data analysis of spontaneous activity**

Spontaneous activity of bladder strips was analyzed by the following methods: *1*) fast Fourier transform (FFT) algorithm, *2*) nonlinear cross-prediction test (NLCP), *3*) renormalized Shannon entropy, and *4*) approximate entropy

(ApEn). For data analysis the following computer programs were applied: WindaqEx, Microsoft Excel, and Microcal Origin.

For frequency analysis the hanning version of FFT was used by collecting 16,384 data points for each analysis and then plotting the magnitude level against the frequency of the spontaneous activity.

For evaluation of the regularity of spontaneous contractions three nonlinear mathematical models were used: the NLCP, the renormalized Shannon entropy test, and the ApEn. The computational method for NLCP was written in Java programming language. The Shannon entropy and ApEn programs were written on C-analytic software. Generally, 4,500 points were used for the nonlinear tests. The change in system complexity was determined by a correlation dimension algorithm or a fractal dimension algorithm. A new mathematical model, the NLCP, was used to determine either linear or nonlinear system activity, or low-dimensional chaos in our preparations using the method of Stam and Pritchard. This model characterizes the time series on the basis of predictability, amplitude asymmetry (*ama*; *A*), or time asymmetry (*tir*; *B*), where *A* and *B* measure the nonlinearity in the system. Because in the case of *A* and *B* the time series used for constructing the model and the time series that has to be predicted are different, the two series can be considered as a method to estimate NLCP. The NLCP determines whether *1*) the time series are asymmetric around their mean values, e.g., the predictability of *A* will be less than that of the original time series, and *2*) the time series are time irreversible, e.g., the predictability of *B* will be less than that of the original time series. Stam et al. obtained simplified quantitative estimates by averaging the correlation coefficient of 20 prediction steps (*pred*). In this context, *ama* signifies the difference between the average correlation coefficient for the original time series and *A*, and *tir* signifies the difference between the average correlation coefficient for the original time series and *B*.

In this work we analyze our data both with a newer method, NLCP, and a more traditional nonlinear method, the Shannon entropy measurement. The latter method has been successfully applied in cardiology, in neurology, as well is in molecular biology. The renormalized version of the Shannon entropy was used because it is amplitude independent. Our data were also analyzed by a new statistical entropy method, the ApEn.

**Statistics**

The data were analysed by one way analysis of variance using the Prism Statistical Program (Graphpad, San Diego, CA, U.S.A.) using the Bonferini test as a post test. A level of $P<0.05$ was considered statistically significant. For statistical analysis, all the data are expressed as means ± SE. For the spontaneous contractility data the two-tailed Student's *t*-test was used to compare unpaired data between dome and base in the same age. For multiple comparisons of the age-dependent changes within either dome or base, ANOVA was used. $P < 0.05$ was taken to indicate statistical significance.

## Results

### Determine of the α adrenergic receptor subtypes in the rat urinary bladder

#### Time-course of the effect of PE

To establish the validity of cumulative concentration response curves which required continuous exposure to PE for 1–2 h, it was necessary to perform preliminary studies to evaluate the effects of prolonged administration of a single concentration of PE. In these experiments the PE effects on: (1) the neurally evoked bladder contractions and (2) basal smooth muscle tone were measured. Bladder strips were stimulated by short (10 shocks) or by long trains of pulses (100 shocks) at 20 Hz every 7 min. After obtaining control responses, 8 μM PE facilitated the contractions by 30–50% and increased the basal tone in the range of 3–6 mN. The strips were further stimulated for 125 min in the presence of PE. The increase in basal tone gradually decreased with time; whereas the facilitation of the neurally evoked contractions evoked by 100 shocks or 10 shocks did not change.

#### Effect of specific inhibitors of $\alpha_{1A}$ adrenoceptors on the PE-induced facilitation of neurogenic contractions and basal tone

After obtaining a cumulative concentration-response curve for PE (concentrations ranging from 0.5–32 μM) in preparations stimulated with trains of 10 shocks at 20 Hz, a selective antagonist for $\alpha_{1A}$ adrenoceptors; 5-methyl-urapidil (5-MU); Rec15/2739 or WB-4101 was applied. The concentration-response curve of PE was repeated in the presence of different concentrations of an antagonist starting with the lowest concentration. When the maximal PE facilitation occurred, both PE and the antagonist were washed out and immediately a higher concentration of antagonist was added to the bath. As shown before, the

concentration-response curves for PE were shifted to the right in a concentration-dependent manner by 5-MU. The $pA_2$ values for 5-MU and the other two antagonists were calculated according to Arunlakshana and Schild (1959). The three antagonists showed slightly different potencies; Rec15/2739 being the most potent and 5-MU the least potent. The slopes of the Schild plot were not significantly different from unity indicating that the three antagonists acted in a competitive manner. 5-MU inhibited the 8 μM PE evoked increase in basal tone with an $IC_{50}$ of 48±12.5 nM ($n$=6), whereas the $IC_{50}$ for the PE-facilitated neurally evoked contractions occurred at significantly lower concentration (10±1.5 nM; $n$=8; $P$<0.05).

**Effect of chloroethylclonidine (CEC) on the pre and postjunctional effects of PE**

In another set of experiments bladder strips were exposed for 30 min to various concentrations (2.5–50 μM) of CEC, an irreversible blocker that is more effective at $\alpha_{1B}/\alpha_{1D}$ adrenoceptors than at $\alpha_{1A}$ adrenoceptors. The facilitating effect of PE on the neurally evoked contractions was not inhibited after CEC treatment, however, the PE-induced increase in basal tone was suppressed by 90%.

CEC in concentrations of 2.5–20 μM also increased the amplitude (by 47.2±8.4 %; $n$=8 in a concentration of 20 μM) and increased the duration and area of the contractile curves (by 261±33%; $n$=8 in a concentration of 20 μM). Atropine (1 μM) suppressed both the amplitude and area of contractile curves in CEC-treated preparations. The effect of atropine was more prominent in CEC-treated than in untreated preparations. 5-MU (10–100 nM) administered 15 min before CEC did not reduce the CEC-increased contractions (data not shown) indicating that the increase in contractile amplitude by CEC is not due to activation of prejunctional $\alpha_{1A}$ receptors.

**Effect of $\alpha_{1A}$ and $\alpha_{1B}/\alpha_{1D}$ adrenoceptor blockade on the PE-evoked facilitation of ACh release**

PE-induced facilitation of ACh release was examined in the presence and absence of CEC (20 μM) and WB-4101 (100 nM). For practical reasons the two stimulation paradigm was used in the CEC experiments. With this stimulation pattern both the effect of PE and CEC could be tested with a smaller number of experiments. The bladder strips were stimulated twice ($S_1$ and $S_2$) and CEC was added to the perfusion solution before $S_2$ for a period of 30 min. CEC was washed out before the addition of PE (2 μM) to the perfusion solution. ACh release was facilitated by PE (2 μM) to the same extent in the presence and absence of CEC. However, ACh release in the absence of PE was significantly increased by CEC itself ($P<0.05$), a finding which is consistent with that obtained in the contractile experiments. On the other hand, WB-4101 (100 nM), an $\alpha_{1A}$ adrenoceptor antagonist, which alone did not alter the evoked release of ACh, inhibited the facilitatory effect of PE on the electrically evoked release of $^3$H-ACh.

## Developmental modifications of the spontaneous bladder activity in neonatal rats

**Amplitude and frequency of spontaneous bladder contractions in the neonatal bladder.**

Spontaneous activity was not detected in bladder strips from 1- to 5-day-old rats. However, small-amplitude, slow spontaneous activity was detected in 50% of strips from 6- to 7-day-old animals ($n = 4$). These bladder contractions had irregular amplitudes (0.1-2 mN) and frequencies. In strips from 2-wk-old rats ($n = 15$) more

regular spontaneous bladder activity occurred with higher peak contraction amplitudes (0.1-4 mN). One dominant peak was detected in the FFT spectrum, which was significantly faster ($P < 0.05$) in the base (0.21 ± 0.03 Hz) than in the dome (0.08 ± 0.01 Hz).

In bladder strips from 3- and 4-wk-old animals ($n = 14$), small-amplitude (<0.5 mN), high-frequency spontaneous contractions (fast component) (0.43 ± 0.07 and 0.41 ± 0.05 Hz, base and dome, respectively, $P > 0.05$) were superposed on high-amplitude (2-7 mN), low-frequency contractions (0.14 ± 0.03 and 0.1 ± 0.01 Hz, base and dome, respectively, $P > 0.05$). The fast component was present in 70% of the base strips but in only 20% of the dome strips. Since there was no difference in the data from 3- and 4-wk-bladders, these two groups were pooled together, and in this way we obtained four age groups: 1 wk old (3-7 day old), 2 wk old, 3-4 wk old, and 5 wk old. In bladder strips from 5-wk-old rats ($n = 13$), the amplitude of the slow activity was reduced to 1-2 mN, and the fast component was present more consistently in the dome (70%). In the FFT spectrum the magnitude of the slow peak was reduced and the fast peak was more prominent.

**Effect of TTX and atropine on the frequency and amplitude composition of spontaneous bladder contraction.**

TTX (1 μM) did not affect the spontaneous activity of bladder strips or the FFT curves from base or dome strips at any age ($n = 6$ in the 2- to 3-wk-old group, $n = 9$ in the 4- to 5-wk-old group). Atropine (1 μM) reduced the amplitude of spontaneous contractions by 65 ± 8% in dome strips and by 39 ± 15% in base strips from 2- to 3-wk-old rats ($n = 9$). In 4- to 5-wk-old bladders atropine was less effective, reducing the amplitude of the contractions in the dome and base by 8 ± 3 and 23 ± 6%, respectively ($n = 11$). Atropine reduced the basal tone of the muscle strips. This effect of atropine was more prominent in 2- to 3-wk-old (63 ± 10%, 58

± 9%, dome and base, respectively) than in 4- to 5-wk-old bladders (22 ± 6%, 44 ± 5%, dome and base, respectively). The magnitude of the slow peak in the FFT spectrum was significantly reduced in the 2- to 3-wk-old rats but not in 4- to 5-wk-old rats.

**Effect of carbachol on the amplitude and frequency of spontaneous bladder contractions.**

Carbachol (1 μM), a cholinergic agonist, induced large tonic contractions of bladder strips from 1- to 5-wk-old animals and also changed the amplitude and frequency of the spontaneous contractions. In strips from 1-wk-old rats carbachol (1 μM) induced vigorous spontaneous activity in 60% of the strips. The peak frequency of slow activity was significantly ($P < 0.05$) increased in the base (from 0.21 ± 0.03 to 0.41 ± 0.08 Hz) and in the dome (from 0.08 ± 0.01 to 0.23 ± 0.03 Hz), and a fast (1.17 ± 0.18 Hz) component was unmasked in the base of 1- and 2-wk-old rats but not the dome after carbachol. After carbachol the frequency of the slow component was significantly ($P < 0.05$) higher in the base than in the dome as noted before carbachol administration. In 2-wk-old rats, carbachol increased the frequency of the slow component in strips from the base but not from the dome. Also, carbachol induced a fast component in the base but not the dome of 1- and 2-wk-old rats. In bladder strips from 3- to 4-wk-old rats, carbachol also significantly stimulated the slow component in the base but not in the dome, whereas in strips from 5-wk-old rats the drug did not significantly change the slow component in the base or the dome (0.19 ± 0.04 and 0.16 ± 0.04 Hz, respectively; $P > 0.05$). However, in strips from 5-wk-old rats after carbachol treatment, the frequency of the fast component was significantly higher in the base than in the dome (0.75 ± 0.08 and 0.58 ± 0.07 Hz, respectively, $P < 0.05$). This difference was not noted in untreated strips.

**Nonlinear analysis of spontaneous bladder contraction.**

The pattern of spontaneous bladder activity was analyzed by the NLCP in tissues from animals of different ages. In the 1-wk-old age group, tissue from a total of 13 animals was studied. Although spontaneous activity was only detected in bladder strips from three animals (5-7 days old), it was clear that the activity was more regular than in older animals. Analysis of records from individual strips showed that both major parameters, i.e., *ama* and *tir*, were near zero and that both dome and bladder strips showed regular contractions [i.e., prediction number (*pred*) was near 1]. In 2-wk-old bladders the "*pred*" of dome strips was decreased (0.75 ± 0.04) compared with the base strips (0.93 ± 0.03), indicating that the regularity of spontaneous activity in dome strips was reduced. The reason for this irregular muscle activity was revealed in the *ama* analysis, which showed higher values in dome strips (0.27 ± 0.07) than in base strips (0.15 ± 0.03). This indicates a greater variability in the amplitude of individual contractions in the dome versus the base. The analysis of individual records showed that 70% of dome-strips had irregular time series (*tir*). In the 3-wk-old animals the *ama* was further reduced both in the dome and the base (0.13 ± 0.03 and 0.1 ± 0.04, respectively), and this reduction was statistically significant for the dome strips ($P < 0.05$). The *tir* values were increased, but this elevation was not significant. A further tendency of *ama* reduction and *tir* elevation was observed in 4- and 5-wk-old bladder strips where the *tir* elevation was more prominent in tissues from the dome. In strips from 5-wk-old rats, the regularity (*pred*) was also reduced in dome strips; however, the reason for this irregular activity was the increased variation in intervals between contractions as measured by the *tir*, which was significantly increased ($P < 0.05$) from 0.07 ± 0.02 in 2-wk-old bladders to 0.15 ± 0.024 in 5-wk-old bladders. The analysis of individual preparations showed that 80% of the dome strips have an irregular time

series, but this was only detected in 25% of base strips. These differences between dome and base were eliminated after treatment with carbachol (1 μM); e.g., there was no difference in *pred*, and the *tir* was not elevated.

The data were also evaluated by a more traditional method, the renormalized Shannon entropy, which is amplitude independent. The frequency-dependent complexity of contractile activity of bladder strips was more regular in the 1-wk-old bladders. The renormalized Shannon entropy was smaller than 2 in both the dome and the base. As noted by Bondarenko, high Shannon entropy indicates a more irregular activity. In the muscle strips from older animals, the Shannon entropy significantly increased, indicating that the low-complexity, synchronous muscle activity disappeared and that the activity in 3- to 4-wk-old and 5-wk-old bladder strips was becoming more complex and more asynchronous. At these ages the renormalized Shannon entropy value was ~3. Generally, there was no significant difference between the dome and base muscle strips at any age. This developmental entropy change was masked in presence of 1 μM carbachol. Similar tends in the results were detected using by the new statistical entropy method, ApEn (data not shown).

## Effect of the cryoinjury on the contractile responses of rat urinary bladders

### Histology and Contractile Function of the Cryoinjured Bladder

Five days after cryoinjury, scar tissue formation could be observed in the bladder wall at the site of the injury. Macroscopically the bladder appeared inflamed and the peritoneum was closely attached to the injured area. Microscopically, there was a consistent area of fibrosis at the site of cryoinjury. When the strips were prepared from the site of the injury, no contraction could be

evoked either by electrical stimulation or $\alpha,\beta$ m-ATP; therefore, the strips were isolated 2–3 mm apart from the injured area.

**Effect of Different Stimulation Frequencies in Intact and Cryoinjured Bladders**

The bladder strips were stimulated at various frequencies (1–40 Hz) with trains of 10 and 80 shocks. As described in previous papers, the cholinergic, in comparison to purinergic, component of the contractions was more prominent during longer train stimulation (80 shocks) than during shorter train (10 shocks) stimulation. The cryoinjured strips generated significantly less contractile force than that of intact bladder strips during both short- and long-train stimulation. Using short-train stimulation (10 shocks), the difference between the intact and injured bladders was similar across a range of frequencies from 10 to 40 Hz. However, during long-train stimulation, the difference between the injured and intact bladders, when calculated on the basis of absolute values ($mN/mm^2$), was frequency dependent and increased as the frequency of stimulation was increased from 20 to 40 Hz.

**Maximal Rate of Contraction and Relaxation in the Intact and Injured Bladder Strips**

The maximal rate of the contractions obtained by stimulation with 10 or 80 shocks at 20 Hz were calculated from the first derivative of the rising phase with a "dt" of 100 ms with the Advanced Codas computer program (DATAQ). The differentiation of the contractile curve produced a biphasic curve, where the positive phase represented the rate of contraction and the negative phase measured the rate of relaxation. Both the rate of contraction and relaxation were significantly slower in the cryoinjured bladders than in intact bladders.

**Effect of Atropine on the Amplitude of the Contractions in Intact and Cryoinjured Bladder Strips**

The muscarinic antagonist, atropine (1 $\mu$M), was added to the tissue bath and the contractile response was measured after 15-min equilibration. The atropine had an inhibitory effect on the neurally evoked (20 Hz, 80 shocks) contractions of both the noninjured and cryoinjured bladder strips. However, the inhibitory effect of atropine was more prominent in the cryoinjured bladders.

**Contractile Effect of α,β m-ATP**

The stable analog of the ATP, $\alpha,\beta$ m-ATP (50 $\mu$M), was added to the tissue bath after atropine administration. The contractile effect of $\alpha,\beta$ m-ATP was significantly lower in cryoinjured bladder strips than in noninjured bladder strips.

## Summary

**Smooth muscle and parasympathetic nerve terminals in the rat urinary bladder have different subtypes of α1 adrenoceptors.**

Neurally evoked contractions and release of $^{3}$H- acetylcholine (ACh) during electrical field stimulation were measured in rat urinary bladder strips. The $\alpha_1$ agonist phenylephrine (PE, 2–8 μM) increased the amplitude of neurally evoked contractions, facilitated the release of ACh and increased the baseline tone of the bladder strips. The PE-induced facilitation of the contractions did not significantly change during a prolonged exposure to PE (120 min), whereas the PE-induced rise in baseline tone gradually decreased to 65% of the initial value. Low concentrations of specific $\alpha_{1A}$ antagonists, 5-methyl urapidil (5-MU), REC15/2739 and WB-4101 competitively inhibited the facilitation of the neurally-evoked contractions ($pA_2$: 8.77; 9.59 and 9.62, respectively), whereas higher concentrations of 5-MU ($IC_{50}$: 48 nM) were required to suppress the PE-rise in baseline. WB-4101 (100 μM) inhibited the PE-induced facilitation of ACh release. The irreversible $\alpha_{1B}$ antagonist chloroethyl-clonidine (CEC, 10–50 μM) inhibited the PE-evoked rise in base line tone, but did not affect the PE-induced facilitation of the neurally evoked contractions nor the facilitation of ACh release. However, CEC increased the area and amplitude of the neurally-evoked contractions by 261±33 and 47.2±8.4%, respectively. Atropine significantly inhibited the CEC evoked increase in area and amplitude of the electrically evoked contractions (76.5±4.8 and 40.8±3%, respectively) indicating that CEC facilitated the cholinergic responses of the electrically stimulated bladder strips.

In summary $\alpha_{1A}$ and $\alpha_{1B}$/$\alpha_{1D}$ adrenoceptors are localized at different sites in the rat bladder. The $\alpha_{1A}$ adrenoceptors mediate prejunctional facilitation at the cholinergic nerve terminals, whereas, $\alpha_{1B}$ or $\alpha_{1D}$ adrenoceptors mediate smooth

muscle contractions. The latter response is more prominent in bladders of older animals. In human bladder body $\alpha_{1A}$ and $\alpha_{1D}$ adrenoceptors have also been identified. Because $\alpha_1$ adrenoceptors may play a significant role in the hyperactivity of obstructed and neurogenic bladders, a more detailed analysis of the relative contribution of prejunctional and postjunctional $\alpha_1$ adrenoceptor mechanisms is warranted. Adrenoceptor antagonists targeting $\alpha_{1A}$ prejunctional and $\alpha_{1B}$ or $\alpha_{1D}$ postjunctional adrenoceptors might have different effects on bladder activity and have different clinical uses.

**Developmental changes in spontaneous smooth muscle activity in the neonatal rat urinary bladder.**

Changes in spontaneous activity of the urinary bladder during postnatal development were examined in muscle strips from the base and dome of bladders from 1- to 5-wk-old rats. Activity was analyzed using fast Fourier transformation (FFT), nonlinear cross prediction, and the Shannon entropy test. Spontaneous activity was not detected in strips from 1- to 5-day-old rats but was observed in 50% of strips from 6- to 7-day-old rats and was prominent in strips from 2-wk-old animals. FFT analysis revealed one peak in activity, which was significantly faster in the bladder base (0.21 ± 0.03 Hz) than in the dome (0.08 ± 0.01 Hz). A second peak at ~0.5 Hz was detected at 3-5 wk of age. Atropine but not tetrodotoxin decreased the amplitude of spontaneous contractions, whereas carbachol, a muscarinic agonist, unmasked or stimulated spontaneous activity. These data suggest that slow rhythmic activity observed previously in neonatal whole bladders is generated by pacemaker cells in the bladder base or dome. The emergence of faster activity in bladders from older animals may reflect the development of multiple pacemaker sites, which would reduce coordination within the bladder wall and improve storage function in the mature bladder.

In summary, the neonatal rat bladder exhibits large-amplitude coordinated contractions that occur in the absence of neural input but which are modulated by spontaneous release of acetylcholine, presumably from cholinergic nerve terminals. FFT analysis revealed that cells in the bladder base have a significantly higher rate of contractile activity than cells in the bladder dome, raising the possibility that this higher level of activity in the base contributes to continence mechanisms by maintaining bladder neck closure or that in spontaneous contractions of the whole bladder might be controlled by pacemaker activity arising in the base and then spreading to the remainder of the bladder. Coordinated, large-amplitude, low-frequency contractile activity declines in strips from older animals and is replaced by low-amplitude, high-frequency, more irregular activity that appears to reflect the emergence of multiple pacemaker sites. This change in intrinsic activity coincides in time with the development of the central neuronal mechanisms that mediate voluntary voiding in adult animals. The change in the intrinsic properties of the bladder probably reflects the appearance of the mature storage function of the organ, which appears to depend at least in part on the disruption of the intercellular smooth muscle communication and emergence of asynchronous, chaotic muscle activity.

**Effect of cryoinjury on the contractile parameters of bladder strips: a model of impaired detrusor contractility.**

In anesthetized Sprague–Dawley rats, the bladder was exposed and cryoinjury was induced by abruptly freezing the serosal side of the bladder wall with a chilled aluminum rod previously placed on dry ice (–40°C). Five days later, the rats were euthanized, and strips were prepared from the area adjacent to the injury. Neurally and $\alpha,\beta$ methylene-ATP ($\alpha,\beta$ m-ATP; 50 $\mu$M)-evoked contractions were measured in bladder strips from cryoinjured or intact bladders prepared from

sham-operated rats. Cryoinjured bladder strips produced significantly lower contractile forces than intact strips to electrical stimulation at higher (10–40 Hz) frequencies. The maximal rate of the neurally evoked contractions was slower in the cryoinjured bladders. The contractile response to $\alpha,\beta$ m-ATP was smaller in the cryoinjured preparations indicating that the changes may have also occurred at the postjunctional site. In addition, atropine was more effective at inhibiting the neurally evoked contractions in the cryoinjured bladder strips suggesting that a cholinergic dominance occurs after cryoinjury. It is concluded that cryoinjury is a viable method of causing a defined, reproducible injury to the urinary bladder resulting in impaired function of both the cholinergic transmission and the smooth muscle. The bladder cryoinjury can be used as a model for studying impaired bladder compliance and detrusor contractility as well as treatments that may improve bladder function such as tissue engineering.

In summary, cryoinjury initiated a number of changes in the bladder contractile system including changing the relative participation of the parasympathetic and NANC nervous system in electrically evoked contractions. As a result, cryoinjured bladders demonstrated a reproducible and persistent model for impaired contractile detrusor function. This new model of impaired detrusor contractility can be a viable model of studying tissue engineering and pharmacological therapy to enhance and restore the contractile function of the bladder detrusor.

## List of publications

***In extenso* publications** (IF based on ISI 2005)

Bánk J., **Széll E.,** Bedő G., Gesztelyi G., Nagy V.: Transcranial Doppler measurements in migraine and tension type headache. *Clin. Neurology* 48, 173-176, 1995. **IF: 0**

**Szell EA**, Yamamoto T, de Groat WC and Somogyi GT: Smooth muscle and parasympathetic nerve terminals in the rat urinary bladder have different subtypes of α1 adrenoceptors. *Br. J. Pharmacol.* 130: 1685-91, 2000. **IF: 3,410 Cit: 25**

Somogyi GT, Yokoyama T, **Szell EA**, Smith CP, de Groat WC, Huard J, Chancellor MB. Effect of cryoinjury on the contractile parameters of bladder strips: a model of impaired detrusor contractility. *Brain Res. Bull.* 59: 23-8, 2002. **IF: 2,481 Cit: 2**

**Szell EA,** Somogyi GT, de Groat WC, Szigeti GP. Developmental changes in spontaneous smooth muscle activity in the neonatal rat urinary bladder. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 285: R809-16, 2003. **IF: 3,802 Cit: 6**

Szigeti GP, Somogyi GT, Csernoch L, **Szell EA.** Age-dependence of the spontaneous activity of the rat urinary bladder. *J. Muscle. Res. Cell M.* 26(1): 23-9, 2005. Invited review. **IF: 1,338 Cit: 0**

**Impact factor: 11,031**

**Number of the independent citations: 33**

## Abstracts

Somogyi GT, **Szell EA**, Yamamoto T, and de Groat WC: The function of alpha-1 adrenoceptors in the bladders of adult and neonatal rats. *Fundam. Clin. Pharm.* 13/Suppl. 1.: 164S, 1999. **IF: 1,717**

Somogyi GT, **Szell EA**, Yamamoto T, and de Groat WC: Smooth muscle and parasympathetic nerve terminals have different subtypes of α1 adrenoceptors in the rat urinary bladder. *Neurosci. Abstr.* 25: 1170, 1999. **IF: 3,410**

**Szell EA,** Somogyi GT, de Groat WC and Szigeti GP: Developmental changes in smooth muscle spontaneous activity in the neonatal rat urinary bladder. *Biophysical J*. 78: 671Pos, 2000. **IF: 4,507**

Szigeti GP, Somogyi GT, Csernoch L, **Szell EA.** Age-dependence of cholinergic and purinergic receptors in urinary bladder. *J. Muscle. Res. Cell M.* 26(1): 2005. **IF: 1,338**

Jenes A, Ruzsnavszky F, **Szell EA,** Varga A, Somogyi GT, Csernoch L, Szigeti GP. The role of the cholinergic and purinergic interaction in the contraction of the cultured human and rat urinary bladder smooth muscle cells. *J. Muscle. Res. Cell M.* 27(5-7): 2006. **IF: 1,338**

**Impact Factor: 12,310**

## Lectures and posters

Somogyi GT, **Szell EA**, Yamamoto T, and de Groat WC: The function of alpha-1 adrenoceptors in the bladders of adult and neonatal rats. *2nd European Congress of Pharmacology*, Budapest, 1999.

Somogyi GT, **Szell EA**, Yamamoto T, and de Groat WC: Smooth muscle and parasympathetic nerve terminals have different subtypes of α1 adrenoceptors in the rat urinary bladder. *29th Annual Meeting, Society for Neuroscience*, Miami Beach, USA, 1999.

**Szell EA,** Somogyi GT, de Groat WC and Szigeti GP: Developmental changes in smooth muscle spontaneous activity in the neonatal rat urinary bladder. *Annual Meeting, Biophysical Society*, New Orleans, USA, 2000.

**Széll E,** Frank M, Jenes Á, Somogyi GT, Szigeti GP. Patkány húgyhólyag kolinerg és purinerg mechanizmusainak korfüggése. *Magyar Élettani Társaság 69. vándorgyűlése,* Budapest, 2005.

Szigeti GP, Somogyi GT, Csernoch L, **Szell EA.** Age-dependence of cholinergic and purinergic receptors in urinary bladder. (felkért előadás) *XXXIV. European Muscle Conference,* Hortobágy, 2005.

Szigeti GP, Jenes Á, Ruzsnavszky F, **Széll E,** Varga A, Csernoch L. A felnőtt patkány hólyag-kontrakcióinak szabályozása, és modellként való felhasználása. *Magyar Élettani Társaság 70. vándorgyűlése,* Szeged, 2006.

Jenes Á, Ruzsnavszky F, **Széll E,** Varga A, Csernoch L, Szigeti GP. A patkány húgyhólyagműködés szabályozásának életkor- és hólyagterület-függése. *Magyar Élettani Társaság 70. vándorgyűlése,* Szeged, 2006.

Jenes A, Ruzsnavszky F, **Szell EA,** Varga A, Somogyi GT, Csernoch L, Szigeti GP. The role of the cholinergic and purinergic interaction in the contraction of the cultured human and rat urinary bladder smooth muscle cells. *XXXV. European Muscle Conference,* Heidelberg, Germany, 2006.