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

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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 (3, 5, 6, 14, 17, 22, 25, 27). During postnatal development this primitive reflex is replaced by supraspinal mechanisms, which underlie the mature bladder-to-blink reflex and voluntary voiding (1, 5, 11, 12, 14). 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 (13, 14, 24, 25). The latter have been demonstrated in whole bladder preparations (14, 15, 22–25) in vitro and in bladder strips (13) 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 (13, 22, 23). In bladder strips, spontaneous activity was absent during the first week but was detectable during the second postnatal week (13).

Excitatory and inhibitory neural mechanisms also change during development (1, 22, 23, 25). 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 (13, 14). Inhibitory neural mechanisms driven by tonic outflow from the spinal cord have been detected in neonatal rat spinal cord-bladder preparations in vitro (22, 23, 25). 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 (10).

The neonatal rat bladder also exhibits prominent changes in activity in response to alterations in temperature (24). 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 (1, 5, 11, 12, 14). 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 (22, 24). 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

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improves the urine storage capabilities but makes voiding entirely dependent on neural mechanisms.

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.

**METHODS**

**Preparation of urinary bladder.** Urinary bladders were removed from 3- to 35-day-old Sprague-Dawley rats that were killed by inhalation of 100% CO2. 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 CaCl2, 1.2 MgSO4, 25 NaHCO3, 1.2 KH2PO4, 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. These two ring-shaped pieces, base and dome, by sectioning the bladder just rostral to the vesicoureteral junction. These two ring-shaped pieces, base and dome, were then cut open and suspended in double-jacketed organ baths of 5 ml in Krebs solution and bubbled with a mixture of 95% O2–5% CO2 at 37°C. Glucose. The bladder was cut transversally into two equal parts, base and dome, by sectioning the bladder just rostral to the vesicoureteral junction. These two ring-shaped pieces, base and dome, were then cut open and suspended in double-jacketed organ baths of 5 ml in Krebs solution and bubbled with a mixture of 95% O2–5% CO2 at 37°C. Glucose.

**Contractile measurement.** The initial tension was set to 5 mN for 1- to 2-wk-old and to 10 mN for 3- to 5-wk-old bladders. The isometric contractions of the strips were measured with strain-gauge transducers (Grass AstroMed, West Warwick, RI) and recorded with a computerized data-acquisition system (WinDaq, DATAQ Instruments, Akron, OH), using a 20-Hz sampling rate.

**Experimental protocol.** When the spontaneous activity was stable, 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. In three experiments we applied TTX and then atropine also. However, the atropine and TTX were not applied in every experiment where the spontaneous activity was detected. It means the numbers of animals are different in Figs. 1 and 2 compared with Fig. 5.

**Drugs.** Atropine sulfate (RBI), carbamylcholine chloride (carbachol, Sigma), and TTX (Sigma) were injected into the Krebs solution in the organ bath.

**Data analysis.** Spontaneous activity of bladder strips was analyzed by the following methods: 1) fast Fourier transform (FFT) algorithm (2), 2) nonlinear cross-prediction test (NLCP; 19, 20), 3) renormalized Shannon entropy (4), and 4) approximate entropy (ApEn; Ref. 16). 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 (7) or a fractal dimension algorithm (8). 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 (20). 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 (19) determines whether J 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 irreversable, e.g., the predictability of B will be less than that of the original time series. Stam et al. (19) 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 paper 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 (18), in neurology (4), as well as in molecular biology (21). The renormalized version of the Shannon entropy was used because it is amplitude independent (4). Our data were also analyzed by a new statistical entropy method, the ApEn (16).

For statistical analysis, all the data are expressed as means ± SE. 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. In Figs. 2, 5, and 6, the asterisk (*) shows the significant differences when Student's t-test was used and the pound sign (#) shows when ANOVA was used for the comparison.

**RESULTS**

**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 (Fig. 1A). 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 (Fig. 1B) 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, Fig. 2).

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) (Fig. 1C and Fig. 2). 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 (Fig. 1D).

**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 (Fig. 3, 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 (Fig. 4, 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 (Fig. 4, A and B) but not in 4- to 5-wk-old rats.

**Fig. 1.** Fast Fourier transform (FFT) analysis of spontaneous contractile activity of urinary bladder strips from base and dome of rats of different ages. In each set of records, the contractile activity of the bladder strip from the base or dome is shown in the top tracing and the FFT analysis fitted curve with 2 Gaussian curves is shown in the bottom tracing. Spontaneous bladder activity was not detected in 3 day-old rats (A) but was prominent in 2-wk-old animals (B). The FFTs exhibited 1 peak (arrow) in 2-wk-old animals, indicating faster activity in the base strips (0.21 ± 0.03 Hz) than in dome strips (0.08 ± 0.01 Hz). In muscle strips from the base of 3- to 4-wk-old animals (C), the FFTs exhibited a 2nd peak (~0.5 Hz). The 2nd peak was also observed in dome muscle strips from 5-wk-old rats (D). In tissue from 5-wk-old animals, the relative magnitude of 1st peak was reduced and the 2nd peak became dominant. The horizontal line represents 30 s and the vertical line indicates 5 mN contractile force. Mag (on ordinate) means magnitude of the FFT power spectra.

**Fig. 2.** Developmental change in the slow and fast peak of the FFT analysis of base and dome contractile activity from 1- to 5-wk-old neonatal rats. A: histogram shows that the peak frequency of the slow component of the spontaneous contractile activity decreases significantly between 1 and 5 wk of age and that the peak frequency in the base is higher than in the dome. B: histogram shows that the fast component is not present at 1–2 wk of age but appears at 3–5 wk of age. Significant differences are illustrated by * and # (see METHODS for more details).
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 (Fig. 5A). The peak frequency of slow activity was significantly ($P < 0.05$) increased in the base (from $0.21 \pm 0.03$ to $0.41 \pm 0.08$ Hz) and in the dome (from $0.08 \pm 0.01$ to $0.23 \pm 0.03$ 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 (Fig. 5, B and C). After carbachol the frequency of the slow component was significantly ($P < 0.05$) higher in the base than in the dome (Fig. 5, B and C) as noted before carbachol administration (to facilitate comparisons the data from Fig. 2 are also shown in Fig. 5, B and C). In 2-wk-old rats, carbachol increased the frequency of the slow component in strips from the base but not from the dome (Fig. 5, B and C). 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 ($0.19 \pm 0.04$ and $0.16 \pm 0.04$ 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 ($0.75 \pm 0.08$ and $0.58 \pm 0.07$ Hz, respectively, $P < 0.05$) (Fig. 5, B and C). This difference was not noted in untreated strips (Figs. 2 and 5).

Nonlinear analysis of spontaneous bladder contractions. The pattern of spontaneous bladder activity was analyzed by the NLCP (Fig. 6A) 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 ($0.75 \pm 0.04$) compared with the base strips ($0.93 \pm 0.03$), 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 ($0.27 \pm 0.07$) than in base strips ($0.15 \pm 0.03$). 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, Fig. 6A). In

Fig. 3. Effect of 1 μM TTX on spontaneous contractions of a bladder strip from 2- and 4-wk-old rats. Data are from bladder dome strips from 2-wk-old (A) and 4-wk-old animals (B) before (left) and after (middle) administration of 1 μM TTX, which did not alter the amplitude of contractile activity in either preparation. The FFT analysis (right) did not reveal any change in contractile activity. The continuous line FFT record represents the control condition, and the dotted line represents the FFT in presence of TTX. The horizontal calibrations represent 20 s, and the vertical calibrations represent 4 mN contractile force.

Fig. 4. Effect of 1 μM atropine on spontaneous contractions of strips from the bladder dome from 2- and 4-wk-old rats. Data are from 2-wk-old (A) and 4-wk-old animals (B) before (left) and after (middle) administration of 1 μM atropine, which reduced the amplitude of contractile activity in the 2-wk-old but not the 4-wk-old tissue. The horizontal calibrations represent 20 s, and the vertical calibrations represent 2 and 4 mN contractile force in A and B, respectively. The FFT records (right) show that the magnitude of the slow peak (continuous lines, the control condition) was reduced after atropine (dotted lines represent the FFT record in presence of atropine) in the 2-wk-old but not the 4-wk-old tissue.
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 (Fig. 6B). The renormalized Shannon entropy was smaller than 2 in both the dome and the base. As noted by Bondarenko (4), high Shannon entropy indicates a more irregular activity. In the muscle strips from older animals, the Shannon entropy significantly increased (Fig. 6B), 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 (Fig. 6B). 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 (Fig. 6B).

**DISCUSSION**

Our findings indicate that during the first five postnatal weeks, the rat urinary bladder undergoes
marked changes in contractile properties. Smooth muscle strips from the bladder dome and base exhibited intrinsic activity that varied in amplitude, frequency, and regularity depending on age. These developmental changes may reflect alterations in the electrical and contractile activity of individual smooth muscle cells as well as changes in the efficiency of intercellular smooth muscle communication and in turn propagation of electrical signals throughout the bladder. In young animals a higher frequency of rhythmic spontaneous activity was detected in the bladder base compared with the dome, suggesting that the level of activity might be linked with the different functions of the base (i.e., bladder neck closure and urine storage) and dome (i.e., urine elimination). This intrinsic smooth muscle activity may be essential for efficient bladder emptying before the maturation of neural voiding mechanisms. On the other hand, elimination of this coordinated activity during postnatal development could be linked with the emergence of the storage functions of the mature bladder smooth muscle.

Previous studies revealed that the whole neonatal rat bladder preparation in vitro during the first 2–3 postnatal weeks exhibited rhythmic spontaneous contractions that were larger in amplitude and slower in frequency than those in adult rat whole bladder preparations (24), suggesting that the immature bladder exhibits synchronized activity that originates at a pacemaker site. The activity then spreads in a coordinated manner throughout the bladder muscle. Our present findings on bladder smooth muscle strips provide evidence that the highest frequency pacemaker is located in the region of the bladder base. FFT analysis of spontaneous contractions of bladder strips from 1- to 2-wk-old animals indicated a peak of activity occurring at slow frequencies ranging from 0.08 to 0.21 Hz. The slow rhythmic activity was detected in both the bladder dome and base but occurred at a higher frequency in the base, indicating that even though cells in both sections of the bladder base and dome respond to carbachol by increasing contractility, the frequency and amplitude of the contractile response are different.
regions are capable of generating coordinated contractions, the cells in the bladder base by virtue of their higher pacing frequency may generate the pattern of rhythmic activity in the whole bladder, assuming that the data obtained in strips are applicable to the intact organ. Although whole bladders and strips are studied under different experimental conditions, it should be noted that the frequency of spontaneous activity in these two preparations at 1–2 wk of age is not markedly different. This is suggested by the fact that intercontraction intervals in strips calculated from the FFT peaks in the present study ranged from 5 to 12 s and the peak intercontraction interval in whole bladders (24) was ~20 s. Another study (15) of neonatal bladder strips (1–3 days old) reported an even better match (e.g., an intercontraction interval of 22 s) with the whole bladder measurements. The difference in results between the two bladder strip studies could be related to differences in the methods used, including orientation and size of the strips as well as the basal tension. Nevertheless, there seems to be a reasonably good correlation between strip and whole bladder studies in regard to the spontaneous contractile activity during the first two postnatal weeks.

The similarity between bladder strip and whole bladder data was also apparent in the changes, which occurred later in development. In the whole bladder the frequency of spontaneous contractions increased at 3–5 wk of age, amplitude decreased, and bladder activity became more chaotic. FFT analysis revealed a similar change in bladder strips from the dome and base. At 3 wk of age a second peak representing faster activity (0.4 Hz) appeared in the FFT. This peak became more prominent in the base strips at 5 wk of age and also was detectable in the FFT of dome strips. It is noteworthy that the intercontraction interval for this fast activity (2.5 s) is very similar to the interval (1.9 s) between small spontaneous movements visible on the surface of the mature bladder (24).

The emergence of faster activity in whole bladders and in bladder strips from older animals may reflect the development of multiple and independent pacemaker sites. This conclusion is consistent with the results of the nonlinear analysis of spontaneous contractile activity. In a small number of strips from the 1-wk age group, both dome and base strips revealed amplitude and time asymmetry measurements near zero and a prediction number near 1, indicating regular contractions. Similar measurements in strips from older animals revealed irregular time and amplitude characteristics, which were more prominent in the dome than in the base. When data were evaluated by a more traditional method, the renormalized Shannon entropy, which is independent of amplitude measurements, the results indicated that activity in muscle strips from 1- to 2-wk-old animals was synchronous and less complex, whereas the activity in strips from older animals was asynchronous and more complex. These observations suggest that contractile activity is more chaotic in older bladders, particularly in the region of the dome, and that intercellular communication mechanisms, possibly via gap junctions, may be downregulated during development. Preliminary studies using optical imaging methods and calcium-sensitive and voltage-sensitive dyes in whole bladder preparations from the neonatal rat have detected electrical activity moving in a coordinated manner from localized regions over the entire bladder (9). On the other hand, only chaotic activity originating at multiple sites was detected in adult bladders.

To determine if the spontaneous activity of the neonatal bladder is modulated by neural mechanisms, the effect of the sodium channel blocker TTX and the muscarinic antagonist atropine was evaluated. TTX did not alter the amplitude or the pattern of spontaneous contractile activity, indicating that neural firing was not important in regulating the contractions of bladder strips. Different results were obtained in the in vitro neonatal spinal cord-bladder preparation in which TTX increased the spontaneous bladder contractions (22). These as well as other experiments (25) indicate that the neonatal rat bladder receives a tonic inhibitory neural input from the spinal cord, which suppresses the intrinsic pacemaker activity of the smooth muscle.

When postjuncturnal muscarinic receptors were blocked with atropine, a significant reduction in the amplitude but not the frequency of the spontaneous contractions of strips from 2-wk-old rats was observed. Maggi et al. (14) also reported a small inhibitory effect of atropine on spontaneous activity in bladder strips from newborn (1–3 days old) but not adult rats. These results suggest that the smooth muscle contractions are modulated by the spontaneous release of acetylcholine from cholinergic nerve endings. Because atropine did not alter the frequency of contractile activity, it seems reasonable to conclude that neurally released acetylcholine does not alter the properties of the pacemaker cells but rather promotes the spread of activity from these cells to other parts of the bladder.

Activation of postjuncturnal muscarinic receptors with carbachol induced a large-amplitude muscle contraction and changed the pattern of spontaneous activity. Carbachol increased the frequency of the slow activity in the base and dome and also unmasked fast activity in the base but not the dome in 1-wk-old rats. This indicates that pacemaker activity can be enhanced either indirectly by raising the tension in the bladder wall or directly by stimulation of muscarinic receptors in the pacemaker cells. The induction of fast activity by carbachol could also be due to an indirect effect to increase wall tension and in turn induce the emergence of multiple pacemaker sites throughout the bladder. However, it is interesting that even in the presence of carbachol, the base exhibited a higher frequency of activity than the dome.

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
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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 (5, 6, 25). 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.

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DISCLOSURES

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