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Properties of spontaneous depolarizations in circular smooth muscle cells of rabbit urethra

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1 Intracellular microelectrode recordings were made from circular smooth muscle of rabbit urethra.

2 The smooth muscle of urethra was spontaneously active exhibiting large, regularly occurring depolarizations, termed slow waves (SWs), 1-3 s in duration, up to 40 mV in amplitude and generated every 3-15 s and small irregularly occurring events (or summations there of) termed spontaneous transient depolarizations (STDs) of <1 s in duration.

3 The SWs and STDs were not sensitive to 10^{-6} M atropine, 10^{-6} M phentolamine, 10^{-5} M guanethidine or 10^{-6} M tetrodotoxin, indicating that they were myogenic in origin.

4 Application of 3×10^{-6} M nifedipine or 5×10^{-5} M $GdCl_3$ did not inhibit the generation of SWs or STDs, indicating that activation of L-type Ca^{2+} channels and non-selective cation channels are not essential for their generation. However, the duration of SWs but not STDs was reduced by nifedipine, indicating L-type Ca^{2+} channels contribute to the plateau-like potential of SWs.

5 Application of low chloride solution (6.4 mM), niflumic acid ($10^{-5}-10^{-4}$ M) or 4,4'-diisothiocyanostilbene-2,2'-disulphonic acid (DIDS, $10^{-4}-5\times10^{-4}$ M) inhibited the generation of SWs and STDs, suggesting an involvement of chloride channels.

6 Application of nominally Ca^{2+} free solution, 5×10^{-5} M BAPTA-AM, 10^{-5} M cyclopiazonic acid, 10^{-2} M caffeine or 10^{-3} M procaine inhibited the generation of SWs and STDs, indicating that Ca^{2+} released from intracellular stores was required for the generation of SWs and STDs.

7 Exogenously applied noradrenaline ($10^{-7}-10^{-5}$ M) increased the frequency of SWs through stimulation of α-adrenoceptors which was inhibited by sodium nitroprusside (SNP, 10^{-4} M). SNP also reduced the frequency of SWs without altering the membrane potential, an effect mimicked by 8-bromocyclic GMP (10^{-3} M) indicating that SNP acted by elevating the production of cyclic GMP.

8 It is concluded that smooth muscle cells of the rabbit urethra exhibit SWs and STDs which are likely to be induced by stimulation of Ca^{2+}-activated chloride channels evoked by release of Ca^{2+} from intracellular stores.

Keywords: Urethra; slow wave; spontaneous transient depolarization; Ca^{2+}-sensitive chloride channel; intracellular Ca^{2+} store

Introduction

Many types of smooth muscle undergo rhythmic contractions with underlying oscillatory depolarizations (slow waves) or spike potentials or both (Tomita, 1981). Such pacemaking activity is often 'myogenic', being generated within the smooth muscle and/or other electrically coupled cells. However, the mechanisms underlying such myogenic activity have in most cases yet to be elucidated. Insight into a basis for myogenic pacemaking has arisen from studies on small lymphatic vessels of the guinea-pig (Van Helden, 1989; 1993). In these tissues myogenic depolarizations which underlie generation of spontaneous constrictions exhibit a pharmacology entirely consistent with the depolarizations arising from release of Ca^{2+} from intracellular stores to generate spontaneous transient inward currents (STICs; Van Helden, 1991; Wang *et al.*, 1992). By comparison much larger tissues such as smooth muscle of the gastrointestinal tract exhibit 'myogenic' pacemaking activity which involves a population of cells termed 'interstitial cells of Cajal (IC)' (Thuneberg, 1982; Liu *et al.*, 1994; Ward *et al.*, 1994). These cells are connected by numerous gap junctions to the smooth muscle. There have been a number of proposals regarding the generation of slow waves including: generation involving sodium pump activity (Connor *et al.*, 1974), a Na^+-Ca^{2+} exchange mechanism (Tomita, 1981), metabolic activity (Huizinga *et al.*, 1991) or a cardiac-like pacemaker cycle involving low threshold, voltage-dependent Ca^{2+} channels (Lee & Sanders, 1993; Publicover, 1994).

The present study considers the origin and ionic basis of myogenic depolarizations observed in circular smooth muscle of the rabbit urethra. This tissue exhibits two types of myogenic depolarizations, small events termed 'spontaneous transient depolarizations (STDs)' and large regularly occurring slow waves (SWs). The response of the membrane to many types of agents indicate that chloride currents are involved in the generation of both STDs and SWs and strongly support a role for Ca^{2+} release from intracellular stores in the generation of both events.

Methods

Male rabbits weighing between 2-3 kg were killed by exsanguination under phenobarbitone anaesthesia. The urethra and bladder were removed and the dorsal wall of the urethra was opened longitudinally and the mucosa, periurethral fat and connective tissues were removed. The longitudinal smooth muscle layer was then removed leaving the circular smooth muscle preparation.

Microelectrode experiments were made on circular muscle preparations 8-10 mm long and 1-2 mm wide mounted with the mucosal side uppermost on a silicon rubber plate fixed at the bottom of recording chamber, and superfused with warmed (35-37°C) Krebs solution at a constant flow rate (about 3 ml min^{-1}). After equilibration for at least 1.5 h, the muscle strip was immobilized on the rubber plate with tiny pins. Electrical responses of urethral circular smooth muscle

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were recorded with glass capillary microelectrodes (diameter 1.2 mm o.d., 0.6 mm i.d. with a glass filament inside, Hilgenberg, Germany) filled with 3 M KCl. The tip resistance of the electrodes ranged between 40–70 MΩ. The electrical responses recorded were displayed on a cathode-ray oscilloscope (SS-9622, Iwatsu, Japan) and also on a pen recorder (Recticorder RJG-4024, Nihon Kohden). Some records were simultaneously recorded on instrumentation tape (LCDR-135, Sony, Japan) by a tape recorder (FE-30A, SONY, Japan) for subsequent playback on a pen recorder or data analysis on a personal computer (Fetchan, Axon instruments, U.S.A.).

The Krebs solution contained in mM: Na^+ 137.5, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134 and glucose 15. The solution was aerated with 95% O_2 and 5% CO_2 with pH of the solution maintained at 7.2–7.3. Low chloride solution containing $[Cl^-]=6.4$ mM was prepared by equimolar replacement of NaCl with sodium isethionate.

Drugs used were: noradrenaline hydrochloride, phentolamine mesylate, sodium nitroprusside, 8-bromoguanosine 3':5'-cyclic monophosphate (8-Br-cyclic GMP), nicardipine, nifedipine, tetrodotoxin, atropine sulphate, guanethidine sulphate, niflumic acid, 4,4'-diisothiocyanostilbene-2,2'-disulphonic acid (DIDS), BAPTA-AM, cyclopiazonic acid, caffeine, procaine and $GdCl_3$ (all from Sigma, St. Louis, MO.). These drugs were dissolved in distilled water, dimethyl sulphoxide (DMSO) or ethyleneglycol at concentrations of 10^{-1}–10^{-4} M as stock solutions, and kept at 4°C for up to 10 days before use. The drugs were then diluted with Krebs solution to obtain the desired concentrations. These procedures did not detectably change the pH of the solution.

The recorded values were expressed as mean ± one standard deviation (s.d.). Statistical significances were determined by Student's t test, and probabilities of less than 5% ($P<0.05$) were considered significant.

Results

General observations

In most recordings, the preparations were spontaneously active, exhibiting large and small transient depolarizations (Figure 1). The former termed slow waves (SWs; Tomita, 1981), occurred periodically (interval between events, 3–15 s, mean 8.7 ± 3.5 s, $n=26$) and were variable in shape and amplitude with a total duration of 1–3 s and amplitudes of up to 30–40 mV (equivalent to absolute membrane potentials of −25 to −15 mV). Other events, termed spontaneous transient depolarization (STDs; Van Helden, 1991), appeared irregularly and were of small amplitude with rapid onset followed by slower decay (e.g. 20–80% rise times 52 ± 11 ms, half decay times 108 ± 20 ms, $n=8$ tissues). Both SWs and STDs were observed in 83% of cells studied and STDs were observed without SWs in the remainder of the cells. No cell exhibiting SWs alone was observed.

The steady potential level at the most negative state was used to estimate the resting membrane potential which was between −40 and −70 mV (mean, −50.6 ± 3.8 mV, $n=48$ tissues, 342 cells). SWs and STDs persisted during application of tetrodotoxin (10^{-6} M; $n=5$), atropine (10^{-6} M; $n=3$), phentolamine (10^{-6} M; $n=5$) and guanethidine (5×10^{-6} M; $n=3$) and thus SWs and STDs were both considered to be of myogenic origin. Application of 5×10^{-5} M $GdCl_3$, an inhibitor of both a caffeine-activated and a stretch-activated non-selective cation channel (Guerrero *et al.*, 1994; Wellner & Isenberg, 1995), failed to inhibit the generation of SWs and STDs ($n=5$), indicating that these non-selective cation channels do not underlie the SWs and STDs.

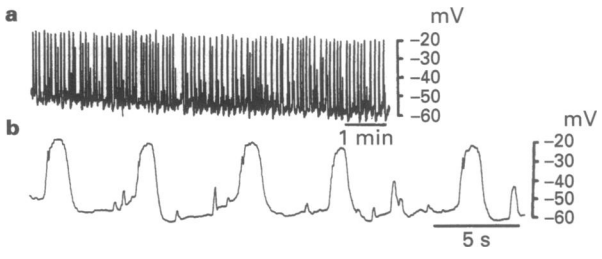

Figure 1 Slow waves (SWs) and spontaneous transient depolarizations (STDs) recorded from the rabbit urethra. (a) and (b), records from the same cell on different time scales.

Modulation of slow waves by nifedipine

As was generally observed, the tissue illustrated in Figure 2 exhibited large rhythmical depolarizations (SWs) and smaller irregularly occurring depolarizations (STDs). Nifedipine shortened the plateau phase of the SWs without obviously decreasing the amplitude (Figure 2d). The histogram in Figure 2c shows the distribution of amplitude of SWs and STDs in control conditions and in nifedipine. The potentials were classified into 3 groups according to amplitude: STDs (amplitude < 20 mV), SWs (amplitude ≈ 40 mV), and 'intermediate' potentials (amplitude ≈ 30 mV). The histogram shows that nifedipine did not modify the distribution of these potentials.

The effects of nifedipine are further exemplified in Figure 2d where averaged SWs are compared before and after 10 min in 3×10^{-6} M nifedipine. This indicates that SWs are composed

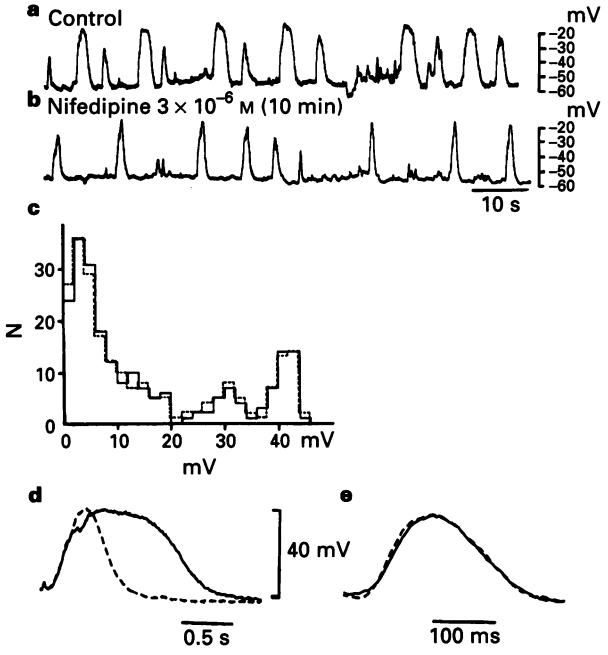

Figure 2 Effects of 3×10^{-6} M nifedipine on spontaneous depolarizations. (a) Spontaneous depolarizations recorded under control conditions. Slow waves (SWs), 'intermediate' depolarizations and spontaneous transient depolarizations (STDs) are shown. (b) A recording obtained from the same impalement as (a) but after 10 min in 3×10^{-6} M nifedipine. (c) Amplitude histograms of all depolarizations during 5 min in control (continuous line) and nifedipine (dotted line) respectively. The distribution of amplitudes was not altered by nifedipine. (d) SWs recorded before (continuous line) and after application of 3×10^{-6} M nifedipine for 10–15 min (dotted line). The traces are the average of 6 and 8 events respectively in each condition. (e) Normalized STDs recorded before (continuous line) and after 10–15 min application of 3×10^{-6} M nifedipine (dotted line). The traces are respectively the average of 4 and 6 events for each condition and potentials are normalized relative to the peak. In (d) and (e) events were synchronized using the time point at which each depolarization achieved peak amplitude.

of two components, an initial component which is unaffected by nifedipine and a nifedipine-sensitive component which underlies the SW plateau. The 20–80% rise times were not significantly altered by nifedipine ($P>0.05$; 208 ± 52 ms in control, $n=6$ and 190 ± 41 ms in nifedipine, $n=8$) while the duration of SWs at half the amplitude was significantly reduced by nifedipine (1129 ± 152 ms in control, $n=6$ and 576 ± 110 ms in nifedipine, $n=8$, $P<0.05$).

The effect of nifedipine (3×10^{-6} M; >10 min exposure) was measured in 3 tissues where the SW duration was reduced to $54\pm12\%$ of control. Nifedipine had no obvious effect on SW amplitude ($100\pm2\%$ of control) and frequency ($99\pm2\%$ of control).

Figure 2e shows a comparison of averaged STDs in control conditions and with nifedipine. As the amplitude of STDs varied widely, they were normalized relative to the peak of individual potentials. The rise times were 47 ± 4 ms in control ($n=4$) and 45 ± 8 ms in nifedipine ($n=6$), and the half-decay times of STDs were 98 ± 7 ms in control ($n=4$) and 102 ± 12 ms in nifedipine ($n=6$). These values were not significantly different ($P>0.05$), thus STDs were not obviously altered by nifedipine.

Effect of low chloride external solution and chloride channel blockers on SWs and STDs

The ionic basis of SWs and STDs was further investigated. It was found that reduction of extracellular chloride ions or application of known inhibitors of chloride channels suppressed both SWs and STDs. The chemicals used were niflumic acid (NFA) and 4,4′-diisothiocyanostilbene-2,2′-disulphonic acid (DIDS), substances which have been shown to inhibit Ca^{2+}-activated chloride channels (Pacaud *et al.*, 1989; White & Aylwin, 1989; Kokubun *et al.*, 1991; Hogg *et al.*, 1994a, b).

Figure 3 shows the effects of low chloride solution on the SWs. Reduction of $[Cl^-]_o$ to 6.4 mM caused a decrease in amplitude of the SWs and after 15 min exposure to the low chloride solution the generation of SWs was completely inhibited (Figure 3a,b; $n=4$). SWs in low chloride solution are shown in Figure 3c–e on a fast time base. After 8 min exposure to low chloride solution, the amplitude of SWs without plateau-like potential were reduced to about 50% of control (Figure 3c and d). At 15 min SWs were abolished (Figure 3e). The low chloride solution suppressed but did not completely inhibit the STDs. Comparison of their amplitude in control and after 15 min exposure to the low chloride solution for each 20 measurable STDs indicated that STD amplitude was reduced by $28.7\pm8.7\%$ ($n=4$).

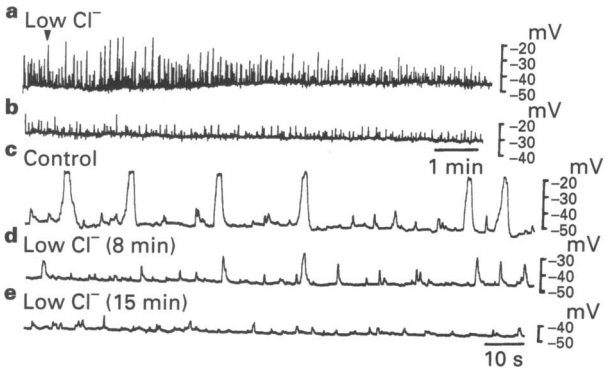

Figure 3 Effects of low chloride solution on slow waves (SWs) and spontaneous transient depolarizations (STDs). (a) and (b) are continuous recordings. The superfused solution was changed to low chloride solution ($[Cl^-]=6.4$ mM, replaced by isethionate) at the arrow. (c), (d) and (e) are continuous recordings shown on a fast time base; (c) control; (d) and (e), 8 and 15 min exposure to low chloride solutions, respectively. (a–b) and (c–e) were recorded from different tissues.

Figure 4 shows the effects of NFA and DIDS on SWs and STDs. Both NFA and DIDS had no significant effect on membrane potential (e.g. -49.8 ± 4.7 mV before and -49.9 ± 5.2 mV in the presence of 5×10^{-5} M NFA, $n=16$; -53.4 ± 4.4 mV before and -53.6 ± 4.7 mV in the presence of 10^{-4} M DIDS, $n=15$). The amplitude of SWs and STDs was determined by an average of 5 to 20 events in control and test conditions. The amplitude of SWs was reduced to $68\pm8\%$ ($n=3$) by 10^{-5} M NFA and application of 5×10^{-5} M NFA reduced the amplitude of SWs to $55\pm5\%$ in 7 cells and completely inhibited SWs in 7 other cells. An example is presented in Figure 4a where 5×10^{-5} M NFA inhibited the generation of SWs within 5 min. In 3 cells 10^{-4} M NFA abolished SWs. Figure 4b shows an example where 10^{-4} M DIDS also suppressed SW amplitude to $58\pm8\%$ ($n=14$) and a higher concentration (5×10^{-4} M DIDS) completely inhibited the generation of SWs ($n=5$).

NFA (5×10^{-5} M) decreased STD amplitude to $53\pm9\%$ of control ($n=10$, see also Figure 4c,d). NFA not only decreased STD amplitude but also appeared to slow the kinetics (Figure 4e). The rise times of STDs were 67 ± 33 ms in control ($n=5$) and 168 ± 70 ms in NFA ($n=9$) and the half-decay times were 145 ± 41 ms in control ($n=5$) and 315 ± 101 ms in NFA ($n=9$). These values were significantly different ($P<0.05$). Application of NFA at higher concentrations (e.g., 10^{-4} M) completely inhibited the generation of STDs. DIDS at 10^{-4} M also reduced the amplitude of STDs to $54\pm6\%$ of control ($n=5$) and higher concentrations (e.g., 5×10^{-4} M) completely abolished STDs.

Role of $[Ca^{2+}]_i/Ca^{2+}$ stores on SWs and STDs

The effects of nominally Ca^{2+}-free solution and drugs which modulate the intracellular Ca^{2+} concentrations and Ca^{2+} stores on SWs and STDs are shown in Figure 5. Nominally Ca^{2+}-free solution depolarized the membrane by 15.2 ± 3 mV ($n=4$) before decreasing the amplitude and frequency of SWs

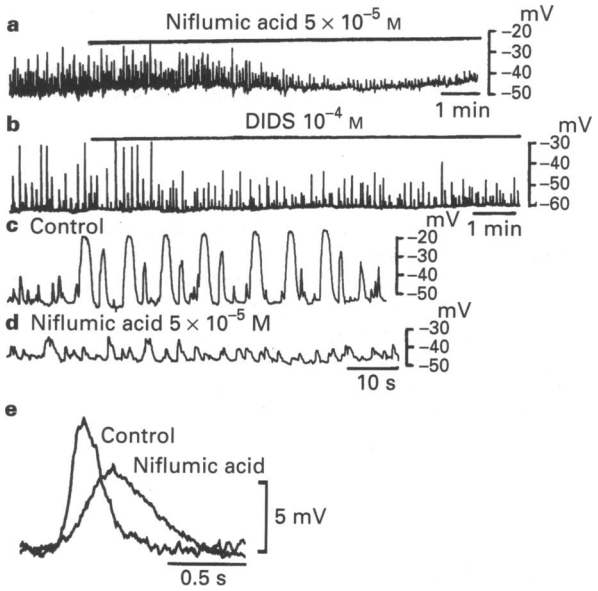

Figure 4 Effects of niflumic acid and 4,4′-diisothiocyanostilbene-2,2′-disulphonic acid (DIDS) on slow waves (SWs) and spontaneous transient depolarizations (STDs). (a) Niflumic acid (5×10^{-5} M) and (b) DIDS (10^{-4} M) were applied during the bar above each record. (a) and (b) were recorded from different tissues. (c) and (d) show the records before and after application of niflumic acid (5×10^{-5} M) on a faster time base. (e) Averaged STDs recorded in control ($n=5$) and after 10 min application of 5×10^{-5} M niflumic acid ($n=9$), with events synchronized with respect to the initiation of the depolarizations.

and STDs (Figure 5a). In nominally Ca^{2+} free solution, the amplitude of SWs and STDs were decreased to 41±17% ($n=4$) and 56±18% of control ($n=4$) respectively, while the frequency of SWs and STDs were decreased to 15±9% ($n=4$) and 23±10% of control ($n=4$), respectively. Application of the calcium chelator BAPTA (5×10^{-5} M, applied in the lipid permeable form, BAPTA-AM) abolished the generation of both SWs and STDs with no detectable change in the membrane potential ($n=4$; Figure 5b). Inhibition of the Ca^{2+}-ATPase of the Ca^{2+} stores by cyclopiazonic acid (CPA, 10^{-5} M) depolarized the membrane by 4.7±1.1 mV ($n=7$) and subsequently abolished the generation of SWs (Figure 5c). CPA at this concentration effectively abolished STDs (e.g., Figure 5c) in 4 out of 7 tissues examined, with STDs inhibited but not abolished in the remaining 3 tissues (frequency 8±3% of control; amplitude 73±9% of control). Caffeine (10^{-2} M) also depolarized the membrane by 7.3±1.5 mV ($n=3$) and abolished the generation of SWs while markedly inhibiting STDs (frequency 109±24% of control; amplitude 10±5% of control, $n=3$, Figure 5d). Application of procaine (10^{-3} M) for 3–5 min decreased SW amplitude and frequency to 56±20% and 36±15% of control respectively ($n=3$) and STD amplitude and frequency to 51±14% and 42±13% of control respectively ($n=3$). Procaine (10^{-3} M) depolarized the membrane by 12.5±4.2 mV ($n=3$) (Figure 5e).

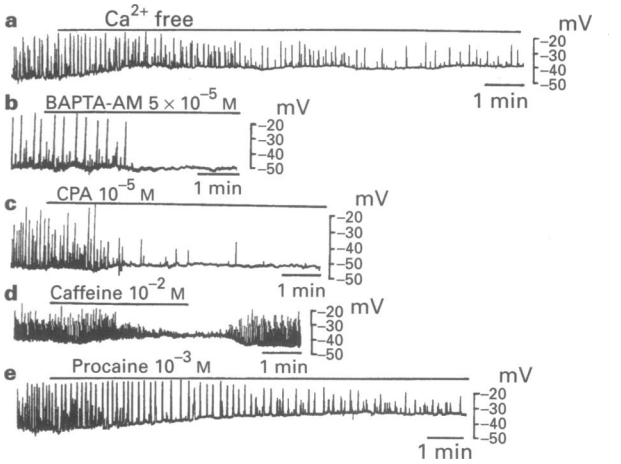

Figure 5 Effects of nominally Ca^{2+} free solution, BAPTA-AM, cyclopiazonic acid, caffeine and procaine on slow waves (SWs) and spontaneous transient depolarizations (STDs). Nominally Ca^{2+} free solution (a), 5×10^{-5}M BAPTA-AM (b), 10^{-5}M cyclopiazonic acid (CPA, c), 10^{-2}M caffeine (d) or 10^{-3}M procaine (e) was applied as indicated by the bar in each trace. All recordings were made from different tissues.

Role of noradrenaline and nitric oxide

The effects of noradrenaline (NA) and the putative neurotransmitter nitric oxide (NO) on SWs and STDs were also examined. Exogenous application of the NO donor, sodium nitroprusside (SNP, 10^{-4} M) reduced, whereas NA (10^{-6} M) enhanced, the generation of SWs in smooth muscle of the rabbit urethra. The effects of SNP on SWs appeared slowly and required 3–5 min to reach a steady reduction in SW frequency to 55±9% ($n=5$) of control (Figure 6a). The effects of NA appeared very rapidly, and markedly increased the frequency of SWs. However, the response to the NA was so marked that it was difficult to distinguish SWs from summated STD activity. This action was accompanied by a mean depolarization of 9.3±2.9 mV ($n=13$). Additional application of phentolamine (10^{-6} M) blocked the actions of NA ($n=5$; Figure 6b). In the presence of NA, additional application of SNP ($n=6$; Figure 6c) or 8-bromo cyclic GMP (10^{-3} M, $n=3$; Figure 6d) slowly antagonized the actions of NA.

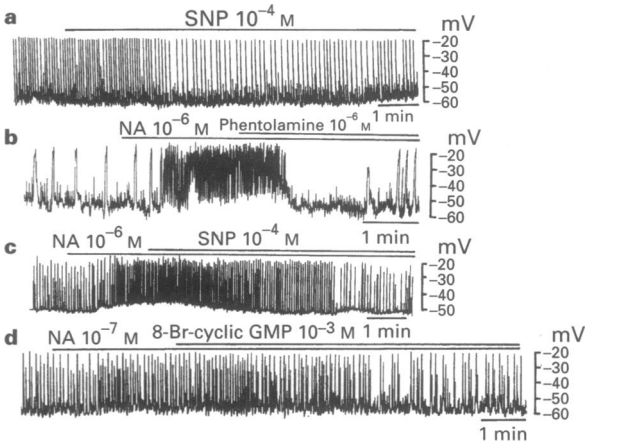

Figure 6 Effects of noradrenaline (NA), sodium nitroprusside and 8-Br-cyclic GMP on spontaneous and NA-induced activities in smooth muscle of the rabbit urethra. (a) Effects of 10^{-4}M sodium nitroprusside (SNP). (b) Effects of 10^{-6}M NA and additional application of 10^{-6}M phentolamine. (c) Effects of 10^{-4}M SNP applied in the presence of 10^{-6}M NA. (d) Effects of 10^{-3}M 8-Br-cyclic GMP applied in the presence of 10^{-7}M NA. Each drug was applied as indicated by the horizontal bars. All recordings were made from different tissues.

Discussion

SWs occur in many smooth muscles but there has been little understanding of the mechanisms which underlie such activity. The findings of the present study provide insight into the basis for SWs in the circular smooth muscle of the rabbit urethra.

The key observation of this study is that SWs appear to be generated by the release of Ca^{2+} ions from intracellular stores possibly through summation of the 'quantal-like' Ca^{2+} release events which underlie spontaneous transient depolarizations (STDs). The findings of our study are consistent with the postulate that SWs are generated by a mechanism similar to that underlying pacemaker potentials in lymphatic smooth muscle (Van Helden, 1993; Van Helden *et al.*, 1996). Here, pacemaker potentials are comprised of STDs which summate to reach threshold, generate a calcium action potential and hence cause constriction to pump lymph. STDs, or more specifically the underlying spontaneous transient inward currents (STICs) which are also observed in venous smooth muscle seem to be 'quantal' events generated by local release of Ca^{2+} from intracellular store site(s) (Van Helden, 1991; Wang *et al.*, 1992). The released Ca^{2+} diffuses to the plasma membrane and causes STICs presumably through opening of calcium-activated chloride channels (as proven in the rabbit portal vein, Wang *et al.*, 1992; Hogg *et al.*, 1994a,b and in the pulmonary artery, Hogg *et al.*, 1993).

STDs in the rabbit urethra share many of these properties. First, STDs are unlikely to be of neurogenic origin as they were not inhibited by atropine, phentolamine, guanethidine or tetrodotoxin. Most importantly they were abolished by BAPTA/AM indicating that they are not due to spontaneous release of transmitter which, as shown in studies on other tissues, is not inhibited by chelation of $[Ca^{2+}]_i$ (e.g., Hunt *et al.*, 1994). Indeed reduction by BAPTA/AM coupled with findings of partial or complete inhibition by nominally Ca^{2+} free solution, CPA, procaine and caffeine of both STDs and SWs, indicates that both arise through release of Ca^{2+} from intracellular stores. A common feature of nominally Ca^{2+} free solution and BAPTA/AM would be a reduction of $[Ca^{2+}]_i$. BAPTA/AM was more effective than the nominally Ca^{2+} free solution possibly through causing a greater reduction of $[Ca^{2+}]_i$. CPA prevents uptake of Ca^{2+} into the intracellular store sites by inhibiting the Ca^{2+}-ATPase, and hence causes reduction of $[Ca^{2+}]$ in the lumen of the Ca^{2+} stores. (Siedler *et al.*, 1989; Uyama *et al.*, 1992). Procaine inhibits the release of Ca^{2+} from store sites, by preventing Ca^{2+}-induced Ca^{2+}-release (CICR;

Itoh *et al.*, 1981). Caffeine is known to activate Ca^{2+} release from stores by activation of ryanodine receptors (e.g., see Kuriyama *et al.*, 1995) or to prevent stored Ca^{2+} release through inositol-1,4,5 trisphosphate (Ins P_3) receptor-operated channels (Parker & Ivorra, 1991). STICs recorded in smooth muscle cells isolated from the rabbit portal vein are also inhibited by caffeine (Wang *et al.*, 1992). The inhibition by caffeine supports the postulate that STDs and SWs arise from Ca^{2+} released from intracellular Ins P_3 receptor-operated Ca^{2+} release channels and not from ryanodine sensitive Ca^{2+} release channels.

The channels underlying STDs and the generation of SWs were inhibited by either low chloride containing solution or the chloride channel blockers NFA and DIDS. Hogg *et al.* (1994a, b) have shown that NFA and DIDS reduce STICs in smooth muscle cells of the rabbit portal vein. Here, the concentrations required to reduce STIC amplitude by 50% (IC_{50}) were 3.6×10^{-6} M and 2.1×10^{-4} M for NFA and DIDS, respectively. In the rabbit urethra, 5×10^{-5} M NFA and 10^{-4} M DIDS reduced the amplitude of STDs by about 50%. Thus, DIDS inhibits both STDs in urethra and STICs in portal vein to a similar extent, while NFA is approximately one order of magnitude weaker as an inhibitor of STDs in urethra than of STICs in portal vein. The differences in the effectiveness of these agents may be related to a different tissue specificity or differences in experimental conditions.

An interesting but difficult aspect of this study was comparing STDs to SWs and their differential sensitivity to the various blockers. The difficulty arose because agents which suppressed SWs reduced the activity so that it became difficult to distinguish SWs from STDs (e.g., see Figures 3 and 4). The weight of our findings indicate that both STDs and SWs are generated by release of Ca^{2+} from intracellular stores. However, in the case of low chloride solution or chloride channel blockers, SWs appeared to be more rapidly inhibited and in the latter case at lower concentrations of NFA and DIDS. These differences need to be clarified. However, in so doing it is to be noted that STDs by analogy to STICs, probably involve Ca^{2+} release from a localized region, within a single smooth muscle cell. By comparison SWs must involve much more distributed calcium release in many coupled smooth muscle cells. It may be that the pharmacological agents or their action to reduce the depolarizations not only suppress STDs but differentially interfere with the intra/intercellular processes (e.g. rhythmical summation of STICs/STDs) which is likely to underlie the generation of SWs.

Stimulation of adrenergic nerves or exogenously applied NA produces α-adrenoceptor-mediated contraction in smooth muscle of the rabbit urethra (Ito & Kimoto, 1985; Kimoto *et al.*, 1987). As stimulation of α_1-adrenoceptors is known to mobilize Ins P_3 in other smooth muscle (Somlyo *et al.*, 1985; Hashimoto *et al.*, 1986), we propose that NA increases the frequency of SWs and STDs by acting on α-adrenoceptors to increase intracellular $[Ins\ P_3]_i/[Ca^{2+}]_i$ and hence cause an increased frequency of oscillatory CICR and subsequent STDs.

An involvement of NO releasing-nerves in the neural regulation of urethral smooth muscles has also been demonstrated (Andersson *et al.*, 1992). NO can relax vascular and non-vascular smooth muscles by increased production of cyclic GMP (Lincoln & Cornwell, 1993). The present experiments demonstrate that SNP can inhibit the generation of SWs with no change in the membrane potential, and these effects of SNP are mimicked by 8-bromo cyclic GMP. An increase in intracellular cyclic GMP content has been shown to reduce $[Ca^{2+}]_i$ through activation of the plasma membrane Ca^{2+} pump (Lincoln & Cornwell, 1993). As demonstrated in Figure 5a, reduction in $[Ca^{2+}]_i$ decreased the frequency of SWs and therefore NO may exert its inhibition of SW activity by reducing $[Ca^{2+}]_i$.

In the present study, we found that SWs but not STDs activated nifedipine sensitive (i.e., L-type) Ca^{2+}-channels. These channels did not contribute to the rising phase of SWs but appeared to be responsible for the plateau component. Therefore, voltage-dependent Ca^{2+} channels contribute to SWs but they do not trigger the SWs (at least under the conditions of this study). In this way they differ from recordings from longitudinal urethral smooth muscle where spontaneous action potentials have been demonstrated (Callaghan & Creed, 1981; 1985). However, while not generating an action potential *per se*, the voltage-dependent Ca^{2+} channels do subserve the role of causing the SW-induced spontaneous constrictions which are completely inhibited by blockers of L-type Ca^{2+} channels (H. Hashitani, unpublished observations).

In conclusion, this study has examined rhythmical depolarizing activity termed SWs and irregularly occurring spontaneous transient depolarizations termed STDs in the circular smooth muscle layer of the rabbit urethra. The STDs shared the characteristics of STICs/STDs observed in other smooth muscles and are likely to reflect localized intracellular release of Ca^{2+} from Ins P_3 receptor operated stores which probably open Ca^{2+}-activated chloride channels. SWs exhibited a similar pharmacology as if they were generated by a more dispersed release of Ca^{2+} which occur through approximate synchronization of STDs within and across coupled smooth muscle cells. The resulting larger depolarizations can activate voltage-dependent L-type Ca^{2+} channels which underlie the plateau component of SWs and activate constriction. This proposed scheme is analogous to the pacemaker mechanism in lymphatic smooth muscle where summations of STDs underlie the generation of voltage-dependent Ca^{2+} entry and phasic constrictions (Van Helden, 1993; Van Helden *et al.*, 1996). At present there is no evidence for interstitial cells in either urethral and lymphatic smooth muscle, yet such cells are mandatory to the generation of SWs in large tissues of the gastrointestinal tract (see Introduction). It may be that interstitial cells with their extensive coupling to smooth muscle cells serve to orchestrate rather than create SWs and that smaller tissues can generate such activity entirely through mechanisms present within smooth muscle.

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