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Role of IP_3 in modulation of spontaneous activity in pacemaker cells of rabbit urethra

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Sergeant, G. P., M. A. Hollywood, K. D. McCloskey, N. G. McHale, and K. D. Thornbury. Role of IP_3 in modulation of spontaneous activity in pacemaker cells of rabbit urethra. *Am J Physiol Cell Physiol* 280: C1349–C1356, 2001.—Isolated interstitial ("pacemaker") cells from rabbit urethra were examined using the perforated-patch technique. Under voltage clamp at −60 mV, these cells fired large spontaneous transient inward currents (STICs), averaging −860 pA and >1 s in duration, which could account for urethral pacemaker activity. Spontaneous transient outward currents (STOCs) were also observed and fell into two categories, "fast" (<100 ms in duration) and "slow" (>1 s in duration). The latter were coupled to STICs, suggesting that they shared the same mechanism, while the former occurred independently at faster rates. All of these currents were abolished by cyclopiazonic acid, caffeine, or ryanodine, suggesting that they were activated by Ca^{2+} release. When D-*myo*-inositol 1,4,5-trisphosphate (IP_3)-sensitive stores were blocked with 2-aminoethoxydiphenyl borate, the STICs and slow STOCs were abolished, but the fast STOCs remained. In contrast, the fast STOCs were more nifedipine sensitive than the STICs or the slow STOCs. These results suggest that while fast STOCs are mediated by a mechanism similar to STOCs in smooth muscle, STICs and slow STOCs are driven by IP_3. These results support the hypothesis that pacemaker activity in the urethra is driven by the IP_3-sensitive store.

D-*myo*-inositol 1,4,5-trisphosphate; spontaneous transient inward currents; spontaneous transient outward currents

INTRACELLULAR RECORDINGS from the urethra reveal that it generates spontaneous electrical slow waves (9, 10) that are thought to be due to summation of smaller electrical events called spontaneous transient depolarizations (STDs). The currents (spontaneous transient inward currents, STICs), which underlie the STDs, are believed to result from activation of Ca^{2+}-dependent Cl^- channels by Ca^{2+} released from intracellular stores (9, 10, 21). At present, it is unclear whether the Ca^{2+} that drives this mechanism is released via D-*myo*-inositol 1,4,5-trisphosphate (IP_3)- or ryanodine-sensitive stores. In smooth muscle cells, STICs appear to be activated the same way as STOCs (spontaneous transient outward currents), via ryanodine-sensitive Ca^{2+}-release channels (3, 14, 32). However, several intracellular microelectrode studies in the gut (22, 29), where a pacemaker mechanism similar to the urethra has been proposed (22, 29), suggest that the Ca^{2+} is released by IP_3. The only way to resolve this issue would be to study the mechanisms underlying STICs and STOCs in the pacemaker cells themselves, where it is conceivable that the Ca^{2+}-activated Cl^- and K^+ channels are activated by different mechanisms.

We have recently isolated cells from rabbit urethra (26) that resemble the interstitial cells of Cajal (ICC) in the gut, where they are widely believed to be the pacemakers (13, 20, 22). These urethral "interstitial cells" differed significantly, both in their morphological and electrophysiological properties, from smooth muscle cells isolated from the same preparation. They expressed large Ca^{2+}-activated Cl^- currents and generated STICs and STDs, while in contrast, the smooth muscle cells expressed little Cl^- current and were electrically quiescent. These properties, together with their resemblance to the gastrointestinal ICCs, make the urethral interstitial cells likely candidates to fulfill the role of pacemakers (26). In the present study, we have investigated the mechanisms activating STICs and STOCs in these cells and have found evidence for differential regulation of these currents. Specifically, IP_3 appears to drive the pacemaker mechanism, while STOCs continue to fire when IP_3-induced Ca^{2+} release is blocked.

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METHODS

Bladder and urethra were removed from both male and female rabbits immediately after they had been killed by lethal injection of pentobarbitone. The most proximal 1 cm of the urethra was removed and placed in Krebs solution, and from this, strips were dissected for cell dispersal.

For cell dispersal, 0.5-cm strips of proximal urethra were cut into 1-mm^3 pieces and stored in Hanks' Ca^{2+}-free solution for 30 min before being incubated in an enzyme medium containing (per 5 ml of Hanks' Ca^{2+}-free solution) 15 mg of collagenase (Sigma, type 1A), 1 mg of protease (Sigma, type XXIV), 10 mg of BSA (Sigma), and 10 mg of trypsin inhibitor (Sigma) for ~5 min at 37°C. They were then placed in Hanks' Ca^{2+}-free solution and stirred for another 5–10 min to release both single relaxed smooth muscle cells and interstitial cells. These were placed in petri dishes containing Hanks' solution (100 μM Ca^{2+}) and stored at 4°C for use within 8 h.

Recordings were made using the amphotericin B perforated-patch method (25). After gigaseals were obtained, the series resistance fell over a 10- to 15-min period to 10–15 MΩ and remained stable for up to 1 h. Voltage-clamp commands were delivered with an Axopatch 1D patch-clamp amplifier (Axon Instruments), and currents were recorded with a 12-bit analog-to-digital/digital-to-analog converter (Labmaster; Scientific Solutions) interfaced to an Intel computer running pCLAMP software (Axon Instruments). During experiments, the dish containing the cells was superfused with physiological salt solution (PSS; solution 2). In addition, the cell under study was continuously superfused with PSS by means of a close delivery system consisting of a pipette (tip diameter 200 μm) placed ~300 μm away. This could be switched, with a dead-space time of around 10 s, to a solution containing a drug. All experiments were carried out at 37°C.

The solutions used were of the following composition (in mM) *1*) Hanks' Ca^{2+}-free solution: 141 Na^+, 5.8 K^+, 130.3 Cl^-, 15.5 HCO_3^-, 0.34 HPO_4^{2-}, 0.44 $H_2PO_4^-$, 10 dextrose, 2.9 sucrose, and 10 HEPES, pH adjusted to 7.4 with NaOH; *2*) PSS: 130 Na^+, 5.8 K^+, 135 Cl^-, 4.16 HCO_3^-, 0.34 HPO_4^{2-}, 0.44 $H_2PO_4^-$, 1.8 Ca^{2+}, 0.9 Mg^{2+}, 0.4 SO_4^{2-}, 10 dextrose, 2.9 sucrose, and 10 HEPES, pH adjusted to 7.4 with NaOH; *3*) Cs^+ pipette solution: 133 Cs^+, 1 Mg^{2+}, 135 Cl^-, 0.5 EGTA, and 10 HEPES, pH adjusted to 7.2 with CsOH; and *4*) K^+ pipette solution: 133 K^+, 1 Mg^{2+}, 135 Cl^-, 0.5 EGTA, and 10 HEPES, pH adjusted to 7.2 with KOH. In current clamp experiments, 80 mM of the KCl was replaced with potassium gluconate to set the Cl^- equilibrium potential to the more physiological value of −24 mV.

The following drugs were used: caffeine, 2-nitro-4-carboxyphenyl *N*,*N*-diphenylcarbamate (NCDC), penitrem A, ryanodine, xestospongin C (all Sigma), 2-aminoethoxydiphenyl borate (2APB; Acros), cyclopiazonic acid (CPA; Calbiochem), and nifedipine (Bayer). Data is presented as means ± SE, and statistical differences were compared using Student's paired *t*-test, taking the $P < 0.05$ level as significant.

RESULTS

Under control conditions, >90% of interstitial cells fired regular STICs and STOCs when held under voltage-clamp conditions using the perforated-patch technique. Figure 1*A* shows a typical recording from a cell held at −30 mV in which both STICs and STOCs may be observed during the control period. Addition of penitrem A, a potent blocker of large conductance Ca^{2+}-activated K^+ channels (BK channels) (18), completely blocked the STOCs over a period of ~1 min, similar to its action on single BK channels in urethral smooth muscle cells (12). It also unmasked larger STICs, suggesting that BK channels were activated during the STICs, partly offsetting them. Fig. 1, *B* and *C*, are expanded records of the activity of the periods marked B (control) and C (penitrem A) in Fig. 1*A*. In Fig. 1*B*, it can be seen that the STICs had a duration in the order of seconds, while the STOCs fell into two broad types, those of small amplitude and short duration (<100 ms)

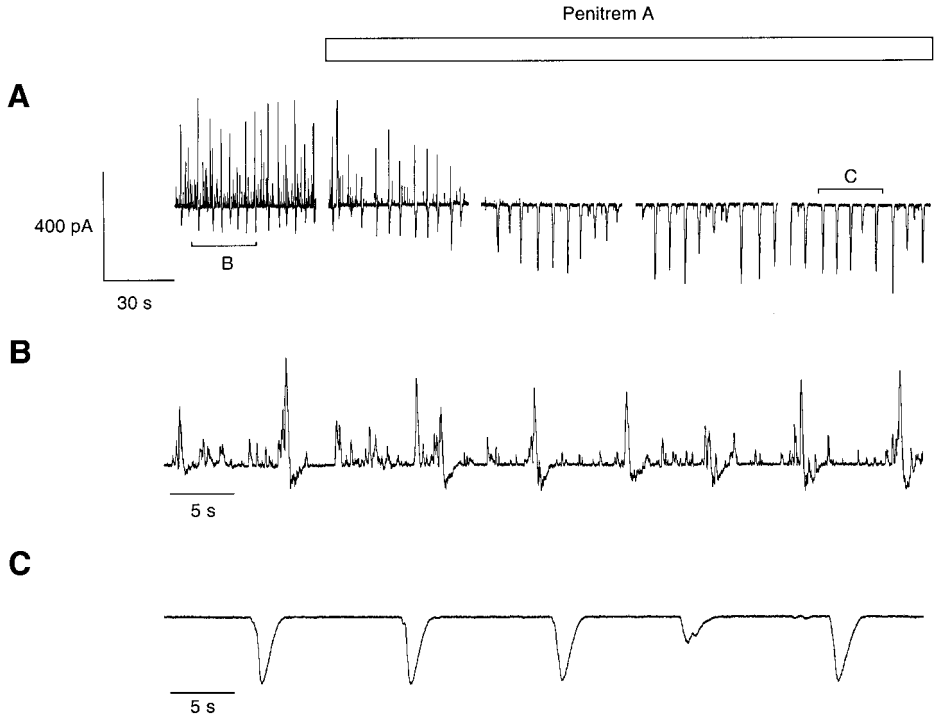

Fig. 1. Spontaneous transient inward currents (STICs) and spontaneous transient outward currents (STOCs) in an interstitial cell from rabbit urethra held at −30 mV. *A*: recording over a 5-min period where the effect of penitrem A (0.1 μM) was studied. Recording was continuous, except for 5-s gaps between records. *B*: record from the control period in *A* was expanded to show the relationship between STICs, fast STOCs, and slow STOCs. *C*: record from *A* in the presence of penitrem A. This drug completely blocked the STOCs to unmask the true amplitude and time course of the STICs.

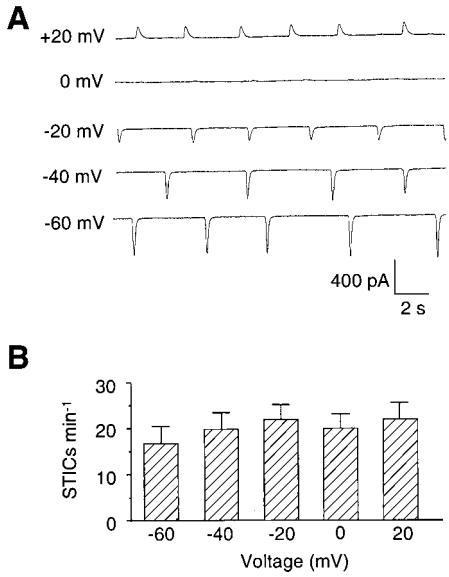

Fig. 2. STIC frequency is not dependent on membrane potential. *A*: spontaneous currents recorded with a Cs^+ pipette in a cell held at various potentials. The currents reversed at 0 mV, as expected for a Cl^- conductance, but their frequency varied little with voltage. *B*: a summary for 8 cells in which STIC frequency was plotted against voltage.

and those of larger amplitude and longer duration. While the smaller fast STOCs bore no obvious relationship to the STICs, the slow STOCs were usually coupled to STICs (Fig. 1*B*), making it difficult to analyze their time course accurately at this potential. Therefore, we also studied them at 0 mV, which was the calculated Cl^- equilibrium potential, E_{Cl} (Fig. 8). In nine cells studied at 0 mV, the mean amplitude of the slow STOCs was 343 ± 89 pA, while their mean duration was 1,087 ± 138 ms. The latter was similar to the duration of STICs studied with Cs^+ pipettes (1,156 ± 120 ms at −60 mV and 1,206 ± 117 ms at −20 mV; n = 10 cells). The mean STIC amplitude was −860 ± 200 pA at −60 mV and −311 ± 74 pA at −20 mV (Cs^+ pipettes; n = 10 cells). Figure 1*C* shows that when the BK channels were blocked, only STICs remained. We have previously demonstrated that these currents are mediated by Ca^{2+}-activated Cl^- channels (26).

We also studied STICs and STOCs at more hyperpolarized membrane potentials than −30 mV. When cells were held at −60 mV (close to the resting potential of rabbit urethra) (11), we never observed STOCs, but STICs were present in ~90% of cells (26). As cells were depolarized to −50 or −40 mV, generally fast STOCs appeared, and at −30 mV, slow STOCs similar to those in Fig. 1 were also present. The effect of depolarization on the frequency of fast STOCs was measured in four cells in which the values were 0 ± 0 min^{-1} at −60 mV, 162 ± 104 min^{-1} at −40 mV, 251 ± 139 min^{-1} at −20 mV, and 262 ± 101 min^{-1} at 0 mV.

Figure 2 shows the effect of changing the membrane potential on STIC frequency using Cs^+ pipette solution to block the BK channels that mediate STOCs. Perhaps surprisingly, the frequency of STICs was found to be little affected by membrane depolarization. Figure 2*A* shows a cell in which STICs were studied at potentials ranging from −60 to +20 mV. At 0 mV (E_{Cl}) they almost disappeared, while at +20 mV they had reversed to become outward currents. It is clear that frequency was little changed throughout this range of depolarization. This was confirmed in the summary data from eight cells shown in Fig. 2*B* where, although there was a slight trend for frequency to increase on depolarization from −60 to −20 mV, the effect was not significant ($P > 0.05$). Under current clamp conditions, these cells fire STDs mediated by the STICs (26). To assess the effect of membrane potential on this activity, STDs were examined using a K^+ pipette with E_{Cl} set to the more physiological value of −24 mV. Figure 3 shows an example of a cell firing STDs at a rate of 6 min^{-1} at rest. These reached a value close to the calculated E_{Cl}. When the membrane was depolarized to −40 mV, by injecting current, both the frequency of the STDs and the level to which they depolarized remained the same. Changing the membrane potential back to −60 mV and then −80 mV also failed to affect either frequency or the maximum depolarization of the STDs. When the cell was depolarized to −20 mV, the STDs disappeared and were replaced by a mixture of fast and slower hyperpolarizations (Fig. 3, *right*).

The effect of caffeine (10 mM) was to completely block STICs and STOCs. An example of the effect on STOCs (recorded at 0 mV = E_{Cl}) is shown in Fig. 4*A*, where caffeine initially evoked a transient outward current after which STOCs were suppressed. These then gradually returned after washout of the drug. This result was confirmed in a total of four cells in which the STOCs were abolished. The effect of caffeine on STICs was similar in that it first evoked a transient inward current followed by abolition of the STICs (Fig. 4*B*). This was confirmed in eight cells firing STICs at a rate of 13 ± 4 min^{-1} in which the effect of caffeine was to stop activity, which then returned to 12 ± 4 min^{-1} after washout. These results are consistent with an

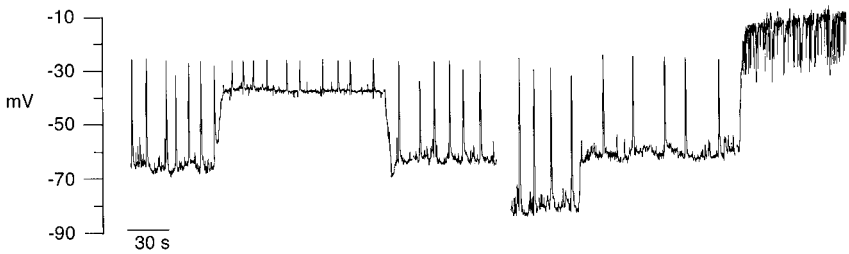

Fig. 3. Frequency of spontaneous depolarizations is not dependent on membrane potential. The trace shows a current clamp record from a cell recorded with K^+ pipette solution [Cl^- equilibrium potential (E_{Cl}) set to −24 mV]. Membrane potential was altered by injecting currents.

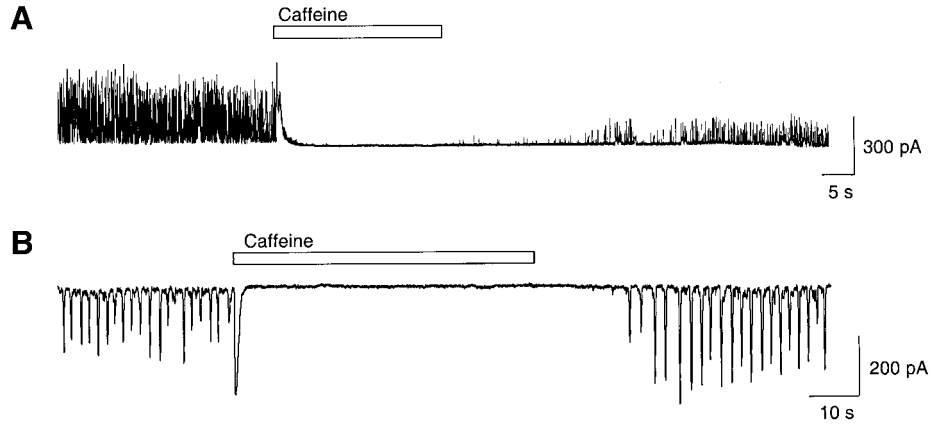

Fig. 4. Caffeine abolishes STICs and STOCs. *A*: STOCs from a cell held at 0 mV using K^+ pipette solution (E_{Cl} set to 0 mV). Caffeine (10 mM) evoked a large transient outward current before abolishing the STOCs. *B*: STICs from a cell held at −60 mV using Cs^+-rich solution. Caffeine (10 mM) reversibly abolished the STICs.

effect of caffeine dumping intracellular stores and suggest that stores are essential for firing STICs and STOCs. This was further supported by demonstrating that ryanodine (30 μM) irreversibly abolished STICs in five cells (Fig. 5*A*), fast STOCs in four cells (Fig. 5*B*), and slow STOCs in four cells (Fig. 5*C*). In Fig. 5, *B* and *C*, ryanodine transiently enhanced the amplitude of the STOCs before suppressing them, possibly by opening ryanodine-sensitive channels before blocking them. CPA also blocked STICs and STOCs, as shown in Fig. 5, *D* and *E*.

To investigate the possible role of IP_3 in mediating STICs, we examined the action of xestospongin C (1 μM), a drug reported to be a membrane-permeant blocker of IP_3 receptors (7). Although its effect was to reduce STIC amplitude by 90% ($n = 6$), it also evoked a large inward current, suggesting that it released intracellular Ca^{2+}, as suggested by Broad et al. (4). Therefore, we did not pursue this substance further. However, two other substances known to interfere with IP_3-mediated responses were found to block STICs. An example of the effect of 100 μM NCDC, a blocker of phospholipase C, is shown in Fig. 6 in which it reversibly blocked the STICs 3 min after it was applied. In six cells, NCDC reduced STIC frequency from 14 ± 3 to 3 ± 2 min^{-1}. The second drug, 2APB, has recently been described as a specific blocker of the Ca^{2+}-release channels coupled to IP_3 receptors (23). Before its effects on STICs and STOCs were tested, a set of control experiments were performed on norepinephrine- and caffeine-evoked Cl^- currents to test its specificity. Figure 7*A* shows that norepinephrine evoked a series of inward currents of diminishing amplitudes. These responses were reproducible if repeated at intervals of >80 s. However, if 2APB (100 μM) was added between norepinephrine applications, it blocked the responses (Fig. 7*A*). After an 80-s wash in normal bath solution, the responses returned, slightly larger than before. The effect of the lower concentration of 10 μM 2APB was also studied in nine cells where it was found to reduce the norepinephrine-induced current from −840 ± 188 pA to −358 ± 112 pA ($P < 0.05$). In contrast, 2APB (100 μM) had very little effect on caffeine-evoked Cl^- currents (Fig. 7*B*). These results are consistent with an action of 2APB on IP_3-sensitive stores, rather than nonspecific actions on either the plasmalemmal Cl^- channels or on ryanodine-sensitive stores.

The effect of 2APB (100 μM) on spontaneous activity is shown in Fig. 7*C*. This cell was held at −40 mV so

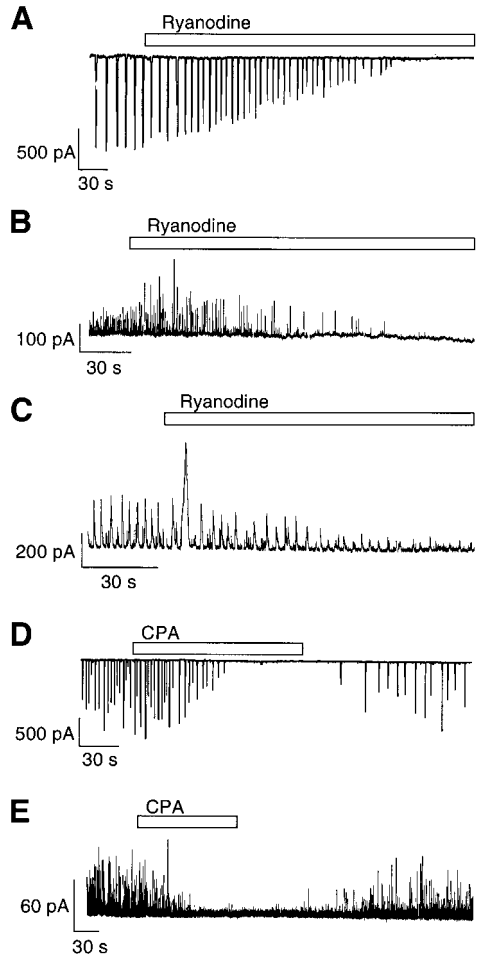

Fig. 5. Depletion of intracellular stores abolishes STICs and STOCS. *A*: ryanodine (30 μM) abolished STICs within 5 min in a cell held at −60 mV (Cs^+ pipette, $E_{Cl} = 0$ mV). *B*: ryanodine (30 μM) abolished fast STOCs within 5 min in a cell held at 0 mV (K^+ pipette, $E_{Cl} = 0$ mV). *C*: ryanodine (30 μM) also abolished slow STOCs in a different cell under similar conditions. *D*: cyclopiazonic acid (CPA; 10 μM) abolished STICs (holding potential = −60 mV; Cs^+ pipette, $E_{Cl} = 0$ mV). *E*: fast STOCs were also reversibly abolished by 10 μM CPA (holding potential = 0 mV; K^+ pipette, $E_{Cl} = 0$ mV).

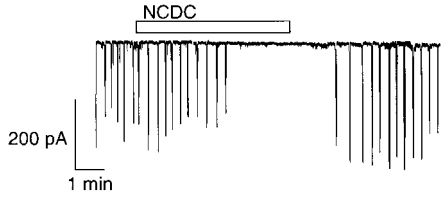

Fig. 6. Inhibition of phospholipase C abolishes STICs. The trace shows a recording of STICs from a cell held at −60 mV recorded with Cs^+ pipette solution. Application of the phospholipase C blocker 2-nitro-4-carboxyphenyl *N*,*N*-diphenylcarbamate (NCDC; 100 μM) slowly abolished the STICs. After washout of NCDC, STIC frequency returned to control levels.

that both STICs and STOCs could be studied simultaneously. It can be seen that 2APB rapidly abolished the STICs while the STOCs remained (although their frequency was reduced by 30%). Summary data for the effect of 2APB (100 μM) on STICs were obtained in another 13 cells studied at −60 mV in which it reduced STIC amplitude from −427 ± 82 pA to −14 ± 9 pA, returning to −431 ± 81 pA after washout. A lower concentration of 2APB (10 μM) was also effective in blocking STICs, reducing the amplitude from −542 ± 71 pA to −75 ± 49 pA (holding potential −60 mV, n = 6, $P < 0.05$). In four cells in which fast STOCs were studied at −30 mV, 2APB failed to block them but reduced their frequency by 27% from 122 ± 17 min^{-1} to 89 ± 19 min^{-1} ($P < 0.05$). When 2APB (100 μM) was studied in cells held at 0 mV to eliminate Cl^- currents, its effect was to preferentially block the slow STOCs compared with the fast STOCs. An example is shown in Fig. 8*A* where, in the control period, slow STOCs can be differentiated from fast STOCs, which occurred either alone or superimposed on the slow STOCs. Exposure to 2APB resulted in abolition of the slow STOCs, while fast STOCs remained. This effect was studied in five cells in which slow STOCs could be readily distinguished from fast STOCs, and, in each case, the slow STOCs were abolished.

Together with the temporal pattern of STOCs and STICs in Fig. 1, the 2APB data suggest that the STICs and slow STOCs are mediated by the same IP_3-dependent mechanism, while the fast STOCs are mediated by a different mechanism. This idea was further investigated by examining the effect of nifedipine (10 μM) on STICs and STOCs. Figure 8*B* shows a cell held at 0 mV (E_{Cl}) to allow examination of the STOCs without the contaminating effect of the STICs. Addition of nifedipine differentially blocked the fast STOCs, while unmasking a series of slow STOCs that were difficult to distinguish during the control (compare expanded records 1 and 2 in Fig. 8*C*). Although the slow STOCs remained, their frequency appeared to be reduced. This trend was observed in a total of four cells in which frequency was reduced by 23% from 31 ± 9 to 24 ± 3 min^{-1}, but this was not statistically significant ($P > 0.05$). However, nifedipine reduced the frequency of the fast STOCs by 79% from 11.2 ± 1.0 s^{-1} to 2.4 ± 1.0 s^{-1} (n = 7, $P < 0.05$).

The effect of nifedipine on STICs was examined at potentials of −60, −40, and −20 mV in cells studied with Cs^+ pipettes to block STOCs. An example is shown in Fig. 9*A* where it can be seen that as the cell was depolarized to −40 and −20 mV, the frequency of the STICs increased. The cell was then exposed to nifedipine (10 μM) and depolarized from −60 to −40

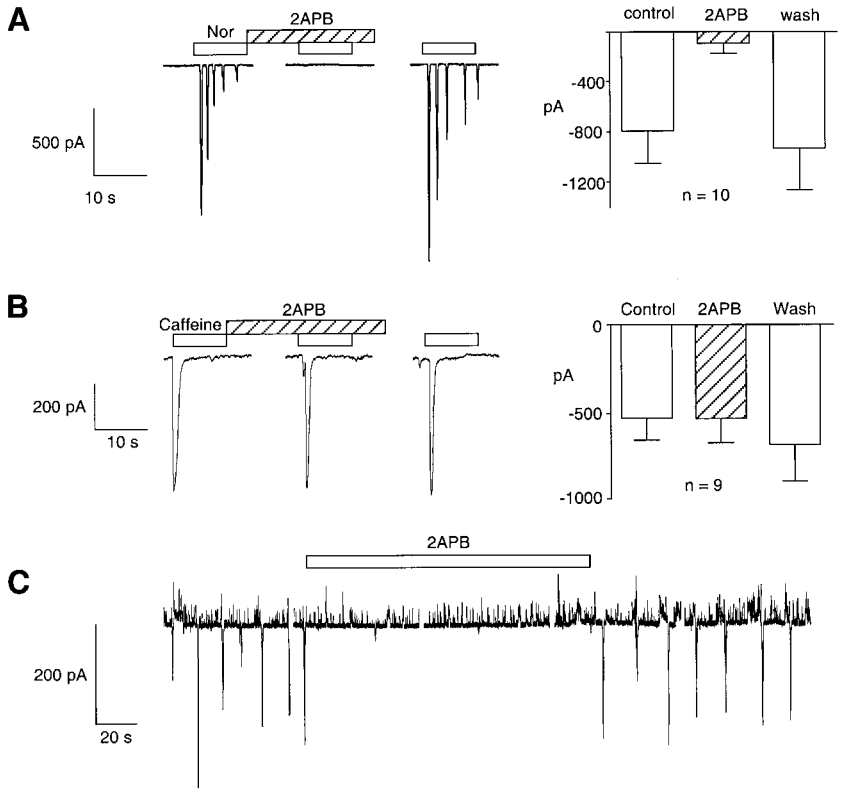

Fig. 7. The effect of 2APB (2-aminoethoxydiphenyl borate; 100 μM). *A*: the effect of 2APB on norepinephrine (Nor)-evoked Cl^- currents. *B*: the effect of the drug on caffeine-evoked Cl^- currents. *C*: 2APB blocked the STICs while leaving the fast STOCs. *A* and *B* recorded at −60 mV using Cs^+ pipettes; *C* recorded at −40 mV using a K^+ pipette.

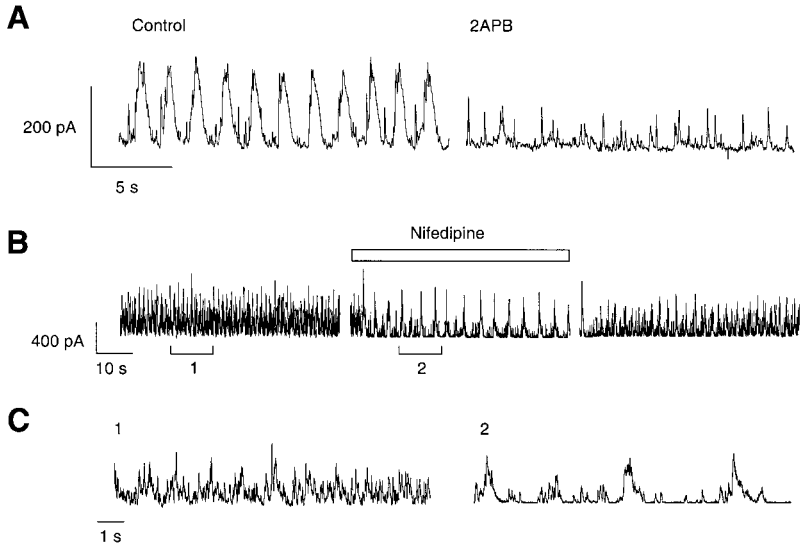

Fig. 8. *A*: records from a cell held at 0 mV (E_{Cl}) to allow STOCs to be observed without contamination with Cl^- currents. This cell was firing both fast and slow STOCs. 2APB (100 μM) abolished the slow STOCs to clearly unmask the fast STOCs. *B*: the effect of nifedipine (10 μM) in a different cell held at 0 mV. Nifedipine had the opposite effect as 2APB, i.e., it unmasked the slow STOCs by reducing the frequency of fast STOCs. *C*: expanded records corresponding with the periods marked 1 and 2 in 8*B*.

and −20 mV again. There was little effect of nifedipine at −60 mV, but it reduced the frequency of the STICs at −40 and −20 mV. Summary data from eight cells is shown in Fig. 9*B* where nifedipine significantly reduced the frequency of STICs at −40 and −20 mV, while having little effect at −60 mV.

DISCUSSION

In the present study, we have shown that 2APB, an agent recently described as a specific membrane-permeant blocker of IP_3-mediated Ca^{2+} release, blocked STICs in rabbit urethral interstitial cells. Because these cells are prime candidates as the urethral pacemakers (26), our results provide support for the hypothesis that the pacemaker mechanism depends on

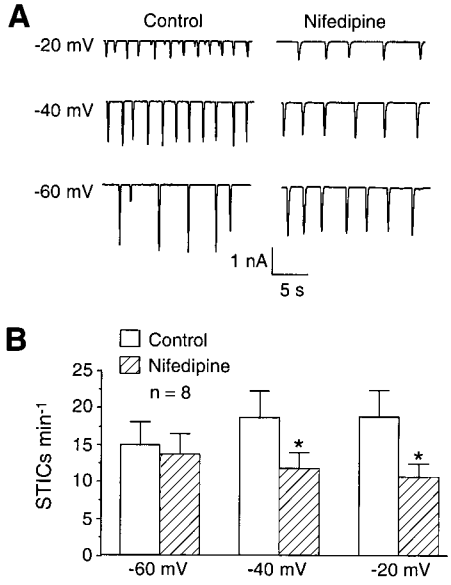

Fig. 9. *A*: the effect of depolarization on STIC frequency before and after nifedipine (10 μM) in a cell studied with a Cs^+ pipette. *B*: summary data from 8 similar experiments. Nifedipine had little effect at −60 mV but significantly reduced the frequency at the other 2 potentials.

IP_3-dependent Ca^{2+} release (see Ref. 10). Recent work has also implicated an IP_3-dependent pacemaking mechanism in gastric smooth muscle where normal slow wave activity was absent in mutant mice that lacked the type 1 IP_3 receptor (27) and in mouse jejunum where pacemaking currents in cultured ICC were suppressed by inhibitors of IP_3-dependent Ca^{2+} release (30).

The urethral interstitial cells also fired STOCs that could be differentiated into two types, fast and slow. When recording conditions were set optimally for simultaneous recording of both STICs and STOCs, it was clear that the STICs and slow STOCs were coupled events, implying a shared underlying mechanism, while the fast STOCs occurred independently at a faster rate. Indeed, the fast STOCs behaved in a very similar fashion to the majority of STOCs widely reported in smooth muscle cells, where they are believed to be activated by localized release of Ca^{2+} (Ca^{2+} sparks) from ryanodine-sensitive release channels located close to the plasma membrane (for reviews, see Refs. 3 and 14).

Sparks have also been implicated in the activation of STICs in several types of smooth muscle (8, 32). Because of the small single-channel conductance of the Ca^{2+}-activated Cl^- channels (2.6 pS) (17), it has been estimated that >600 of these would have to open to produce an average STIC of approximately −90 pA in a smooth muscle cell (14, 21). Taking into account the Ca^{2+} sensitivity of the Cl^- channels, the total number of Cl^- channels per cell (~10,000), and the membrane area (0.25%) over which the required Ca^{2+} concentration is delivered by a spark, it has been estimated that significant clustering of the Cl^- channels over the Ca^{2+} release sites is a prerequisite for sparks to evoke STICs (14). While sparks might be able to evoke the relatively small STIC characteristic of some smooth muscle cells (21), it is unlikely that the much larger STICs described in the present paper could be evoked by single Ca^{2+} sparks. The STICs in rabbit urethral interstitial

cells often exceeded −900 pA, which would involve activation of around 6,000 channels. This is likely to reflect activation of channels over a much greater area of membrane than could be affected by a single Ca^{2+} spark. However, it is interesting that in pancreatic acinar cells, large Cl^- currents are activated by local Ca^{2+} events that are confined to the apical membrane (15, 28). This appears to depend on a combination of clustering of the Cl^- channels on this membrane (31) and close apposition of the endoplasmic reticulum to the membrane maintained by the microtubular network (6). When the latter was disrupted, STICs disappeared, but the response to a supramaximal concentration of carbachol remained. While it is possible that a similar situation exists in urethral interstitial cells, several factors may suggest otherwise. First, these cells resemble smooth muscle cells that do not have well-defined polar membranes like acinar cells. Second, the local Ca^{2+} events that activate the Cl^- currents in acinar cells are still very large compared with the sparks seen in smooth muscle.

The situation in urethral interstitial cells may more closely resemble that in rat portal vein myocytes, where sparks were observed to activate STOCs, but Ca^{2+}-activated Cl^- currents were only observed during a propagating Ca^{2+} wave (evoked by 10 mM caffeine) (24). Propagating waves in smooth muscle are also evoked by agonists that are coupled to IP_3 as the second messenger (2, 8) and may occur spontaneously in a variety of cells where there is basal IP_3 production (9). It is possible, therefore, that the STICs in urethral interstitial cells are generated by Ca^{2+} waves in response to spontaneous production of IP_3. In support of this argument, NCDC, a phospholipase C inhibitor, and xestospongin C and 2APB, both of which have been reported to block the action of IP_3 (7, 23), abolished the STICs. Because xestospongin C has been reported to have certain nonspecific actions, including causing Ca^{2+} release (4) and blockade of the sarcoplasmic Ca^{2+} pump (5), we concentrated the most effort on the action of 2APB. This drug was first shown to inhibit IP_3-mediated Ca^{2+} release in rat cerebellar microsomes and human platelets where it appeared to inhibit opening of the channel domain of the IP_3 receptor without affecting either IP_3 binding or IP_3 production (23). This action seemed to be specific as it failed to block caffeine-induced Ca^{2+} release from cardiac and skeletal sarcoplasmic reticulum vesicles (23). It also blocked agonist-induced contractions in both rabbit aorta (23) and rat myometrium (1) over the concentration range of 10–100 μM. In high concentrations (generally >200 μM), it developed some tendency to cause Ca^{2+} release and to block Ca^{2+} uptake into stores (23). The concentrations used in the present study did not induce inward or outward currents, suggesting that it was unlikely to have caused significant Ca^{2+} release. It was also unlikely to have blocked Ca^{2+} uptake, because we found that it had no effect on caffeine response, although it blocked norepinephrine-induced Cl^- currents. Also, although it blocked STICs, it only slightly reduced the frequency of fast STOCs. Again, this suggests that its effect was unlikely to have been due to blockade of Ca^{2+} uptake since the Ca^{2+}-ATPase blocker CPA abolished STOCs.

Several complicating factors, however, remain to be explained. First, 2APB produced a 30% reduction in the frequency of fast STOCs, and second, ryanodine blocked all of the responses. The first observation may be explained by the fact that IP_3 has been shown to increase spark frequency in vascular myocytes (8). This probably arises because the Ca^{2+} released from the IP_3-sensitive store causes Ca^{2+} release from the ryanodine-sensitive store by Ca^{2+} induced-Ca^{2+} release (CICR). Indeed, there have been several studies (16, 19) in which heparin or xestospongin C reduced STOC frequency in smooth muscle cells, suggesting that IP_3 may modulate these currents. Interaction between IP_3- and ryanodine-sensitive stores also helps to explain the second observation, since it has been demonstrated that CICR at the ryanodine receptor is a requirement for the propagation of an IP_3-induced Ca^{2+} wave (2). Thus norepinephrine-induced Ca^{2+} waves were abolished if either the IP_3- or ryanodine-sensitive channels were blocked (2). This result could explain how ryanodine blocked the STICs in the present study, even though the IP_3-sensitive store was the driver of the pacemaking mechanism.

Finally, it is interesting to speculate the functional significance of STICs and STOCs in the rabbit urethral interstitial cells. We propose that IP_3-dependent Ca^{2+} waves initiate the STICs and slow the STOCs in these cells. At potentials below −40 mV, the Cl^--mediated STICs predominate over the slow STOCs, probably because at these potentials the voltage- and Ca^{2+}-sensitive properties of the BK channel determine that open probability is low (see Refs. 8 and 32). This would allow the cell to fire STDs from the resting potential under physiological conditions. However, the cell can still fire fast STOCs at potentials as negative as −50 mV. These would arise if BK channels were activated in areas of plasma membrane overlying the ryanodine-sensitive release channels that discharge localized sparks of extremely high Ca^{2+} concentrations (14), but we speculate that a lack of clustering of Cl^- channels in these regions means that very little Cl^- current is activated concurrently. In this case, the activation of BK channels by sparks is allowed to go unchecked, generating a net outward current. This opens up the intriguing possibility that fast STOCs set the membrane potential in interstitial cells, similar to their proposed action in arterial smooth muscle cells (14), whereas large STICs are responsible for the generation of rhythmic depolarizations. Such a subtle mechanism could explain how two Ca^{2+}-sensitive channels, which produce opposite effects on membrane potential, could be independently regulated by different Ca^{2+}-release events to determine the overall electrical activity of the pacemaker cells. However, these ideas must remain speculative until further studies characterize the Ca^{2+} events that underlie STICs and STOCs in these cells and until the anatomical relationship among the Cl^-

channels, the BK channels, and the Ca^{2+} stores is defined.

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