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K^+ channel blocker modulation of the refractory period in spontaneously active guinea-pig ureters

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Abstract The effects of various K^+ channel blockers on the spontaneous electrical activity of the smooth muscle cells of the ureter still attached to its primary pacemaker regions were investigated using standard intracellular microelectrode recording techniques. Spontaneous action potentials in the ureter were complex, consisting of an initial rapidly rising spike which was followed by a period of membrane oscillation, a quiescent plateau phase and terminated by an abrupt repolarisation and an after-hyperpolarisation with a peak "diastolic" potential of −66 mV. This after-hyperpolarization decayed slowly over 5–20 s until the underlying triggering potentials achieved threshold for another action potential discharge. Application of the Ca^{2+}-entry blocker, nifedipine (1 μM), blocked action potential discharge within 2–5 min, after which the membrane settled at a potential of −55 mV. 4-Aminopyridine (4-AP)(1 mM for 2 min) and Ba^{2+} (100 μM for 2 min) both depolarized significantly the diastolic potential. In 4-AP, this membrane depolarisation was associated with a decreased amplitude of the initial spike and an increase in the half-amplitude duration. In contrast, tetraethylammonium (TEA) (0.5 mM for 2 min) only increased the frequency and half-amplitude duration of these ureteric action potentials. Apamin (200 nM), Cs^+ (1 mM) and glibenclamide (1 μM) had no significant effects on any parameters of the ureteric action potential. It was concluded that the refractory period of the spontaneous action potentials in the whole-mount preparation of the upper urinary tract was determined by the opening of at least three K^+ channel populations: large conductance ('maxi K') Ca^{2+}-activated K^+ channels; Ca^{2+}-insensitive transiently opening K^+ (I_{Kto}) channels and K^+-selective inward rectifier channels.

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Introduction

The spontaneous contractions recorded in the human ureter in vitro appear to be maintained by the endogenous release of both sensory nerve tachykinins and prostacyclins as both capsaicin, the depletor of sensory nerve neuropeptides, and inhibitors of prostaglandin (PG) production, indomethacin and diclofene, can readily block the spontaneous contractility. Moreover, spontaneous phasic contractions can be restored upon exposure to the tachykinins, neurokinin A, neuropeptide K and substance P, or the prostaglandins, $PGF_{2\alpha}$ and PGE_2 [3, 4, 6]. In contrast, the ureters of most other mammals only display spontaneous contractions in vitro if the pelviureteral junction is left intact, or if pharmacologically induced. To date, most investigations of the membrane conductances or channels underlying ureteric electrical activity in mammals have been carried out in either quiescent preparations dissected free of the pelviureteral junction using both intracellular microelectrode and extracellular sucrose gap recording techniques [5, 9, 21, 22], or in single ureteric myocytes under voltage or current clamp [7, 10, 18]. Previous studies of the effects of K^+ channel blockers on the electrical activity in intact quiescent ureters [9] and single ureteric myocytes arrested with various Ca^{2+}-entry blockers [7, 8, 14] have generated a number of controversies with respect to the physiological role that particular K^+ channel populations may play in determining both the time course of, and the refractory period following, an electrically evoked action potential. For example, it has been recently suggested that 4-aminopryidine (4-AP)-sensitive K^+ channels, which are readily recorded in single ureteric myocytes [7, 8, 10], may well have little role in controlling ureteric function [19].

Recently, we have developed a whole-mount preparation of the guinea-pig upper urinary tract (renal pelvis

and proximal ureter) which displays spontaneous migrating contractions. These spontaneous contractions originate mostly in the proximal renal pelvis and propagate distally the length of the ureter [20]. These migrating contractions occur at the same frequency as the contractions recorded in circumferentially cut strips of the proximal renal pelvis [12, 23], confirming the long-held view that the atypical smooth muscle cells, located in the proximal renal pelvis, generate the pacemaker mechanisms which trigger pyeloureteral motility and ureteral peristalsis [12, 13, 22, 23]. In this report, we have characterised the spontaneous action potentials recorded in the guinea-pig ureter still attached to the pacemaker regions of the renal pelvis. Moreover, we have examined the effects of applying Ca^{2+} and K^+ channel blockers on the frequency and time course of these spontaneous action potentials, allowing a more physiological examination of the role of specific K^+ channel populations. In particular, we have established that 4-AP (1 mM) has dramatic effects on the rising phase and half-amplitude duration of the spontaneous action potentials in the ureter, as well as the refractory period between action potentials.

Materials and methods

Dissection

Guinea-pigs (250–400 g) were killed by stunning and exsanguination and the kidney and attached ureter removed through an abdominal incision. The upper urinary tract (renal pelvis and 2–3 cm of proximal ureter) was dissected free of the kidney and loosely pinned out in an organ bath (volume 1 ml). The ureteral end of the preparation was cut along its longitudinal axis (1 cm) and pinned firmly, serosal surface uppermost, to the bottom of the organ bath. The organ bath was then mounted on an inverted microscope and perfused with a physiological saline (see below) at 3–4 ml/min (at 34–35°C). The effects of the K^+ channel blockers on the spontaneous action potentials were generally examined in preparations not pre-treated with capsaicin, as capsaicin pre-treatment invariably resulted in a time-dependent development of irregular action potential discharge over several hours [15, 20]. However, the effects of K^+ channel blockade on the other action potential parameters were essentially the same if preparations were pre-treated with capsaicin (10 μM for 15 min; $n = 3$).

Electrical recordings

Electrophysiological recordings were made from the pinned region of the ureter (5 mm × 5 mm), using standard intracellular recording techniques, and glass microelectrodes with tips < 1 μm and resistances of 50–80 MΩ. Changes in the membrane potential were recorded with a standard unity-gain pre-amplifier and stored digitally using a MacLab 4000/e analog-to-digital converter (sample rate 400–1000 Hz) and a Powermac 1600/60. Membrane potential recordings were analysed and displayed using Chart and Sigmaplot software. Various parameters of the spontaneous action potentials were measured: the frequency of action potential discharge; the amplitude and rate of rise of the initial spike; the duration of the action potential measured from the time the initial spike was half maximal; the peak negative (''diastolic'') potential reached during the after-hyperpolarisation and the membrane potential 600 ms before the peak of the initial spike [12]. In each experiment, the parameters of 3 or 4 action potentials were averaged and compared with those measured after 2–10 min exposure to a test drug. A number of similar experiments were then averaged as indicated. In most experiments a paired Student's t-test was used for tests of significance, $P < 0.05$ was considered to be statistically significant.

Solutions and drugs used

The physiological saline was of the following composition (in mM): NaCl 120, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1, NaH_2PO_4 1, $NaHCO_3$ 25, glucose 11 and bubbled with a 95% O_2 : 5% CO_2 gas mixture to establish a pH of 7.3–7.4. The following drugs were used in the present experiments: 4-aminopyridine (4-AP), apamin, $BaCl_2$, CsCl, capsaicin, tetraethylammonium (TEA), (Sigma, St Louis, Mo.). The concentration of all stock solutions ranged between 0.1 mM and 10 mM. Most drugs were dissolved in filtered distilled water and diluted with physiological saline to their final concentrations as indicated. Capsaicin and nifedipine (Bayer) were dissolved in absolute ethanol. Before use, solutions were vigorously bubbled with the gas mixture to restore any changes of pH.

Results

Spontaneous action potentials in the ureter

The frequency of the spontaneous action potentials in the ureter was 3.7 ± 0.2 ($n = 165$) spikes per minute. In general, the time course of the action potentials recorded in the ureter consisted of a rapidly rising initial spike, followed by a period of membrane oscillation and a plateau phase (0.2–5 s in duration) (Fig. 1Ai, iii), essentially similar to action potentials electrically evoked in quiescent ureters (Fig. 1Bii) [5, 9, 17]. The relative duration of the oscillation and plateau phases varied between tissues and cells within each tissue; in some cells, the membrane oscillations continued throughout the plateau phase. At the end of the plateau, the membrane abruptly repolarised to create an ''under shoot'' or after-hyperpolarisation which slowly decayed over 5–20 s until the next action potential discharge. In 165 ureteric cells, the mean amplitude and rate of rise of the initial spike, the half-amplitude duration, the most negative ''diastolic'' potential reached during the after-hyperpolarisation and the membrane potential 600 ms before each spontaneous event were 60.9 ± 0.8 mV, 4.5 ± 0.1 V/s, 1088 ± 50 ms, −66.5 ± 0.4 mV and −60.1 ± 0.4 mV, respectively. In contrast to circumferentially cut strips of the proximal renal pelvis, action potentials with a more simpler time course, which we have previously termed ''pacemaker'' or ''intermediate'' action potentials [12, 13, 23], were never recorded in over 160 successful impalements of the ureter.

The spontaneous action potentials recorded in the ureter were invariably followed by a distinct refractory period, during which an action potential could not be evoked either upon the invasion of a subsequent triggering event (Fig. 1Aii, iii) or upon the application of a depolarising electrical stimulation (10 V, 200 ms) (Fig. 1Bi–iii). During this refractory period, the underlying triggering potential in either a spontaneously active preparation (Fig. 1Aiii) or evoked electrically (Fig. 1-Biii) could often be clearly seen. The duration of the refractory period in ureteric cells was quite variable be-

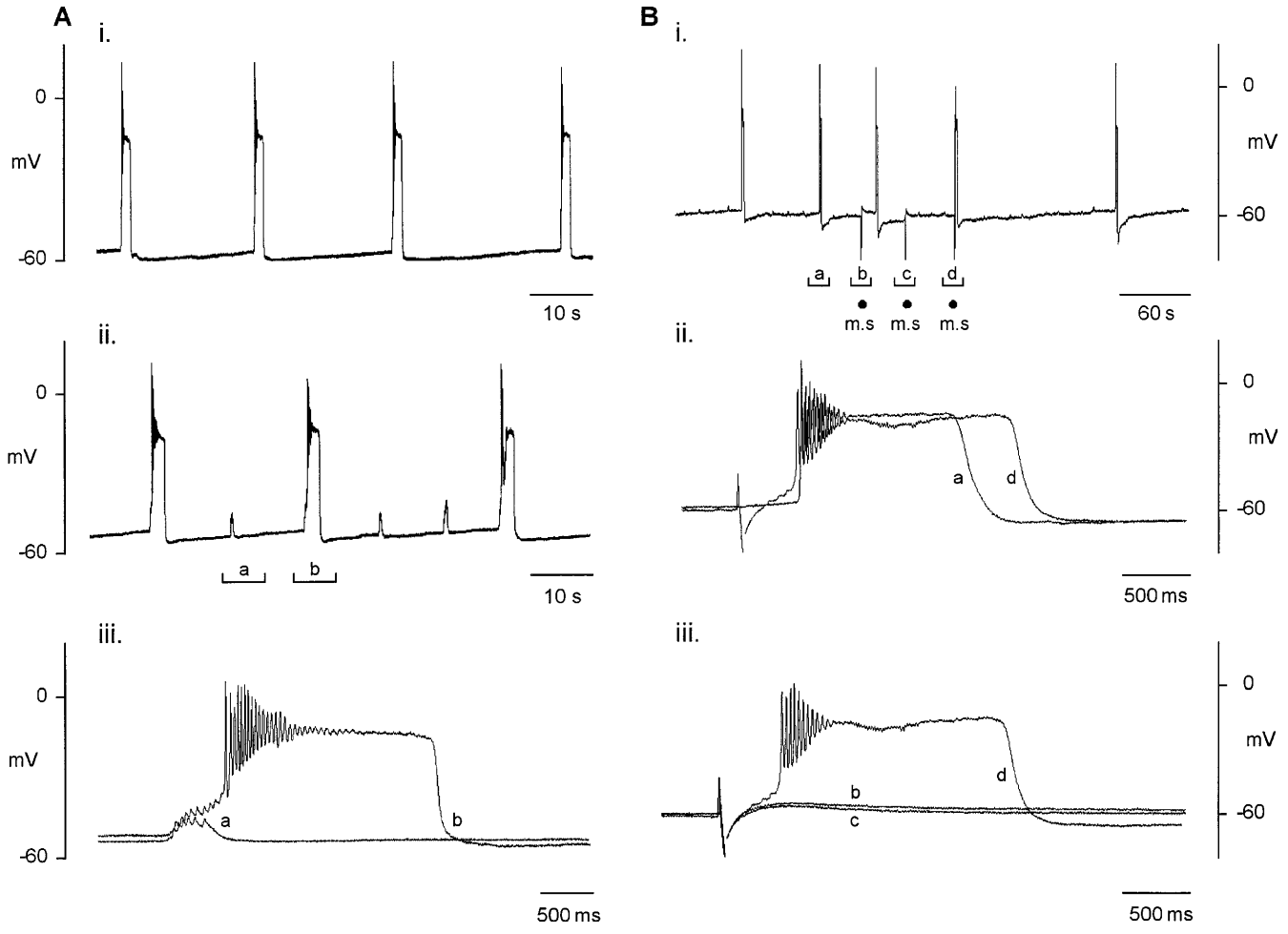

Fig. 1A, B Spontaneous action potentials recorded in the guinea-pig ureter when the primary pacemaking regions of the proximal renal pelvis remain attached. **Ai, ii** Examples of action potentials with different refractory periods recorded in different smooth muscle bundles of the same guinea-pig ureter showing that not every invasion by a triggering potential evokes an action potential (**Aii**). **Aiii** The time course of the triggering potential (a) and the resulting action potential (b), indicated by the horizontal brackets, have been compared on an expanded time course. **B** The refractory period of ureteric action potential discharge was determined by attempting electrically (10 V and 200 ms) to evoke an action potential at various periods after a spontaneously occurring action potential (a) using two large transmurally placed silver–silver chloride electrodes. **Bii, iii** comparisons of the spontaneously occurring ureteric action potentials (a) with the membrane responses (b–d) to electrical stimulation on an expanded time course

tween cells within, and between, tissues. For example, the spontaneous action potentials and their differing refractory periods illustrated in Fig. 1A were recorded in the same preparation.

Effects of nifedipine

The application of the "L-type" Ca^{2+} channel blocker, nifedipine (1 μM), caused a time-dependent decrease in the frequency of the action potentials recorded in the spontaneously discharging ureter (Fig. 2i). This was accompanied by a time-dependent membrane depolarisation and a decrease in the amplitude of the initial spike, as well as a decrease in the duration of the plateau phase and the number of superimposed membrane oscillations (Fig. 2ii, iii). Within 2 min of applying nifedipine, action potential discharge had generally ceased and the membrane potential settled at its "true" resting membrane potential of −55.3 ± 3.4 mV ($n = 5$), approximately 7 mV more depolarised than the membrane potential measured 600 ms preceding each action potential in the absence of nifedipine (−62.6 ± 3.0 mV; $P < 0.05$, $n = 5$; Fig. 2iii). Nifedipine (1 μM) also abolished action potential discharge evoked by directly stimulating the ureteric muscle with depolarising pulses of 10–20 V and 200 ms duration (data not shown).

Effects of K^+ channel blockers

We have previously applied a number of K^+ channel blockers, at relatively specific concentrations, to examine the contribution of particular K^+ channel populations to the differing phases of the "driven" action potentials recorded in the proximal renal pelvis [12]. In the present experiments, the role of any small conductance ("SK")

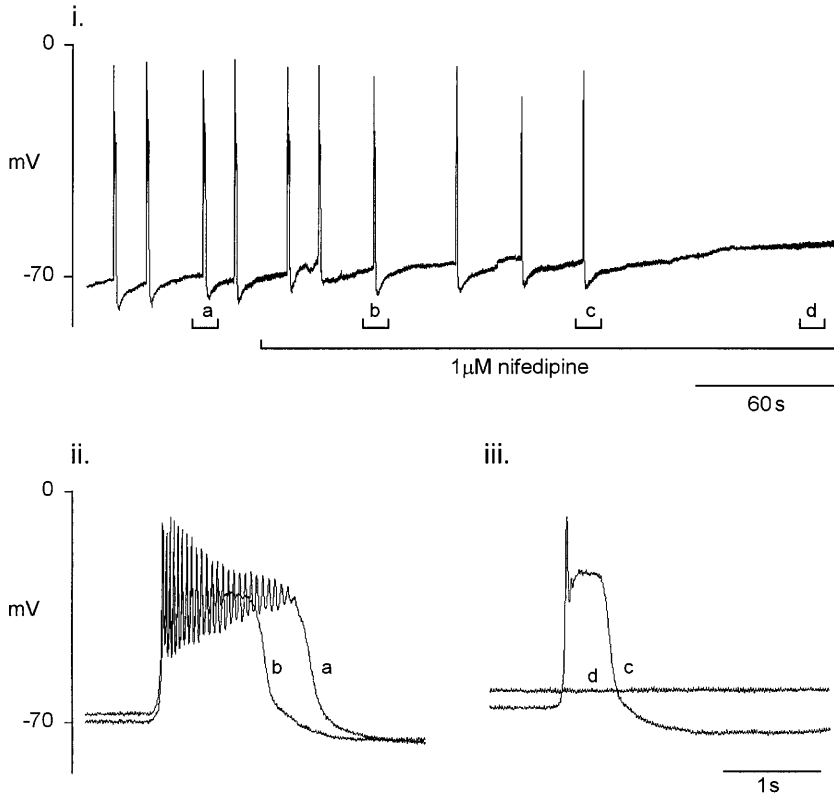

Fig. 2i–iii Effects of (**i**) nifedipine (1 μM) on the spontaneous action potentials recorded in the guinea-pig ureter. **ii, iii** The effects of nifedipine on the action potentials indicated by the horizontal brackets (a–d) illustrated on an expanded time course

or large conductance ("maxi K") Ca^{2+}-activated K^+ channels on the time course of, and refractory periods between the spontaneous action potentials recorded in the ureter were investigated using apamin (200 nM), TEA (0.5 mM) and charybdotoxin (30 nM). The role of ATP-dependent or cromakalim-activated K^+ channels, K^+-or cationic-selective inward rectifier channels and Ca^{2+}-insensitive transiently opening K^+ (I_{Kto}) channels in the time course of ureteral action potentials was also examined using glibenclamide (1 μM), Ba^{2+}(100 μM), Cs^+ (1 mM) and 4-AP (1 mM), respectively (see [12]).

In Fig. 3, it can be seen that the frequency of action potential discharge in the ureter, was increased by both 4-AP (1 mM applied for 2 min; Fig. 3Ai) and Ba^{2+} (100 μM for 2 min; Fig. 3Bi; Table 1). These changes in frequency of action potential discharge in 4-AP and Ba^{2+} (100 μM) were associated with a significant depolarization of the peak "diastolic" potential to -62.5 ± 1.3 and -58.0 ± 0.9 mV, respectively (respective control values of -66.9 ± 1.5 ($n = 8$) and -61.5 ± 1.5 ($n = 4$) mV; both $P < 0.05$; Fig. 3Ai, Bi); the membrane potential recorded 600 ms before each action potential, however, was not significantly affected by 4-AP or Ba^{2+} (Table 1). In the presence of 4-AP (1 mM), the amplitude and maximum rate of rise of the initial spike of the ureteric action potentials were significantly reduced to 90% and 81% of control values (both $P < 0.05$, $n = 8$; Fig. 3Ai, ii). Ba^{2+} (100 μM) did not significantly affect any other of the parameters of the action potential (Table 1). However, both 4-AP (1 mM for 2 min; $n = 8$) and higher concentrations of Ba^{2+} (1 mM for 2 min; $n = 4$) significantly increased the half-amplitude duration of the ureteric action potentials 160% and 319%, respectively, over the control durations (Fig. 3Aii, Bii; both $P < 0.05$; Table 1). In preparations in which action potential discharge was infrequent, or absent, even though the spontaneous triggering events were present, the application of either 4-AP (1 mM; Fig. 3C) or Ba^{2+} (100 μM; Fig. 3D) readily promoted action potential discharge suggesting that both K^+-selective inward rectifier and I_{Kto} channels contributed to the refractory period in the spontaneously active ureter. In these preparations, a "wash out" period of these drugs for >30 min was usually required before the irregular discharge of the action potentials returned (data not shown).

The role of any "SK" or "maxi-K" channels in determining the time course of the ureteric action potential was investigated using TEA (0.5 mM; Fig. 4A) and apamin (200 nM; Fig. 4B). The application of TEA (0.5 mM) for 2–5 min; ($n = 9$) increased the frequency and half-amplitude duration of the spontaneous action potentials by 15% and 48%, respectively, above control values ($P < 0.05$, $n = 9$; Table 1). As in the guinea-pig proximal renal pelvis [12], charybdotoxin (30 nM; $n = 1$), another more specific blocker of maxi K channels, had essentially the same effects on the duration and frequency of the ureteric action potentials as 0.5 mM TEA (data not shown). TEA (2 mM; $n = 7$) evoked a larger (98%; $P < 0.05$) increase in the half-amplitude duration which was associated with a 12% ($P < 0.05$) increase in the amplitude of the initial spike, but no

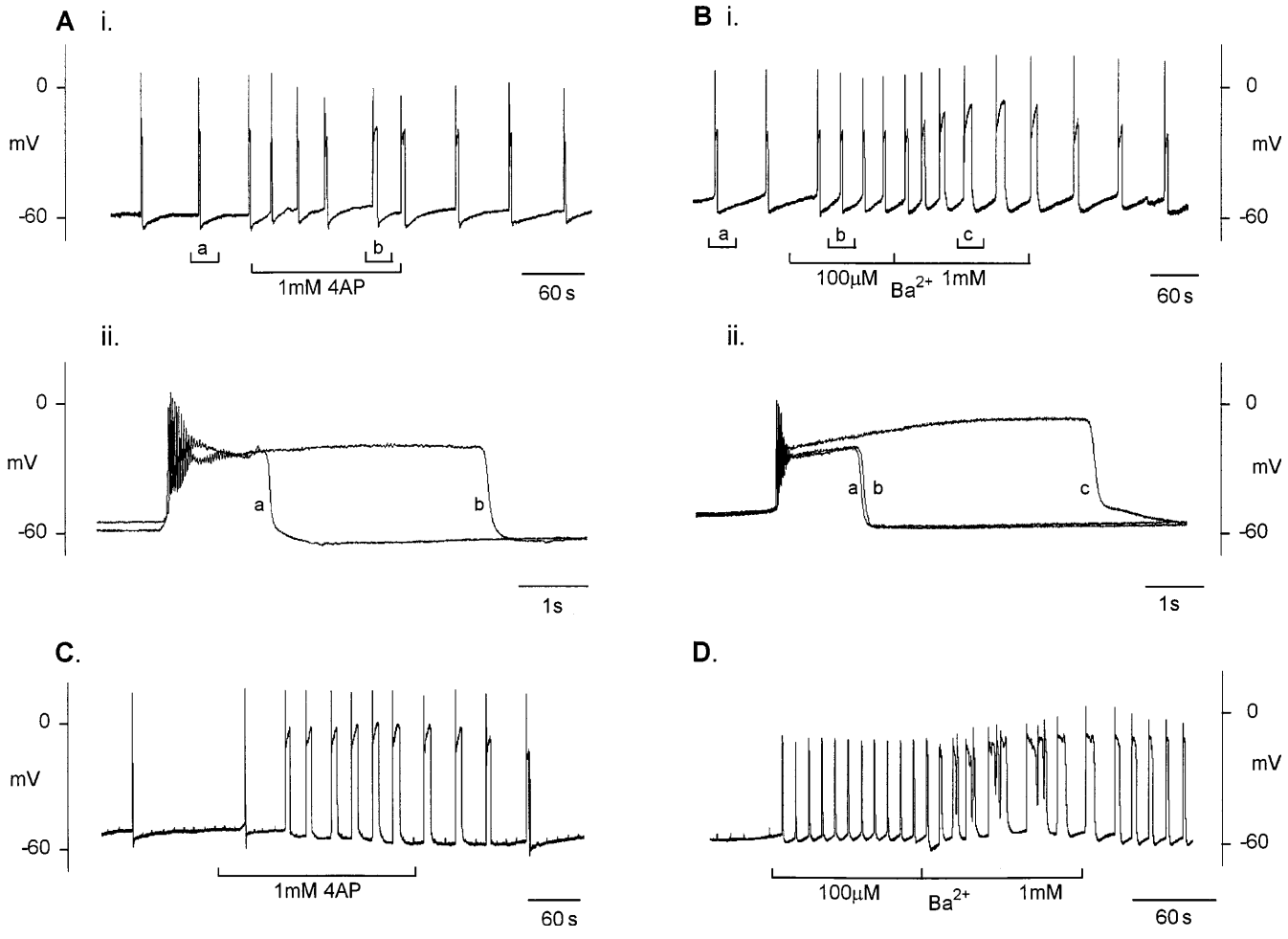

Fig. 3A–D Effects of 4-AP (**A, C**) and Ba^{2+} (**B, D**) on the spontaneous electrical activity in the guinea-pig ureter. Two-min exposure to (**A**) 4-AP (1 mM) or (**B**) Ba^{2+} (100 μM) induced a significant increase in the frequency of action potential discharge. Both 4-AP (1 mM) and Ba^{2+} (1 mM) also caused a significant increase in the half-amplitude duration of these spontaneous action potentials (**Aii, Bii**). When action potential discharge was irregular and not occurring on every invasion of a triggering potential, the application of (**C**) 4-AP (1 mM) or (**D**) Ba^{2+} (100 μM) caused every triggering potential to evoke an action potential

other significant changes in the action potential parameters (Table 1). TEA (0.5–2 mM) also decreased the amplitude, but not the frequency of the membrane oscillations superimposed on the action potential plateau (Fig. 4Aii, Bii). In contrast to the proximal renal pelvis [12], exposure to apamin (200 nM for >2 min; Fig. 4B) had no significant effects on the action potentials recorded in the ureter ($P > 0.05$, $n = 6$; Table 1). Moreover the presence of apamin did not prevent the effects of the subsequent addition of 0.5 mM TEA (Fig. 4B).

Finally, as in the proximal renal pelvis, Cs^+ (1 mM; Fig. 5Ai, ii) and glibenclamide (1 μM; Fig. 5Bi, ii) did not significantly affect any of the action potential parameters measured (Table 1).

Discussion

The ureteric membrane potential recorded in the presence of nifedipine (−55.3 ± 3.4 mV, $n = 5$; Fig. 2) is 5 mV positive of the membrane potential (−60.1 ± 0.4 mV, $n = 167$) recorded 600 ms prior to each control spontaneous action potential and some 5 mV more negative than the membrane potential recorded in intact ureters dissected free of the renal pelvis [9] or enzymatically isolated single ureteric myocytes (at room temperature) [7, 19]. This ureteric membrane potential in the presence of nifedipine is also some 10–15 mV more negative than the membrane potential recorded in nifedipine-arrested driven cells of the proximal renal pelvis [14] consistent with the notion that there is a gradient in the membrane potential along the upper urinary tract arising from an increased expression of membrane K^+ channels [12].

The action potentials recorded in the spontaneously active preparations used in the present experiments are similar in time course to the electrically evoked action potentials in quiescent intact ureters [9, 17] or single ureteric myocytes [7, 10, 19]. It is well established that the rising phase of the action potential in the intact ureter arises from the influx of Ca^{2+} through "L-type"

Table 1 Effects of K^+ channel blockers on the electrical activity recorded in the guinea-pig ureter

K^+ channel blockers	Membrane potential (mV)	Initial spike amplitude (mV)	Half-amplitude duration (ms)	Maximum rate of rise (V/s)	After-hyper-polarisation (mV)	Frequency (×/min)	Number of cells (*n*)
Control	−56.0 ± 2.5	59.8 ± 4.4	1615 ± 533	5.8 ± 1.1	−61.5 ± 1.5	2.2 ± 0.7	4
Barium 100 μM	−53.7 ± 1.3	54.3 ± 5.2	2148 ± 364	5.3 ± 1.1	−58.0 ± 0.9*	3.1 ± 0.5	
Control	−53.3 ± 3.7	53.8 ± 2.7	1530 ± 456	4.9 ± 1.0	−60.1 ± 3.2	2.9 ± 0.2	4
Barium 1 mM	−50.5 ± 3.1	54.3 ± 4.6	6417 ± 1822*	4.3 ± 1.3	−53.1 ± 3.3	4.1 ± 0.4	
Control	−61.1 ± 1.9	60.6 ± 1.4	1110 ± 111	4.8 ± 0.5	−66.9 ± 1.5	2.7 ± 0.5	8
4-AP 1 mM	−58.5 ± 1.0	54.4 ± 1.6*	2893 ± 655*	3.9 ± 0.4*	−62.5 ± 1.3*	3.4 ± 0.5†	
Control	−59.1 ± 2.1	60.6 ± 2.6	759 ± 171	4.1 ± 0.8	−66.4 ± 2.8	3.6 ± 0.7	5
Caesium 1 mM	−63.0 ± 3.1	64.1 ± 2.5	814 ± 176	4.7 ± 0.9	−66.2 ± 3.0	3.1 ± 0.8	
Control	−52.9 ± 4.1	59.9 ± 2.5	1704 ± 559	4.8 ± 1.0	−60.1 ± 4.1	4.0 ± 1.7	4
Glibenclamide 1 μM	−52.4 ± 4.2	59.6 ± 2.2	1647 ± 508	4.8 ± 0.9	−59.9 ± 4.0	4.3 ± 1.3	
Control	−59.0 ± 1.9	61.9 ± 2.5	900 ± 218	4.1 ± 0.5	−65.9 ± 1.9	3.4 ± 0.5	9
TEA 0.5 mM	−60.2 ± 1.8	65.1 ± 3.2	1336 ± 302*	4.0 ± 0.5	−66.2 ± 1.9	3.9 ± 0.05*	
Control	−55.8 ± 0.6	62.0 ± 3.7	1180 ± 283	4.8 ± 0.4	−61.9 ± 0.8	3.6 ± 0.7	7
TEA 2 mM	−57.1 ± 1.6	69.4 ± 3.9*	2334 ± 477*	4.4 ± 0.6	−60.5 ± 1.9	3.9 ± 0.9	
Control	−60.7 ± 2.1	60.5 ± 6.1	780 ± 118	4.1 ± 0.7	−68.6 ± 1.8	4.2 ± 0.4	6
Apamin 200 nM	−59.5 ± 2.7	57.6 ± 6.1	885 ± 168	4.2 ± 0.8	−67.3 ± 2.5	4.3 ± 0.4	

* $P < 0.05$, † $P = 0.05$

Ca^{2+} channels. Removal of Ca^{2+} from the bathing saline or the application of the Ca^{2+}-entry blockers inhibits action potential discharge in the intact ureter, while raising the extracellular Ca^{2+} concentration increases the amplitude of the initial spike/s and reduces, if not completely abolishes, the plateau phase [21]. In single ureteric myocytes, membrane depolarisation to potentials positive to −40 mV generally evokes a small inward current which activates rapidly and decays slowly over 200–400 ms [7, 10, 18]. This current is increased in amplitude in the presence of Bay K8644 [18] or when the external Ca^{2+} is replaced with Ba^{2+} [10, 18] and blocked in the presence of the Ca^{2+} entry blockers (Cd^{2+} or nifedipine) [7, 11, 16].

The potential oscillations during the initial phase of the spontaneous action potentials in the intact ureter correspond in time with the repeating transient outward currents recorded upon membrane depolarisation in single ureteric myocytes under voltage clamp. These outward current oscillations in the single ureteric myocytes and membrane potential oscillations in the intact tissue [9] are readily reduced in amplitude by TEA [10, 19] and are likely to arise from the increased activity of the "B-K" or "maxi-K" channels previously observed in excised ureteric membrane patches which have a single channel "slope" conductance of 220 pS in a symmetrical high K^+ (126 mM) gradient. Ureteric maxi K channels are readily blocked by TEA and charybdotoxin [7, 19] and are activated by both membrane depolarisation and rises in the internal Ca^{2+} concentration [7]; for example, raising the Ca^{2+} concentration 10-fold (from pCa 7 to 6) shifts the activation curves of these maxi-K channels approximately 60 mV in the negative direction [19]. Recently, we have demonstrated in the proximal renal pelvis that the duration of the driven action potentials was increased by TEA (0.5 mM), charybdotoxin, or apamin, the specific blocker of SK channels [12]. In the present study, TEA (0.5 mM) and charybdotoxin, but not apamin (200 nM), increased the half-maximal duration of the ureteric action potential suggesting that the termination of the plateau phase occurs upon the activation of maxi-K channels alone. Moreover, there have been no reports indicating that apamin-sensitive SK channels are indeed active in single ureteric myocytes [7, 10, 19]. The peak of the after-hyperpolarisation of the spontaneous ureteric action potentials was significantly depolarised by both 4-AP (1 mM) and Ba^{2+} (100 μM), but not by apamin nor TEA (0.5 mM; Table 1). In contrast, TEA and charybdotoxin both significantly reduced the after-hyperpolarisation of the driven action potentials recorded in the proximal renal pelvis. The frequency of action potential discharge in the ureter was, however, increased by 4-AP, Ba^{2+} (100 μM) and TEA (0.5 mM) which may well be expected if 4-AP and Ba^{2+} were increasing the excitability of the proximal regions of our whole mount preparation of the upper urinary tract [12]. We would like to suggest that there is an increased expression of 4-AP and Ba^{2+}-sensitive K^+ channels in the distal regions of the upper urinary tract and that these K^+ channels are increasingly more important in setting both the membrane potential and the refractory potential in the ureter, compared with any Ca^{2+}-activated K (SK or maxi-K) channels present.

The 4-AP-sensitive K^+ channel currents underlying the effects of 4-AP above have been characterised in single ureteric myocytes at the whole cell and single channel current level [8]. In single ureteric myocytes bathed in a Ca^{2+}-entry blocker, membrane depolarisation evokes a transient outward whole-cell current (I_{Kto}) which rapidly activates (within 5 ms) to a peak amplitude and then decays within 200 ms and is readily blocked by 1–2 mM 4-AP [8, 10]. The voltage range of activation and inactivation of I_{Kto} is influenced by both the nature of the Ca^{2+}-entry blocker used and on the relative concentrations of the divalent cations due to the

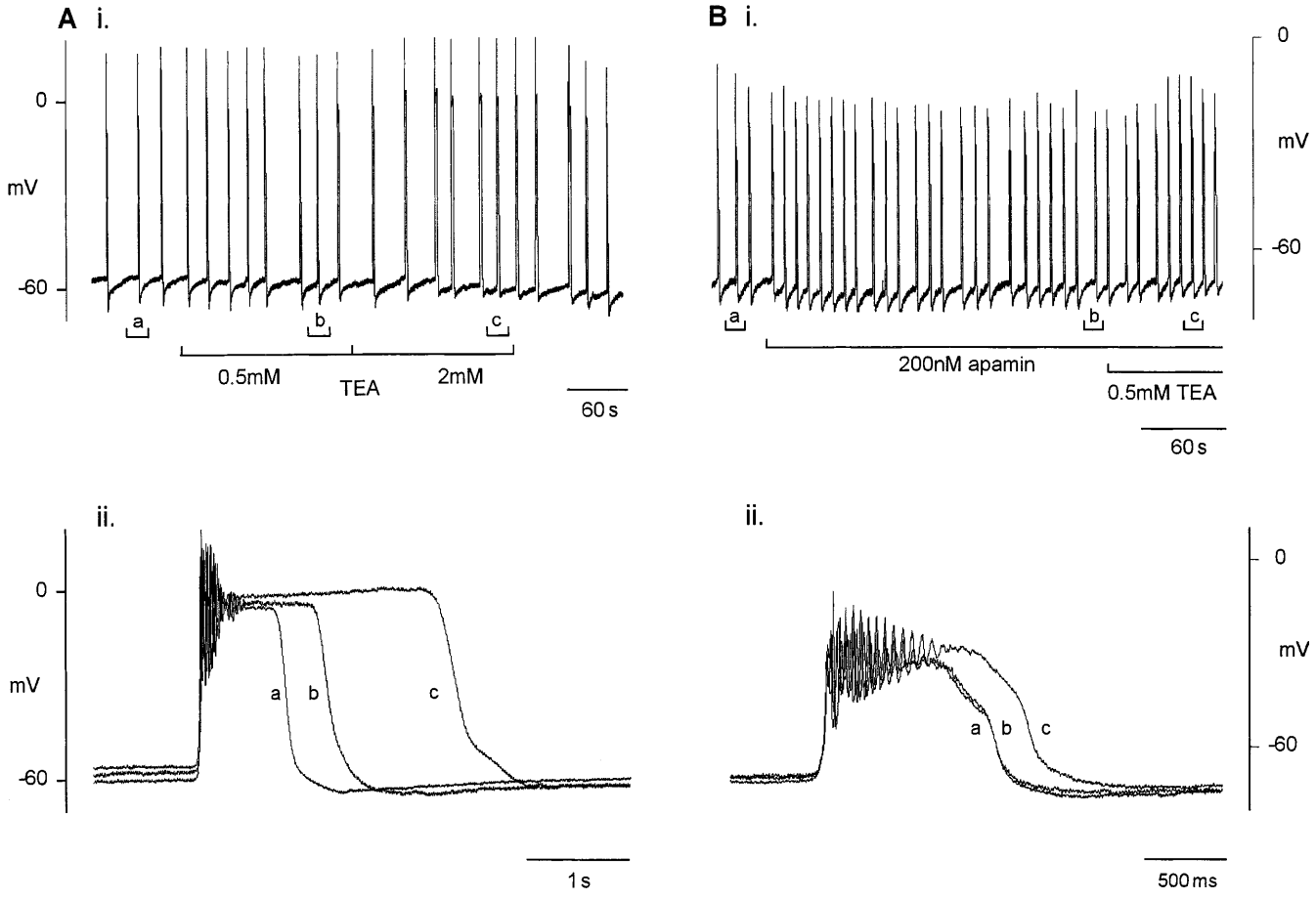

Fig. 4A, B Comparing the effects of (**A**) TEA (0.5–2 mM) and (**B**) apamin (200 nM) on the spontaneous action potentials recorded in the ureter. Two-min exposures to TEA (0.5–2 mM) significantly increased the half-amplitude duration of the ureteric action potential in a concentration-dependent manner (**Aii**). (**B**) In contrast, apamin had little effect on the spontaneous ureteric action potentials, nor did apamin prevent the actions of TEA (0.5 mM; **Bii**)

masking of surface charges [8]. In contrast, the Ba^{2+}-sensitive, presumably inward rectifier, K^+ channels have yet to be identified or characterised in single ureteric myocytes, either at the whole cell or single channel current level.

In the intact ureter, 4-AP (1 mM) decreased both the amplitude and rate of rise of the initial spike of the spontaneous action potentials. These reductions are presumably associated with the significant depolarisation of the peak after-hyperpolarisation which would reduce the number of available Ca^{2+} channels and therefore reduce the Ca^{2+} influx during the upstroke of the subsequent action potential. An extrapolation of the steady-state kinetics of the Ca^{2+} and I_{Kto} channels in single ureteric myocytes to quiescent intact ureters, suggests that only some of the Ca^{2+} channels, but most of the 4-AP-sensitive I_{Kto} channels, would be inactivated at the resting membrane potential of a quiescent ureter [8, 11]. 4-AP would therefore have little effect on the rising phase of an action potential electrically-evoked from −50 mV. However, at the peak of the "diastolic" potential in spontaneously discharging ureters, more I_{Kto} channels would de-inactivate and become available for opening during the "diastolic" depolarisation. The opening and closing of these I_{Kto} channels would slow the diastolic depolarisation and therefore contribute to the refractory period between successive action potentials [14]. In support of this suggestion, blockade of I_{Kto} by 4-AP in single ureteric myocytes under current clamp is associated with an acceleration of the upstroke of action potentials triggered from −70 mV [8], but not of those evoked from −50 mV [19].

The effects of 4-AP on the absolute amplitude and duration of the plateau phase of the ureteric action potential were somewhat surprising, but can be explained if the curves describing the activation and inactivation of I_{Kto} channels in the intact ureter show a considerable degree of overlap. Such an overlap would create a little-inactivating or constant "window" current over the potential range of the plateau phase, which would tend to hyperpolarise the absolute level, as well as decrease the duration, of the plateau phase. Blockade of these I_{Kto} channels would therefore lead to the observed effects in Fig. 3A. Alternatively, the relatively small Ca^{2+} and K^+ currents activated by membrane depolarisation in ureteric myocytes has led to the suggestion that the dominant membrane current underlying the plateau

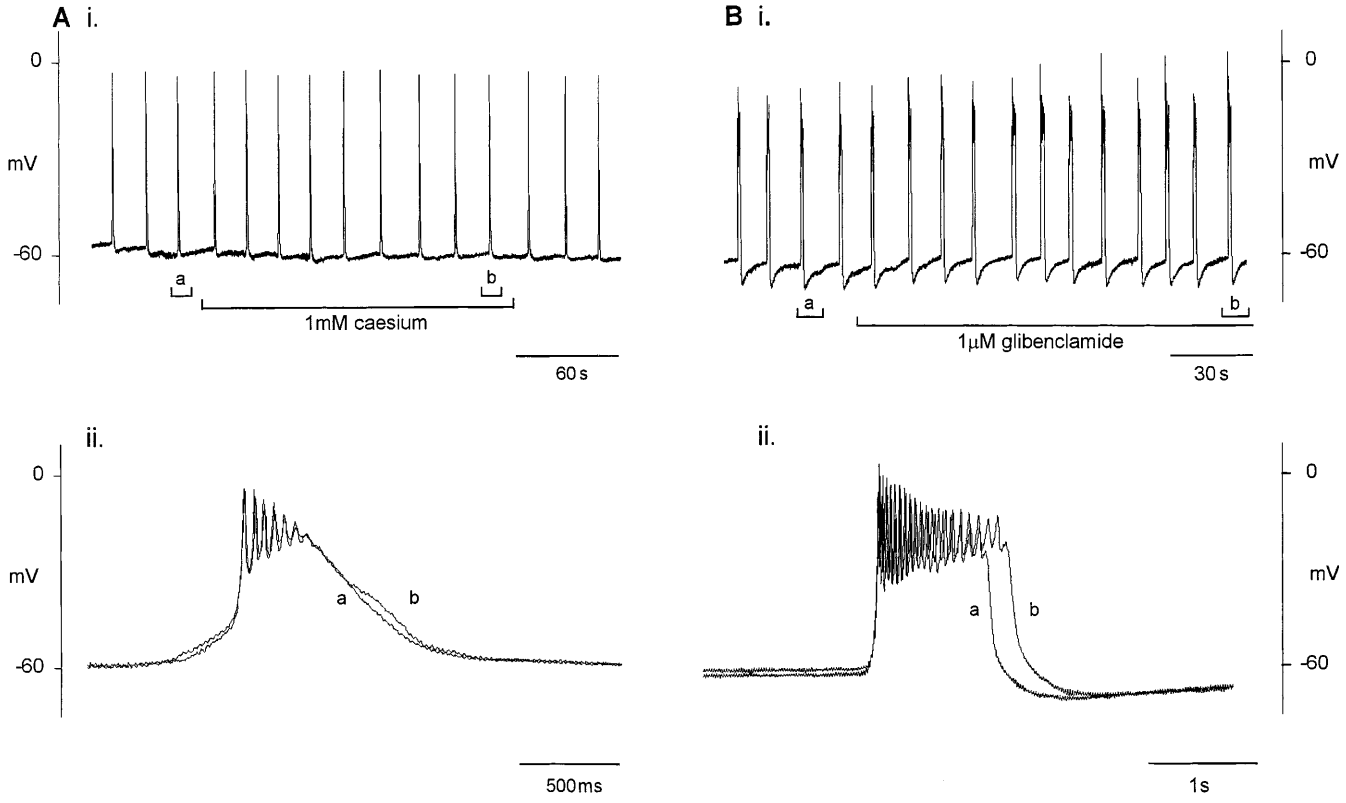

Fig. 5A, B Neither (**A**) caesium (1 mM), nor (**B**) glibenclamide (1 μM) had any significant effects on the frequency or time course (**Aii, Bii**) of the spontaneous ureteric action potentials

phase of the ureteric action potential, in fact arises from the activation of a Na^+–Ca^{2+} exchanger as Ca^{2+} entry during the initial phase of the action potential raises the internal concentrations of Ca^{2+} [14]. Such a Na^+–Ca^{2+} exchanger current has been demonstrated in single ureteric myocytes [1] and would explain the well-documented reduction of the plateau phase of the electrically evoked action potentials in quiescent ureters bathed in low-Na^+ saline [2, 17]. Blockade of I_{Kto} channels may well lead to a greater Ca^{2+} influx, resulting in a greater rise in the internal Ca^{2+} which would, in turn, enhance the activity of the Na^+–Ca^{2+} exchanger to generate a relatively larger depolarising current. This increased inward current would sum with the slowly inactivating Ca^{2+} current to prolong the plateau phase of the action potential (Fig. 3Aii). A similar effect of an increased inward current on the absolute level and the duration of the plateau phase of the ureteric action potential can be demonstrated when cells are bathed in 1 mM Ba^{2+} (Fig. 3Bi). Under these conditions, the increased Ba^{2+} entry through Ca^{2+} channels has created a larger inward depolarising current, perhaps even blockade of both maxi-K and I_{Kto} channels to generate the marked effects on the plateau observed in Fig. 3Bii.

In summary, we have developed a preparation of the guinea-pig ureter which allows us to record the spontaneous electrical activity in the absence of excitatory agonists or Ca^{2+} agonists [15]. Altogether, our results suggest that Ca^{2+} entry through "L-type" Ca^{2+} channels during the early oscillating phase of the ureteric action potential is opposed by K^+ leaving through maxi-K channels. The plateau phase arises from the depolarising influences of Ca^{2+} continued influx through Ca^{2+} channels and, although not yet demonstrated, via the activation of an inward Na^+–Ca^{2+} exchanger current as the internal Ca^{2+} rises [1]. Repolarisation occurs by internal Ca^{2+} activating maxi-K channels, while the peak after-hyperpolarisation and refractory phase may well involve the opening of K^+-selective inward rectifier channels, maxi-K and I_{Kto} channels. Using this whole-mount preparation of the upper urinary tract, we are now able to investigate directly the effects of exogenously applied agents such as K^+ channel blockers, as well as endogenously released sensory neuropeptides and prostaglandins on the time course of and the refractory periods between these spontaneously occurring ureteric action potentials; thus more directly investigating the physiological roles of these agents on ureteric function.

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