Urol Res (1999) 27:328–335

ORGINAL PAPER

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Effects of nerve stimulation on spontaneously active preparations of the guinea pig ureter

Received: 22 December 1998 / Accepted: 14 April 1999

Abstract The effects of intrinsic nerve stimulation on the spontaneous electrical activity of the smooth muscle cells of the guinea pig ureter still attached to its renal pelvis were investigated using standard intracellular microelectrode techniques. Action potentials discharged spontaneously at a frequency of 3.3 ± 0.2 min^{-1} (n = 67) and consisted of an initial rapidly rising spike, followed by a variable period (0.2–5 s) of membrane potential oscillation and a quiescent plateau phase which was terminated by an abrupt repolarisation and after-hyperpolarisation to −66 mV. Transmural electrical stimulation (20–50 Hz for 2 s) transiently decreased the frequency of action potential discharge; the half-amplitude duration of the following action potentials, however, was transiently increased to 156 ± 12% of control. Substance P (1 μM applied for 2 min) or neurokinin A (100 nM for 2 min) transiently increased the frequency of action potential discharge to 155 ± 19% and 142 ± 21%, respectively, of control. The excitatory actions of nerve stimulation or agonist application were reduced by the tachykinin antagonist, MEN 10,627 (1–3 μM), while the inhibitory actions of nerve stimulation were enhanced by MEN 10,627 (1 μM) or thiorphan (1 μM). Capsaicin (10 μM for 10–15 min) also evoked a transient increase in the frequency and half-amplitude duration of the ureteric action potentials, in a manner blocked by MEN 10,627 (3 μM), which was followed by a long period of membrane potential quiescence. Human calcitonin gene related peptide (hCGRP) (100 nM applied for 2–5 min) induced a time-dependent decrease in the frequency amplitude and duration of the spontaneous action potentials, in a manner blocked by glibenclamide (1 μM). It was concluded that the nerve-evoked excitatory and inhibitory changes in the parameters of the spontaneous ureteric action potentials arise from the release of the sensory neuropeptides, tachykinins and CGRP, respectively.

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Key words Guinea pig upper urinary tract · Calcitonin gene related peptide · Tachykinins · Capsaicin-sensitive sensory nerves · Ureteric peristalsis · Action potentials

Introduction

It is well established that ureteric peristalsis in the mammalian upper urinary tract is predominantly myogenic in origin, being little affected by extrinsic denervation or the application of blockers of autonomic nerve function such as atropine, guanethidine or tetrodotoxin [15, 21]. However, ureteric peristalsis is maintained, though not necessarily initiated, by the endogenous release of prostaglandins and sensory neuropeptides, as both capsaicin and blockers of prostaglandin synthesis reduce the frequency and amplitude of the contractions in the renal pelvis and ureter in vitro [2, 12, 28, 29]. Distension of the guinea pig ureter in vivo induces an increase in the activity of two populations of afferent nerves, responding to either small (< 5–8 mmHg) or large (> 8 mmHg) changes in intraluminal pressure [3, 24]. In the rat, ureteral distension or exposure to bradykinin and substance P evokes an increase in afferent nerve activity in the ipsilateral kidney and a reflex decrease in sympathetic nerve activity in the contralateral kidney, in a manner blocked by capsaicin, tachykinin antagonists or indomethacin [9, 10]. Immunohistochemical and retrograde labelling techniques have also revealed that these sensory nerves contain both tachykinin- and calcitonin gene related peptide (CGRP)-like immunoreactivity, originate mostly from the ipsilateral dorsal root ganglia and are present both within and between the muscle and epithelial layers of the renal pelvis and ureter [8, 27]. In the guinea pig and rat, activation of these sensory nerves generally has a net excitatory effect on motility in spontaneously active preparations of the renal pelvis [21, 29] and an inhibitory action in the isolated ureter [20].

In most mammals, except man [4, 8], the isolated ureter is electrically and mechanically quiescent in vitro.

Therefore, most studies on the effects of sensory nerve stimulation or applied agonists have been performed on ureters artificially driven to produce contractions of constant amplitude using either repetitive electrical stimulation in the presence of the Ca^{2+} agonist, Bay K8644 [17, 22, 25], or a maintained exposure to raised K^+ saline [15] or endothelin-1 [16]. Recently, we have developed a whole-mount preparation of the guinea pig upper urinary tract (renal pelvis and ureter) which readily displays spontaneous contractions that originate in the proximal renal pelvis and propagate distally into and along the ureter [28]. In this report, we have examined the electrical activity underlying these migrating contractions, recording action potential discharge with intracellular microelectrodes in the ureteric portion of the whole-mount preparation used in the contractile studies. Moreover, we have compared the effects of electrical nerve stimulation on the frequency and time course of these spontaneous action potentials with the effects of applied sensory neuropeptide mimetics, substance P, neurokinin A and hCGRP. Some of these results have been presented previously in abstract form [6].

Materials and methods

Dissection

Guinea pigs (250–400 g) were killed by stunning and bleeding, 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. The ureteral end of the preparation was cut along its longitudinal axis (1 cm) and pinned firmly, serosal surface uppermost, to the bottom of an 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).

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 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 analogue-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 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 membrane potential 600 ms before the peak of the initial spike, the peak negative (''diastolic'') potential reached during the after-hyperpolarisation, and the frequency of action potential discharge [13]. In each experiment, the parameters of three or four action potentials were averaged and compared with those measured after 2–5 min of exposure to a test drug or after a short period of electrically stimulating (2 s at 20 V and 20–50 Hz) the intrinsic sensory nerves. 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. Bubbling with a 95% O_2:5% CO_2 gas mixture established a pH of 7.3–7.4. The following drugs were used in the present experiments: capsaicin (Sigma, St Louis, Mo.), neurokinin A (Auspep, Australia), substance P (Auspep, Australia), tetrodotoxin (Sigma, St Louis, Mo.), thiorphan (Auspep, Australia), human calcitonin gene related peptide (hCGRP 1–37) (hCGRP) and the hCGRP receptor antagonist, hCGRP (8–37) (both Auspep, Australia). MEN 10,627 was a kind gift from Dr. C.A Maggi (Menarini Ricerche). The concentration of all stock solutions ranged between 0.1 mM and 10 mM. Most drugs were dissolved in filtered distilled water and were diluted with physiological saline to their final concentrations as indicated. MEN 10,627 and neurokinin A were dissolved in a mixture of filtered distilled water and absolute ethanol. Capsaicin was dissolved in absolute ethanol. Before use, solutions were vigorously bubbled with the 95% O_2:5% CO_2 gas mixture to restore any changes of pH.

Results

Spontaneous action potentials in the upper urinary tract

The time course of the spontaneous action potentials recorded in the ureter of the whole-mount preparation of the guinea pig upper urinary tract was always of the ''driven'' type. ''Pacemaker'' or ''intermediate'' action potentials, identified previously in the renal pelvis [13, 14, 29], were never recorded in over 160 successful impalements of the ureter. These ''ureteric'' action potentials were characterised by an initial spike, a period of membrane oscillation and a quiescent plateau phase of the action potential. The duration of the oscillation and plateau period varied between and within preparations, being 0.2–5 s in duration. The termination of the action potential was characterised by a rapid repolarisation to a peak after-hyperpolarisation which slowly decayed over 2–15 s. In 67 ureteric cells, the amplitude and rate of rise of the initial spike, the half-amplitude duration, the most negative (''diastolic'') potential reached during the after-hyperpolarisation, the ''resting'' membrane potential (recorded 600 ms before each spontaneous event) and the frequency of the action potential discharge were 61.9 ± 1.6 mV, 4.7 ± 0.2 V/s, 1172 ± 83 ms, −65.5 ± 1.2 mV and −59.9 ± 1.3 mV, 3.3 ± 0.2 min^{-1}, respectively.

Effects of nerve stimulation

The application of repetitive transmural electrical stimulation (0.2 ms duration, 50–100 V, 20–50 Hz for 2–5 s) evoked a transient decrease in the instantaneous frequency of the action potential discharge to 2.6 ± 0.3 min^{-1}, from a control frequency of 3.9 ± 0.4 min^{-1} ($n = 15$, $P < 0.05$) (Table 1), corresponding to a 120 ± 6% increase in the mean interval between action potentials immediately following the nerve stimulation (Fig. 1Ai). A transient excitatory effect of nerve stimulation was also observed as the half-amplitude duration of the first few action potentials, recorded immediately after a period of nerve stimulation was increased to 156 ± 12% ($n = 15$, $P < 0.05$) of control (Table 1; Fig. 1Aii).

Table 1 Modulation of the action potentials recorded in the smooth muscle cells of the guinea-pig ureter

	Membrane potential (mV)	Initial spike amplitude (mV)	Half-amplitude duration (ms)	Maximum rate of rise (V/s)	After hyper-polarisation (mV)	Frequency (x/min)	No. of cells (n)
Control	−61.7 ± 1.4	66.0 ± 1.7	937 ± 128	4.4 ± 0.3	−68.2 ± 1.6	3.9 ± 0.4	15
Nerve stimulation	−60.9 ± 1.7	65.0 ± 1.9	1144 ± 170*	4.7 ± 0.4	−67.4 ± 1.8	2.6 ± 0.3*	
Control	−58.2 ± 2.8	62.6 ± 2.9	1251 ± 292	5.5 ± 0.9	−63.7 ± 2.7	3.1 ± 0.9	4
Substance P 1 μM	−54.7 ± 1.8	59.6 ± 3.4	1860 ± 462	5.1 ± 1.2	−59.1 ± 1.1*	4.3 ± 0.7*	
Control	−59.8 ± 3.7	70.7 ± 3.9	1304 ± 325	6.4 ± 0.2	−65.1 ± 3.1	3.7 ± 1.2	3
Neurokinin in A 100 nM	−55.1 ± 2.3	68.3 ± 2.8	2678 ± 855*	6.5 ± 0.8	−56.3 ± 1.8*	4.7 ± 1.2*	
Control	−60.9 ± 2.8	64.9 ± 2.9	1062 ± 263	5.5 ± 0.5	−66.7 ± 2.6	4.4 ± 1.0	5
MEN 10,627 1μM	−58.8 ± 2.6*	63.2 ± 2.0	745 ± 144*	5.8 ± 0.4	−64.7 ± 2.7	3.2 ± 0.9	
Control	−60.1 ± 1.5	59.3 ± 2.0	766 ± 127	3.6 ± 0.6	−67.0 ± 1.6	3.5 ± 0.3	8
hCGRP (1–37) 100 nM	−59.1 ± 1.2	56.9 ± 2.9	606 ± 132*	3.7 ± 0.6	−66.6 ± 1.3	2.3 ± 0.6*	
Control	−62.1 ± 2.6	61.5 ± 1.3	582 ± 78	3.0 ± 0.5	−69.8 ± 4.4	2.9 ± 0.6	3
hCGRP (8–37) 1 μM	−59.5 ± 1.2	58.7 ± 1.0	557 ± 81	2.8 ± 0.5	−67.3 ± 3.7	3.0 ± 0.6	

*$P < 0.05$

Effects of MEN 10,627 and capsaicin

Application of the tachykinin antagonist, MEN 10,627 (1 μM), significantly depolarised the ureteric smooth muscle 2.1 mV from −60.9 ± 2.8 mV to −58.8 ± 2.6 mV ($P < 0.05$, $n = 5$) and decreased the half-amplitude duration of the spontaneous action potentials to 75 ± 6% of control ($P < 0.05$, $n = 5$), but only tended to decrease the frequency of discharge to 75 ± 9% of control ($P = 0.053$, $n = 5$) (Fig. 1Bi–iii; Table 1). In two of three other ureteric preparations, however, 3 μM MEN 10,627 completely abolished the spontaneous action potential discharge. MEN 10,627 (1 μM) also prevented the nerve-evoked increase in half-amplitude duration of the action potentials recorded immediately after nerve stimulation (to 99 ± 6% of control) ($P > 0.05$, $n = 3$) (Fig. 1Biv).

The role that intrinsic sensory nerves may play in maintaining action potential discharge and conduction in the ureter was assessed by comparing action potential discharge before (Fig. 2Ai–ii), during (Fig. 2Ai–ii) and after (Fig. 2Aiii) exposing the whole-mount preparations to capsaicin (10 μM) for 10–15 min. In three of four successful experiments in which the microelectrode impalement was maintained, action potential discharge was completely abolished either during the capsaicin exposure or within 5–10 min after the washout of capsaicin (Fig. 2Ai). The membrane potential (−57.2 ± 1.8 mV) recorded in these capsaicin-arrested ureters (5–10 min after washout) was not significantly different from control (−57.1 ± 2.0 mV) ($n = 3$, $P > 0.05$). Pretreatment of two preparations with MEN 10,627 (3 μM) prevented the excitatory actions of sensory nerve tachykinins released by capsaicin (10 μM) exposure (Fig. 2B). It was not possible to maintain the impalement long enough (1 h) to establish whether action potential discharge returned with time, as might be expected from our recent contractile experiments using the whole-mount preparation of the upper urinary tract [28].

Thiorphan and tetrodotoxin

The neutral endopeptidase inhibitor, thiorphan, has been used previously to enhance the duration and intensity of the inhibitory actions of sensory nerve stimulation or applied hαCGRP in the guinea pig ureter driven with endothelin-1 [16]. In two experiments, thiorphan (1 μM applied for 2–5 min) mimicked the effects of MEN 10,627, preventing the nerve-evoked increase in the action potential duration and prolonging the interval between action potentials recorded immediately following sensory nerve stimulation (data not shown). In contrast, the application of tetrodotoxin [1.5 μM ($n = 5$) or 10 μM ($n = 2$)] blocked the effects of nerve stimulation in all ureteric preparations, but had no significant effects on the time course or frequency of the spontaneous action potentials (data not shown).

Effects of applied substance P and neurokinin A

Both substance P (1 μM) ($n = 4$) and neurokinin A (100 nM) ($n = 3$) significantly increased the frequency of the ureteric action potentials to 156 ± 19% and 142 ± 21% (both $P < 0.05$), respectively, of the control frequency (Fig. 3i–iii). Substance P and neurokinin A also increased the half-amplitude duration of these action potentials to 152 ± 16% ($P > 0.05$) and 195 ± 20% ($P < 0.05$), respectively, of control; other action potential parameters were little affected by these agents (Table 1) [23]. In addition, the peak "diastolic" or after-hyperpolarisation potential of these action potentials was significantly depolarised 4.6 and 8.8 mV, respectively (both $P < 0.05$) by substance P and neurokinin A (Table 1). In the presence of MEN 10,627 (1 μM), the excitatory effects of both substance P (1 μM) ($n = 2$) and neurokinin A (100 nM) ($n = 1$) were prevented (Fig. 3i, iv).

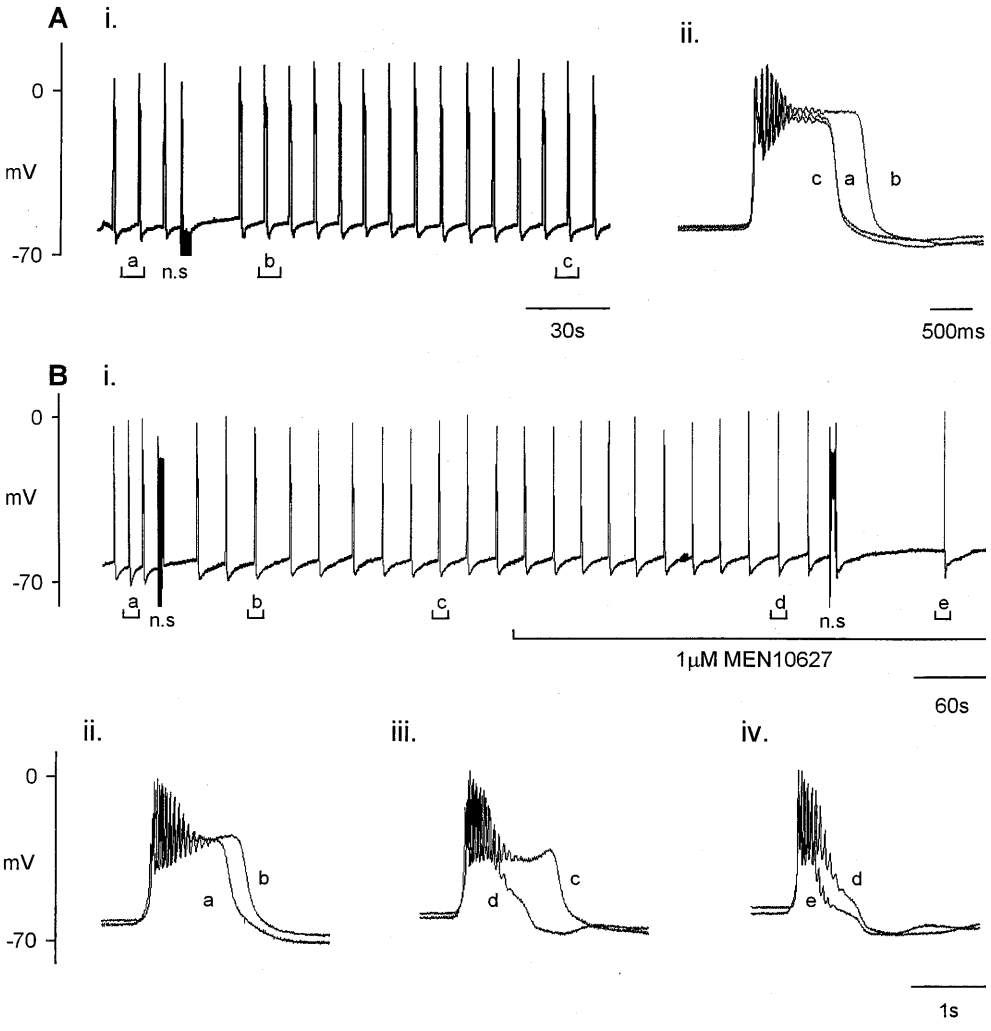

Fig. 1 **A** Effects of transmural electrical stimulation (*n.s*) on the spontaneous action potentials recorded in the ureteric region of a whole-mount preparation of the guinea pig upper urinary tract. **Bi** The negative chronotropic effects of nerve stimulation (*n.s*) in the ureter can be enhanced by MEN 10,627 (1 μM), the NK2 receptor antagonist. **Aii**, **Bii–iv** the time course of the action potentials indicated by the *horizontal brackets* (*a–e*) have been compared on an expanded time base

Effects of hCGRP

In the spontaneously active ureter, hCGRP (100 nM applied for 2–5 min) decreased the frequency of action potential discharge to 67 ± 13% of control ($P < 0.05$, $n = 8$) (Table 1; Fig. 4Ai, Bi). In addition, hCGRP caused a time-dependent decrease in the duration of the spontaneous action potentials which was often associated with a hyperpolarisation of the plateau potential. After 5 min the half-amplitude duration of the ureteric action potential was decreased to 77 ± 4% of control ($P < 0.05$, $n = 8$) (Table 1; Fig. 4Aii, Bii). However, other properties of the action potentials were little affected by hCGRP (100 nM) (Table 1).

The effects of hCGRP were readily reversible upon wash-out of the agent (Fig. 4Ai–ii), or upon the addition of the blocker of ATP-dependent K channels, glibenclamide (1 μM) ($n = 2$) (Fig. 4Bi–ii). Moreover, the effects of hCGRP were prevented by the previous pretreatment with the CGRP receptor antagonist, hCGRP (8–37) (1 μM applied for 10 min) (data not shown). hCGRP (8–37) did not have any significant effects on the time course or frequency of the spontaneous ureteric action potentials ($n = 3$) (Table 1).

Finally, comparisons of the inhibitory effects of sensory nerve stimulation and hCGRP revealed a considerable variability between preparations. In two preparations, nerve stimulation and hCGRP (100 nM) both evoked a strong inhibition of the electrical activity in the ureter, while in two preparations both interventions had relatively minor effects. In contrast, in three other preparations nerve stimulation had little effect, while applied hCGRP strongly inhibited ureteric action potential discharge.

Discussion

Using a whole-mount preparation of the guinea pig upper urinary tract in vitro, we have recently reported that the simultaneous recording of tension in the proximal and distal renal pelvis and the ureter reveals the migration of spontaneous contractions, which originate (in 79% of preparations) in the proximal renal pelvis. These contractions occur at a frequency of 4.5 min^{-1}

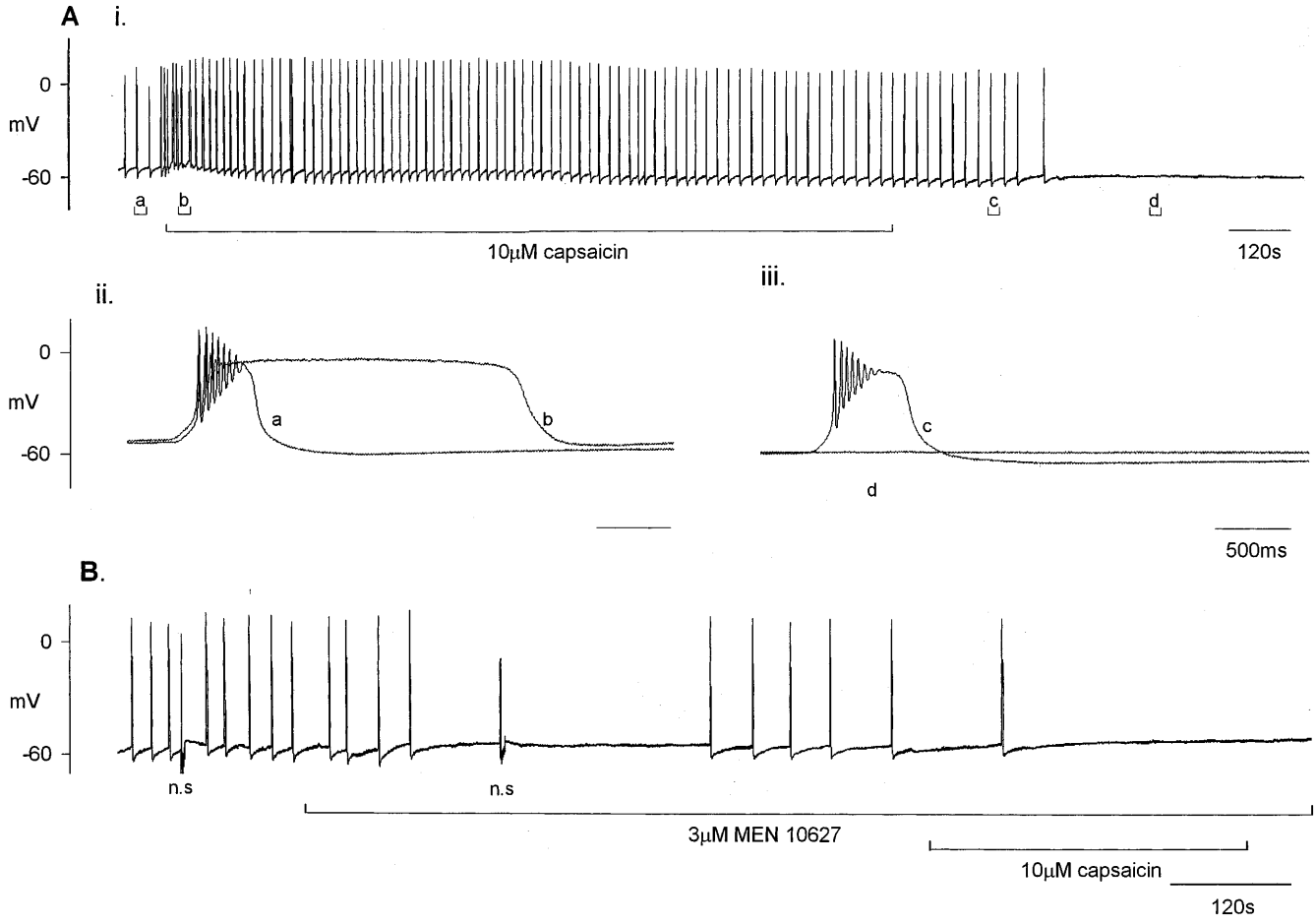

Fig. 2 **Ai** Effects of capsaicin (10 μM) on the spontaneous action potentials recorded in the ureter. **Aii–iii** the action potentials indicated by the *horizontal brackets* (*a–d*) are displayed on an expanded time base for comparison. The positive inotropic and chronotropic effects of capsaicin (10 μM) were blocked by pretreatment with MEN 10,627 (3 μM)

and travel at a velocity of 1–3 cm^{-1} [28], rates similar to those obtained during previous recordings of pyeloureteral peristalsis [5]. Even though the membrane potential measured 600 ms prior to each spontaneous action potential recorded in the present experiments (−60 mV) (Table 1) was generally 5–10 mV more negative than the membrane potential recorded in quiescent preparations of the isolated ureter, the time course of the spontaneous and evoked action potentials recorded in the two preparations were essentially similar [1, 11]. The frequency (3.3–3.9 min^{-1}) of the spontaneous ureteric action potentials in the whole-mount preparation was also similar to the frequency of the propagating contractions in this preparation (see above), but was smaller than the discharge frequency in circumferentially-cut strips of proximal renal pelvis (4.7–7.1 min^{-1}) [13, 14, 29].

Repetitive electrical stimulation has often been demonstrated to produce a net excitatory action on the contractile and electrical activity in the renal pelvis [21] and a net inhibitory action in the ureter (Fig. 1) [15, 20], both of which are blocked by tetrodotoxin or capsaicin pre-treatment (Fig. 2) [28]. Nerve stimulation has a negative inotropic effect on the guinea pig ureter, reducing the amplitude of the spontaneous contractions induced previously by a high K^+ saline or endothelin-1, in a manner blocked by the CGRP antagonist, hCGRP (8–37) [15, 18]. The positive inotropic effects of sensory nerve stimulation on the spontaneous contractions in the proximal renal pelvis are blocked by the tachykinin antagonist, MEN 10,376, to reveal an underlying hCGRP (8–37)-sensitive negative inotropic action as seen in the ureter [21].

In the present experiments, the frequency and half-amplitude duration of the spontaneous action potentials in the ureter in the *absence* of nerve stimulation were also readily reduced by MEN 10,627 (1 μM) (Fig. 3i, iv), but not by tetrodotoxin (1–3 μM). This suggests that tachykinins, released tonically from sensory nerve varicosities in control saline, maintain in some way the action potentials in the ureter in a manner not dependent on the conduction of the nerve action potential along the sensory nerves. This notion is supported by the fact that tetrodotoxin has little effect on the amplitude and frequency of the contractions recorded in the whole-mount preparation of the upper urinary tract, while ω-conotoxin GVIA, a blocker of Ca^{2+} entry through neuronal "N-type" Ca^{2+} channels, reduces the amplitude but not

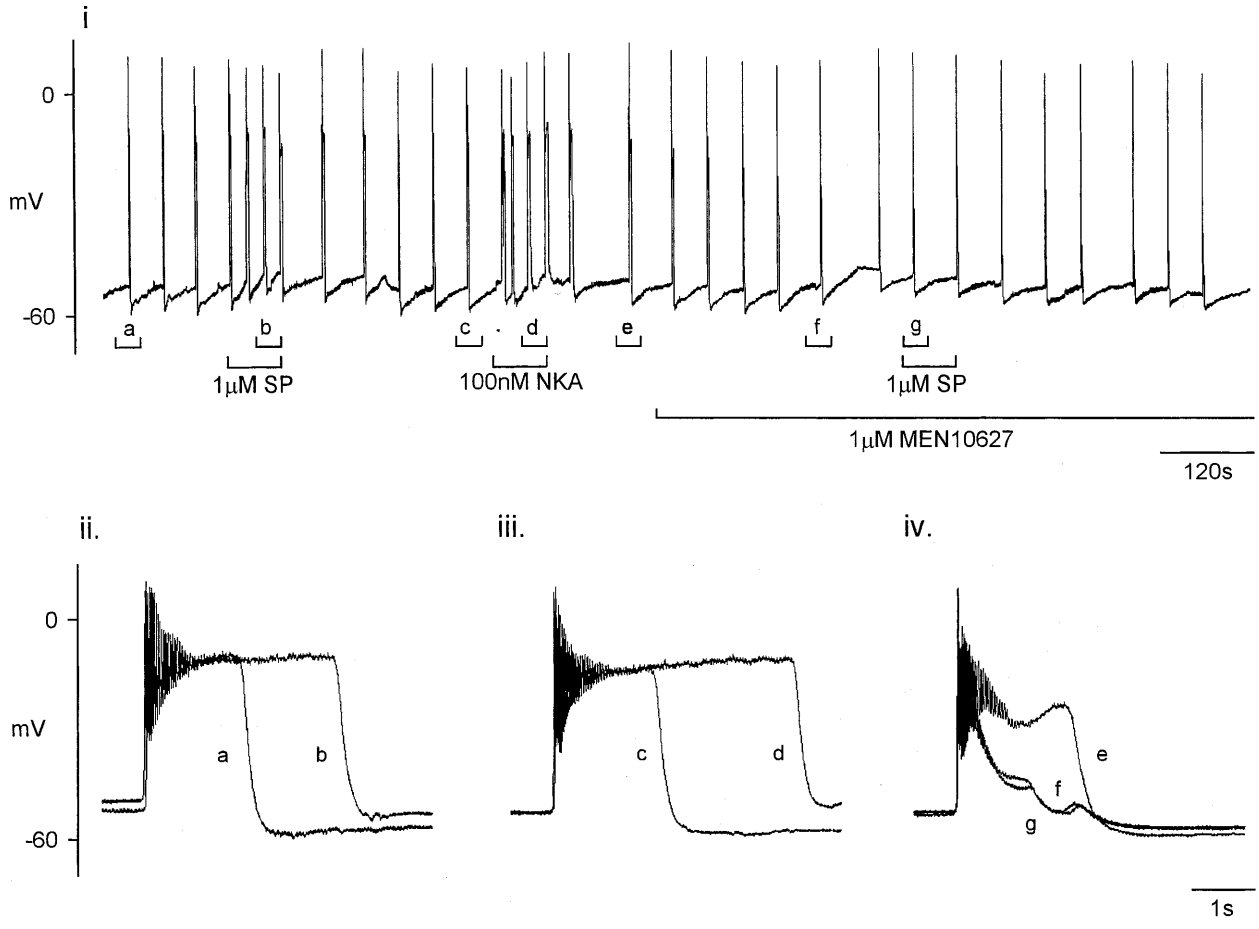

Fig. 3 The excitatory effects of substance P (*SP*; 1 μM) and neurokinin A (*NKA*; 100 nM) on the frequency **(i)** and time course **(ii–iv)** of the spontaneous ureteric action potentials were prevented by MEN 10,627 (1 μM) **(i)**

the frequency of these contractions [28]. In contrast, in the spontaneously contracting guinea pig ureter depolarized with high-K saline, the amplitudes of the contractions are increased by ω-conotoxin GVIA or hCGRP (8–37), suggesting that the net effect of the tonic release of tachykinins and CGRP from "depolarized" sensory nerves is inhibitory [15] rather than facilitating action potential discharge as in the present experiments.

A delayed-onset excitatory action of repetitive nerve stimulation was also observed in the ureter, as evidenced by the increase in the half-amplitude duration of the action potentials recorded immediately after the nerve-evoked period of membrane potential quiescence (Fig. 1Aii, Bii). This increase in half-amplitude duration following nerve stimulation was prevented by MEN 10,627 (Fig. 1B), or pretreatment with capsaicin (10 μM for 15 min) or tetrodotoxin (1–3 μM) (data not shown), suggesting that sensory-nerve-released tachykinins are responsible for this increase. The application of either neurokinin A or substance P mostly mimicked the excitatory effects of nerve stimulation, increasing the frequency and half-amplitude duration of the spontaneous action potentials recorded in the ureter (Fig. 3i–iii). Similar activation of tachykinin (NK2) receptors has recently been reported to increase the duration of electrically evoked action potentials in quiescent ureters of the guinea pig and human [23]. As has been reported in the renal pelvis [21], neurokinin A had a more potent excitatory effect than substance P in the ureter (Table 1); however, the actions of both agents were readily prevented by MEN 10,627.

In the whole-mount preparation of the upper urinary tract, exposure to capsaicin led to a transient increase in the frequency and duration of ureteric action potentials which was followed by a longer period of membrane quiescence, consistent with the positive and delayed negative inotropic and chronotropic effects on the spontaneous contractions in this preparation [28]. In addition, capsaicin can either inhibit (low doses) or enhance (high doses) the peristaltic contractions of a constantly perfused guinea pig ureter in vivo [7], the spontaneous contractions in human ureter in vitro [8] or the neurokinin-induced contractions in the rat ureter [20]. In the whole-mount preparation of the guinea pig upper urinary tract, it is likely that the overall initial excitatory effect seen upon the addition of capsaicin is initiated in the proximal regions of the renal pelvis, where the effects of nerve-released tachykinins predominate. These activated action potentials and contractions propagate distally into the ureter, presumably overriding

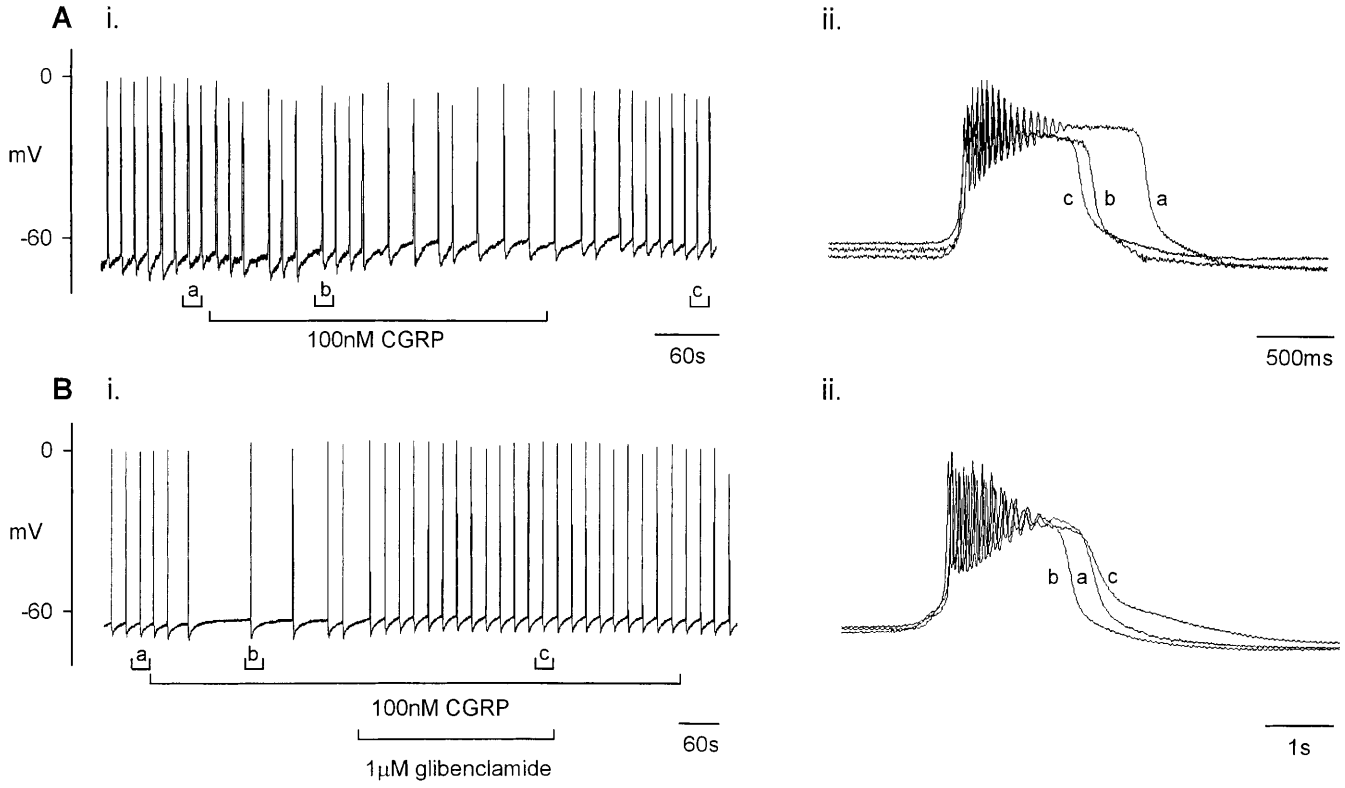

Fig. 4 **Ai** Effects of human calcitonin gene related peptide (*CGRP*; 100 nM) on the spontaneous action potentials recorded in the ureter. **B** The inhibitory action of CGRP (100 nM) were readily reversed by glibenclamide (1 μM). **Aii**, **Bii** The time course of the action potentials indicated by the *horizontal brackets* (*a–c*) in **Ai**, **Bi** have been compared on an expanded time base

the inhibitory effects of CGRP released locally in the ureter by capsaicin. Finally, these present experiments confirm our previous suggestion that the positive inotropic effects of nerve stimulation, K^+ channel blockers or capsaicin in the proximal renal pelvis and ureter can be directly correlated with the increase in the half-amplitude duration of the spontaneous "driven" action potentials (Fig. 2Aii) [13, 14, 28].

Applied hCGRP has a minor effect on the spontaneous contractile activity in the proximal regions of the renal pelvis, compared with a strong inhibitory action in the distal renal pelvis and ureter [15, 17, 22, 25, 28]. Moreover, the relatively poor inhibitory action of CGRP in the proximal renal pelvis is little affected by glibenclamide, the specific blocker of cromakalim-activated or ATP-dependent K^+ channels [19]. In contrast, the effects of hCGRP in the distal regions of the upper urinary tract are readily blocked by glibenclamide, as are the inhibitory actions of cromakalim in all regions of the upper urinary tract [15, 17, 22, 25]. In the present experiments, hCGRP decreased both the frequency and the half-amplitude duration of the action potentials in the ureter (Fig. 4) in a manner readily blocked by glibenclamide, suggesting that ATP-dependent K^+ channels are indeed being activated by hCGRP [15, 17, 22, 25, 28]. Moreover, in some cells, the propagating trigger potential underlying action potential discharge was still evident during the quiescent period following nerve stimulation (e.g. Fig 1Ai) or hCGRP application (data not shown). This perhaps suggests that these treatments are: (i) having little inhibitory action in the proximal pacemaker regions of the upper urinary tract; (ii) keeping the membrane potential of the ureteric smooth muscle cells negative of threshold; and (iii) having little effect on the electrical coupling or conduction between the proximal and distal regions of the upper urinary tract.

In summary, we have investigated the electrical activity underlying the peristaltic contractions in the ureteric region of a spontaneously active whole-mount preparation of guinea pig upper urinary tract. We have established that only "driven" action potentials are recorded in the guinea pig ureter. "Pacemaker" or "intermediate" action potentials [12, 13, 29] were never recorded in the ureter, consistent with the quiescent membrane potentials previously recorded in ureters dissected free of the renal pelvis. This spontaneously active whole-mount preparation of the guinea pig upper urinary tract may well be a particularly useful model for the study of the function of the human ureter, as the human ureter in vitro also demonstrates spontaneous contractile activity which can be blocked by inhibitors of prostaglandin synthesis [4] and evoked electrical activity which can be enhanced upon tachykinin receptor activation [23]. In addition, the propagation of action potentials along the ureter can be modified by the stimulation of intrinsic capsaicin-sensitive sensory afferents, to cause a predominantly inhibitory chronotro-

pic effect. A delayed-onset positive inotropic effect could possibly arise from the effects of nerve stimulation on the duration of the action potentials following the quiescent period after nerve stimulation, perhaps reminiscent of the effects of β-adrenoceptor activation in the heart. However, it is not likely that this effect arises from the regional-dependent post-junctional cyclic-AMP-dependent mechanisms linked to activated CGRP receptors [26], as this small excitatory action of nerve stimulation was mimicked by substance P, neurokinin A, or exposure to capsaicin and blocked by MEN 10,627, suggesting that nerve-released tachykinins were responsible.

Acknowledgement This work was supported by the NHMRC (Australia).

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