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Pharmacological characterization of the inwardly-rectifying current in the smooth muscle cells of the rat bladder

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1 In freshly-isolated single cells of the rat bladder detrusor, outwardly-rectifying and inwardly-rectifying membrane currents were identified by the whole-cell voltage-clamp technique.

2 The inwardly-rectifying current (I_{IR}) exhibited features of a cation current permeable to both K^+ and Na^+ but it was unaffected by changes in extracellular Ca^{2+}. It had an activation threshold close to −60 mV and an estimated reversal potential of −29 mV.

3 I_{IR} activated slowly with a voltage-sensitive time-constant of 69 ms at −140 mV and 209 ms at −100 mV but it did not exhibit time-dependent inactivation.

4 I_{IR} was unaffected by tetraethylammonium (up to 20 mM) but it was reduced by extracellular Ba^{2+} (1 mM) and by extracellular Cs^+ (1 mM).

5 I_{IR} was reduced by terikalant (100 μM) and markedly inhibited by ciclazindol (100 μM) although at these concentrations, both agents also reduced outward currents.

6 I_{IR} was inhibited by ZD7288 (10–100 μM) in a concentration-dependent manner. At concentrations up to 30 μM, ZD7288 did not reduce the magnitude of outward currents but these were inhibited by 100 μM ZD7288.

7 In strips of bladder detrusor, spontaneous mechanical activity was increased by ZD7288 (0.3–100 μM) and by ciclazindol (0.3–100 μM) but was unaffected by glibenclamide (1–10 μM).

8 It is concluded that I_{IR} closely resembles the hyperpolarization-activated current, I_h, previously described in the smooth muscle of rabbit jejunum and in a variety of other cell types. This current may play an important role in modulating detrusor excitability but this could not be confirmed using the inhibitors ZD7288 and ciclazindol.

Keywords: Whole-cell voltage-clamp; rat bladder detrusor; smooth muscle; inward-rectifier; I_h; ZD7288; ciclazindol; terikalant; glibenclamide; tetraethylammonium; Ba^{2+}; Cs^+

Introduction

Inwardly-rectifying conductances are absent from most types of smooth muscle (see Bolton & Beech, 1992). However, in the gastro-intestinal tract (rabbit jejunum: Benham *et al.*, 1987), in certain arterioles (submucosal and cerebral: Edwards & Hirst, 1988; Edwards *et al.*, 1988; Quayle *et al.*, 1993) and in guinea-pig detrusor (Inoue & Brading, 1990), inwardly-rectifying currents have been described.

In rabbit jejunum these show some characteristics of the hyperpolarization-activated cation current (I_h) described in the cells of the sino-atrial node (DiFrancesco *et al.*, 1986), in salamander rod photoreceptors (Wollmuth & Hille, 1992) and in a variety of neurones (Pape, 1996). I_h (also called I_f or I_Q) is typically more selective for K^+ than Na^+ with a reversal potential in the range −25 to −40 mV. It is inhibited by extracellular Cs^+ but is relatively insensitive to blockade by external Ba^{2+} (Pape, 1996). In contrast, the arteriolar inwardly-rectifying current is K^+-selective and is inhibited by external Ba^{2+} in the range 1–10 μM (Quayle *et al.*, 1993). In guinea-pig detrusor, neither the purinoceptor-gated nor the stretch-activated inwardly-rectifying currents exhibit selectivity for monovalent over divalent cations (Inoue & Brading, 1990).

In preliminary studies, a current (I_{IR}) with inwardly-rectifying properties has been detected in freshly-isolated smooth muscle cells from rat detrusor (Green *et al.*, 1996). The objective of the present investigation was to determine whether this conductance was K^+-selective (Quayle *et al.*, 1993) or if it more closely resembled I_h (Pape, 1996) or the cation current of guinea-pig detrusor (Inoue & Brading, 1990). In addition to determining the sensitivity of I_{IR} to Cs^+ and Ba^{2+}, the effects of ciclazindol (an inhibitor of $I_{K(ATP)}$ and of outwardly-rectifying smooth muscle K-currents; Noack *et al.*, 1992b), terikalant (a blocker of the cardiac inward rectifier; Escande *et al.*, 1992) and of ZD7288 (an inhibitor of I_h in guinea-pig sino-atrial node; BoSmith *et al.*, 1993) were also investigated.

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Methods

All experiments were performed on bladders removed from male Sprague-Dawley rats (100–125 g body weight), previously killed by stunning and bleeding.

Production of isolated cells

A segment of bladder detrusor muscle was cut into small pieces and the smooth muscle cells were dispersed by incubation in a low-Ca^{2+} physiological salt solution (PSS) containing collagenase and pronase (see Drugs and solutions) for approximately 15 min. The tissue was then triturated in Kraftbrühe (Klöckner & Isenberg, 1985) with a wide bore, smooth-tipped pipette. The cells were used for experiments within 10 h of separation, during which time they were stored at 6°C in Kraftbrühe. All experiments were performed at 23°C.

Single-cell electrophysiology

The whole-cell configuration of the patch-clamp technique (Hamill *et al.*, 1981) was used in all experiments. Patch pipettes were pulled from Pyrex glass (687–055, Jencons, U.K.) and had resistances of 3–4 MΩ when filled with the internal (intracellular) solution. Voltage commands were performed as

described by Noack *et al.* (1992a). For cell stimulation and for recording and analysing the data the pCLAMP 5.5 programme was used (Axon Instruments, U.S.A.). Data acquisition and storage were as described by Ibbotson *et al.* (1993). In each experiment the currents evoked by voltage steps from the stated holding potential were measured at the end of the test pulse.

The effects of K-channel modulators were investigated by adding the appropriate amount of the agent to the main reservoir containing the control external solution (Ca^{2+}-free PSS) to ensure that responses were obtained under steady-state conditions. Ca^{2+}-free PSS was used because preliminary experiments showed that I_{IR} was Ca-insensitive and this allowed the contaminating effects of Ca-sensitive outward currents to be avoided. The bath (volume: 1 ml) was continuously perfused (0.7 ml min^{-1}) with fresh external solution using a peristaltic pump (Microperpex, Pharmacia LKB, Freiburg, Germany); a second identical pump was used to remove excess solution from the recording chamber.

Tissue bath experiments

Longitudinal strips of detrusor were dissected from each bladder and these were mounted for isometric tension recording under a resting tension of 10 mN. Tissues were allowed to equilibrate for 1 h in Krebs solution at 37°C, gassed with 95% O_2: 5% CO_2, pH 7.4. During this period the tissues were frequently washed and their resting tension continuously adjusted until it was maintained in the range 6–8 mN. Tissues were exposed to either ZD7288 (10–100 μM) or to glibenclamide (1–10 μM) using a cumulative protocol and a contact time of 30 min for each drug concentration. Mechanical responses of the bladder strips were recorded and the activity was integrated with respect to time using an Apple Macintosh computer in conjunction with MacLab (MacLab 8) and software (Chart, version 2.5) supplied by Analog Digital Instruments.

Drugs and solutions

The low-Ca^{2+} PSS used for the cell separation comprised (mM): KOH 130, $CaCl_2$ 0.05, taurine 20, pyruvate 5, creatine 5, HEPES 10, fatty acid-free albumin 1 mg ml^{-1}, pronase (Calbiochem) 0.2 mg ml^{-1} and collagenase (Type VIII, Sigma) 1 mg ml^{-1}, buffered with methanesulphonic acid to pH 7.4. Kraftbrühe comprised (mM): KCl 85, KH_2PO_4 30, $MgSO_4$ 5, Na_2ATP5, pyruvate 5, creatine 5, taurine 20, β-OH-butyrate 5, fatty acid-free albumin 1 mg ml^{-1}, pH adjusted to 7.2 with KOH. The Ca-free PSS in the bath, which had the following composition (mM): NaCl 124.7, KCl 4.8, $MgCl_2$ 3.7, KH_2PO_4 1.2, glucose 11, HEPES 10, EGTA 1.0, was buffered with 4 M NaOH to pH 7.30 and was gassed with O_2. The pipette (internal) solution contained (mM): NaCl 5, KCl 120, $MgCl_2$ 1.2, K_2HPO_4 1.2, HEPES 10, EGTA 1.2, glucose 11, oxaloacetic acid 5, sodium pyruvate 2, sodium succinate 5, buffered to pH 7.30 with 4 M KOH. In some experiments the Ca-free PSS was replaced with a Ca-containing, EGTA-free external solution which had the following composition (mM): NaCl 125, KCl 4.8, $MgCl_2$ 1.2, KH_2PO_4 1.2, glucose 11, $CaCl_2$ 2.5, HEPES 10. In these experiments, EGTA was also omitted from the pipette solution. The Krebs solution had the following composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.1.

Ciclazindol (Pfizer), terikalant (Rhône-Poulenc Rorer) and glibenclamide were each first dissolved in dimethyl sulphoxide to produce a concentrated stock solution (20 mM) from which dilutions were prepared with bath solution immediately before they were required. ZD7288 (Zeneca) was dissolved in the external solution to produce a 10 mM stock solution. Tetraethylammonium chloride (TEA) was dissolved in twice-distilled water to produce a stock solution and external solutions containing TEA were isosmotically adjusted by omission of an equimolar concentration of NaCl. Unless otherwise stated, all compounds were obtained from Sigma.

Data analysis

To determine the significance of drug effects on current-voltage relationships, a one-way, within subject (repeated measures) analysis of variance with multiple dependent measures (Manova) test was used (Statistica v.3.0a: Statsoft). P values less than 0.05 were assumed to indicate significance.

Results

Whole-cell membrane currents under nominally Ca-free conditions

In the whole-cell configuration, the voltage-step protocols used (Figure 1) elicited several types of membrane current which were reproducible for at least 40 min. On stepping from a holding potential of −100 mV to test potentials positive to −60 mV, a current with fast activation and inactivation kinetics typical of an A-current ($I_{K(A)}$, see Bolton & Beech, 1992) was generated. At test potentials more positive than 0 mV, this current usually became masked by a more prominent current with slower activation and inactivation characteristics (Figure 1a). This current exhibited low electrical noise and was totally unaffected by the presence of 100 nM iberiotoxin in the bath solution ($n=6$, data not shown). It was thus typical of a delayed rectifier current ($I_{K(V)}$, see Bolton & Beech, 1992) and there was no evidence of the involvement of the large conductance, Ca-sensitive K-channel (BK_{Ca}). Both $I_{K(A)}$ and $I_{K(V)}$ became inactivated at a holding potential of −10 mV and on stepping from this potential to test potentials from −50 mV to +40 mV, voltage-insensitive, time-independent currents were observed (Figure 1b).

When the cells were stepped from a holding potential of −10 mV to test potentials in the range −60 to −140 mV, inwardly-rectifying currents (I_{IR}) with time-dependent activation were generated (Figure 1b). Inwardly-rectifying currents of similar magnitude were also activated on stepping from −100 mV to more negative potentials ($n=6$, data not shown).

Reversal potential, voltage-dependence, kinetics and ion-dependency of I_{IR}

Tail current reversal could not be used alone to estimate the reversal potential due to the activation of outwardly-rectifying K-currents in the membrane potential range over which the reversal potential of the inward current was estimated (see Mayer & Westbrook, 1983). This was therefore determined graphically from the voltage corresponding to the intersection of current-voltage plots of the instantaneous current generated at test potentials ranging from −120 mV to −60 mV and from the instantaneous tail currents produced at test potentials ranging from −90 mV to −60 mV. Using this method, the mean reversal potential of I_{IR} determined in 6 cells was -29 ± 4 mV (Figure 2).

The voltage-dependence of I_{IR} was determined in 5 cells by plotting the normalized steady-state conductance (G/G_{max}) against the appropriate test potential (Figure 2d). Conductance values were determined at each test potential after subtraction of the leak and background currents and assuming a reversal potential for I_{IR} of 29 mV. The continuous line fitted through the data using a least squares method described a Boltzmann distribution with half-maximal activation at −74 mV, and a slope factor of 0.021. The activation time-course of I_{IR} could be described by a single exponential (correlation coefficient, $R>0.95$). At test potentials of −140 mV, −120 mV and −100 mV, the activation time-constants were 69 ± 3 ms, 91 ± 4 ms and 209 ± 46 ms, respectively (each $n=12$).

To investigate the ionic basis of I_{IR}, the concentration of either K^+ ($[K^+]_o$) or Na^+ ($[Na^+]_o$) in the Ca-free PSS was altered in some experiments. The $[K^+]_o$ was increased to 21.2 mM by replacement of NaCl with KCl or reduced to

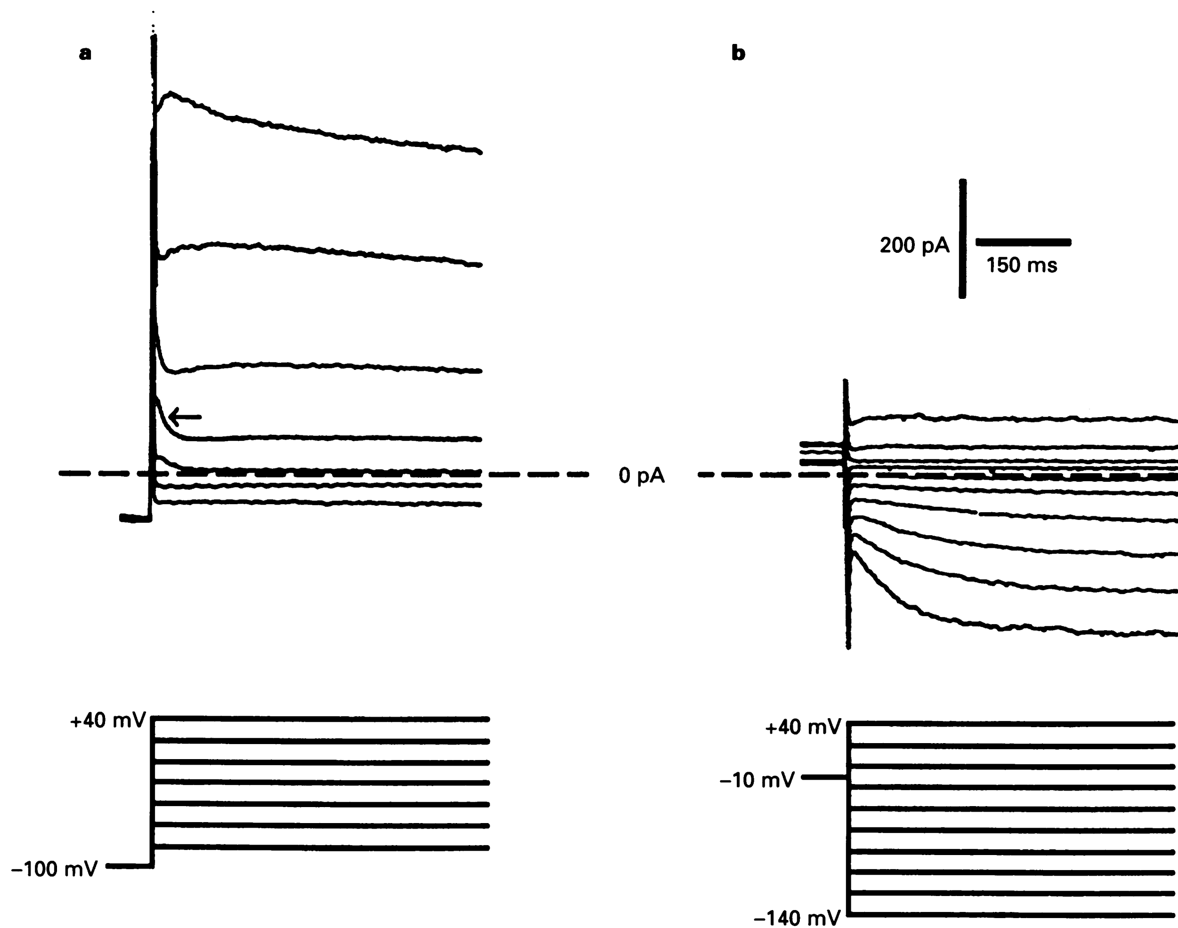

Figure 1 Whole-cell currents in rat detrusor cells under control conditions. (a) Cells were held at −100 mV and stepped for 500 ms to a series of test potentials from −80 mV to +40 mV in 20 mV increments. A rapidly-activating and -inactivating current (indicated by arrow) was evident over the range of test potentials from −40 mV to 0 mV. At more positive test potentials this current was masked by the more slowly-activating and -inactivating current. (b) After holding at −10 mV to inactivate these outward current components, cells were stepped for 500 ms to a series of potentials from −140 mV to +40 mV. At potentials positive to 0 mV a further outwardly-rectifying current component was observed. At potentials more negative than −60 mV there was clear evidence of activation of an inwardly-rectifying current. The horizontal dashed line in this and all following figures indicates the zero current level. Each trace is the computer-generated mean derived from 5 cells from different animals.

2.2 mM by substitution with Tris chloride. When the cells were bathed in Ca-free PSS containing the higher $[K^+]_o$, the magnitude of inward current generated at potentials negative to −60 mV was increased, whilst under conditions of lowered $[K^+]_o$ this current was reduced. In contrast, the voltage-dependence of I_{IR} was not affected by these changes in $[K^+]_o$ (Figures 3a, b). Decreasing $[Na^+]_o$ from 128 mM to 13 mM by substitution of NaCl with Tris chloride also reduced the magnitude of I_{IR} but to a lesser extent than that produced by reducing $[K^+]_o$ (Figure 3c). In contrast, the inclusion of Ca^{2+} (2.5 mM) in the bath solution (and omitting EGTA from both bath and pipette solutions) had no effect (Figure 3d).

Pharmacological characterization of I_{IR}

TEA, Ba^{2+} and Cs^+ The detrusor inwardly-rectifying current was inhibited by 1 mM Ba^{2+} and by 1 mM Cs^+ (Figure 4) but it was insensitive to 100 μM Ba^{2+} ($n=4$, data not shown). After 10 min exposure to 20 mM TEA, I_{IR} was unaffected (Figure 5a) although the amplitude of outward currents generated from a holding potential of −100 mV was greatly reduced (Figure 5b).

Ciclazindol This imidazoline derivative inhibits both K_V and K_{ATP} in rat portal vein cells (Noack *et al.*, 1992b). In the rat detrusor, ciclazindol (100 μM) produced a marked inhibition of I_{IR} which appeared to be due to a shift in the voltage-dependence of activation of this current in a hyperpolarizing direction (Figure 5c). Ciclazindol also inhibited the outward currents induced at test potentials positive to −30 mV. (Figure 5d).

Terikalant This benzopyran derivative is a selective inhibitor of I_{K1}, the cardiac inwardly-rectifying K-current (Escande *et al.*, 1992). In the detrusor, terikalant produced a slight but significant inhibition of I_{IR} (Figure 5e). Like ciclazindol, 100 μM terikalant also inhibited the outward currents generated on stepping from a holding potential of −100 mV to test potentials positive to −30 mV (Figure 5f). At a concentration of 10 μM, terikalant had no effect on I_{IR} although some inhibition of outward currents was obtained (data not shown).

ZD7288 The inhibition of I_h in cells of the guinea-pig sino-atrial node and the resulting bradycardia has been described by BoSmith *et al.* (1993). A 15 min exposure to ZD7288 (10 μM–100 μM) produced a concentration-dependent inhibition of I_{IR} in the rat detrusor and at the highest concentration, the current was virtually abolished (Figure 6a–c). As reported in cells of the sino-atrial node (BoSmith *et al.*, 1993), the time-course of inhibition of the detrusor inward current was slow. With a longer incubation period of 30 min, ZD7288 (1 μM) also produced a significant inhibition of I_{IR} ($n=3$, data not shown). At none of the concentrations tested did ZD7288 modify the peak outward current generated on stepping to test potentials from

a holding potential of −100 mV (data not shown). However, at a concentration of 100 μM, ZD7288 exerted a small but significant inhibitory effect on the magnitude of outward currents measured at the end of the 500 ms test pulse (Figure 6d). The effects of ZD7288 could not be reversed by washing for up to 30 min.

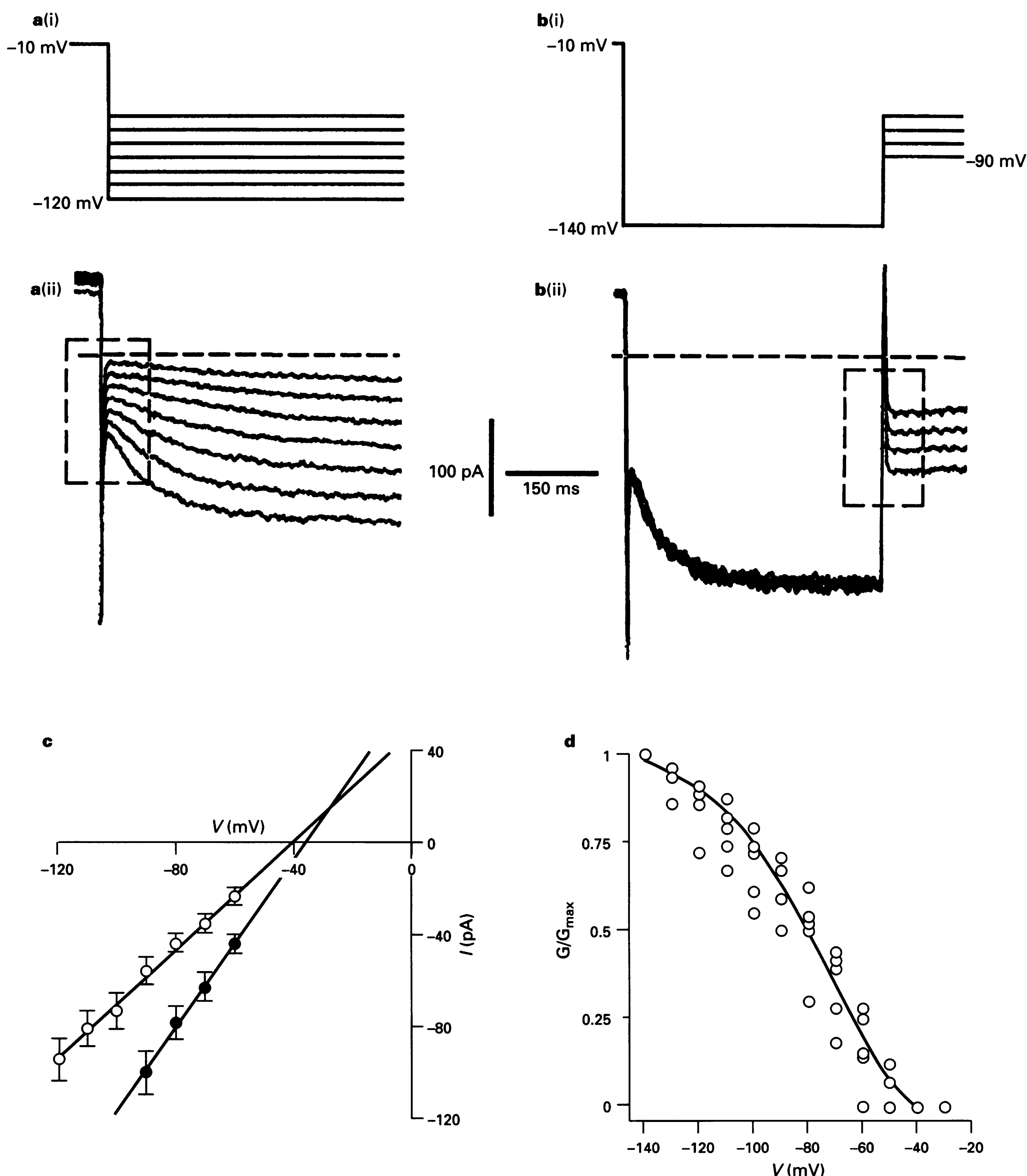

Figure 2 Estimation of reversal potential and activation of the inwardly-rectifying current in rat detrusor cells. (a) Stepping from −10 mV to a series of hyperpolarizing test potentials ranging from −120 mV to −60 mV in 10 mV increments (i) activated inwardly-rectifying currents. The instantaneous currents (indicated by the dashed box in a (ii)) at each test potential represented the background currents with an unknown contribution of I_{IR}. (b) In the same cells, the channel underlying I_{IR} was maximally opened by stepping the cells to −140 mV. When the cells were stepped to a series of depolarizing test potentials ranging from −90 mV to −60 mV (in 10 mV increments), the instantaneous tail currents (indicated by the dashed box in b (ii)) represented the maximum I_{IR} at that potential plus the background currents. Each trace is a computer-generated mean derived from 4 cells from different animals. (c) Current (I)-voltage (V) relationships used to derive the reversal potential of the I_{IR} which was estimated as the voltage at the point of intersection of the two lines. (○) Represents the instantaneous current generated from a holding potential of −10 mV and (●) denotes the instantaneous tail currents. Each point represents the mean ± s.e. mean, $n=6$. (d) Activation curve for I_{IR} determined in 5 cells. Points were generated by plotting the normalized steady-state conductance (G/G_{max}) against test potential (mV). The continuous line was fitted to a Boltzmann distribution by a least squares method.

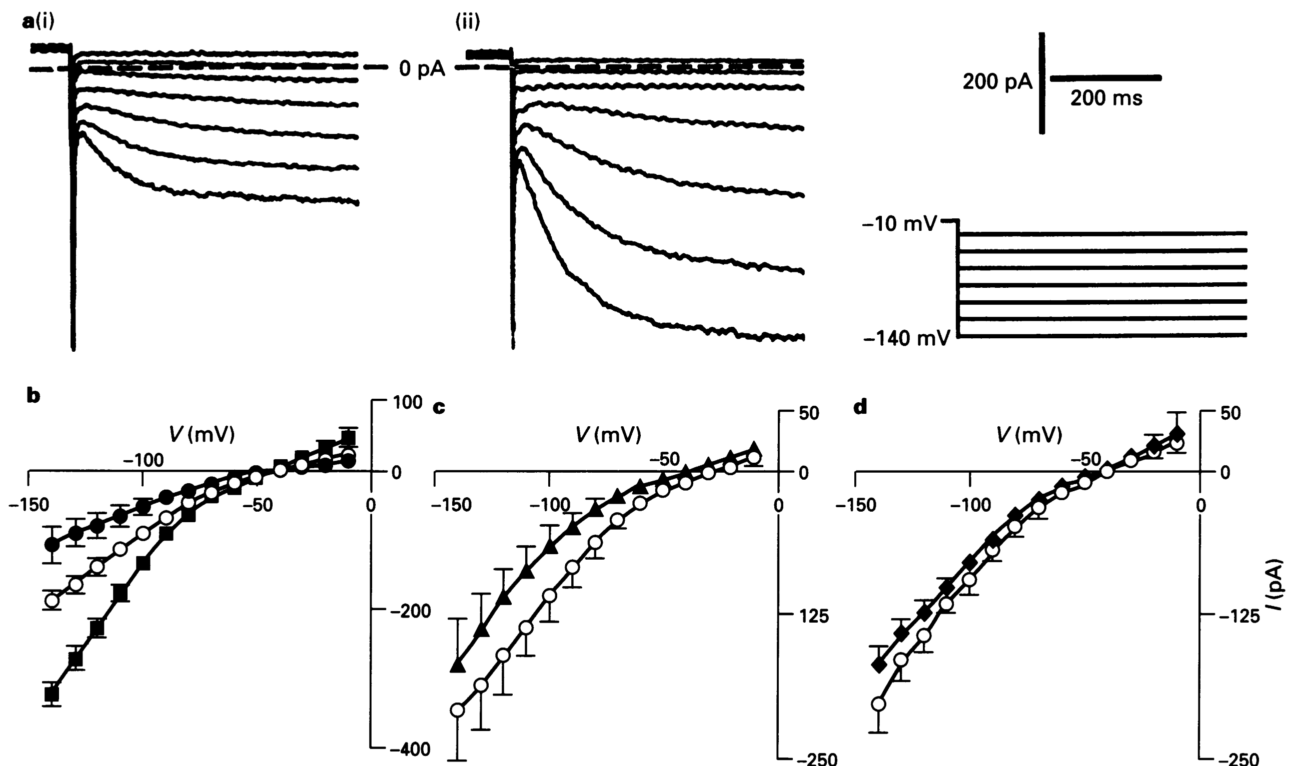

Figure 3 Effects of modification of the extracellular K^+, Na^+ and Ca^{2+} concentrations on inwardly-rectifying currents (I_{IR}) in rat detrusor cells. (a(i)) Shows computer-generated mean traces obtained from 4 cells which were held at −10 mV and then stepped for 500 ms to a range of test potentials from −140 mV to −20 mV under control conditions. (a(ii)) When the extracellular K^+ concentration ($[K^+]_o$) was increased to 20 mM there was an increase in the magnitude of I_{IR} at potentials negative to −60 mV. (b) Full current (*I*)-voltage (*V*) relationships for I_{IR}, measured at the end of each 500 ms step, under control conditions ($[K^+]_o$ = 6 mM, ○) or in the presence of a low (2.2 mM, ●) or high (21.2 mM, ■) $[K^+]_o$ (c) *I-V* relationships for I_{IR} under control conditions ($[Na^+]_o$ = 128 mM, ○) or when $[Na^+]_o$ was reduced to 13 mM, (▲). (d) The magnitude of I_{IR} was similar under calcium-free conditions (○) or when the bath solution contained 2.5 mM calcium (◆). In the graphs shown in (b)–(d), each point represents the mean ± s.e. mean, n = 4–8.

Effects on spontaneous mechanical activity in detrusor strips

In strips of detrusor muscle, ZD7288 (0.1–100 μM) produced a concentration-dependent enhancement of total (integrated) mechanical activity which consisted of an increase in both the amplitude and frequency of spontaneous phasic contractions (Figure 7a,c). Thus, after exposure to 100 μM ZD7288 the spontaneous activity had increased to 431 ± 82% of the initial level (mean ± s.e. mean, n = 4). Experiments were also performed using either ciclazindol, which inhibits both $I_{K(V)}$ and $I_{K(ATP)}$, or the inhibitor of K_{ATP}, glibenclamide. Glibenclamide apparently inhibited the spontaneous contractions of rat detrusor (Figure 7b) although a similar reduction was seen in time-matched control experiments suggesting that the inhibition was not an effect of glibenclamide itself. Ciclazindol increased spontaneous contractions in a manner similar to ZD7288 (Figure 7d).

Discussion

I_{IR} in rat detrusor cells

The characteristics of the inactivating currents generated in rat detrusor were qualitatively similar to those of the A-current ($I_{K(A)}$) and of the delayed-rectifier current ($I_{K(V)}$) described in other types of smooth muscle (see review by Bolton & Beech, 1992). Atypically, however, an inwardly-rectifying current (I_{IR}) was always present in rat detrusor cells. I_{IR} was slowly-activating, it exhibited a reversal potential of −29 mV and it was sensitive to changes in both the K^+ and Na^+ gradients. Interestingly, the decrease in I_{IR} produced by reduction of the Na^+ gradients was not as marked as might have been predicted suggesting that the underlying channel was permeable to the substituent ion, $Tris^+$. However, in other tissues $Tris^+$ does not permeate the I_h channel (Pape, 1996) and it is possible that the small effect of Na^+ removal simply reflects the higher permeability of the channel to K^+ than to Na^+. I_{IR} could be elicited in the Ca^{2+}-free conditions of the experiment (under which no iberiotoxin-sensitive current was present) and it was not modified when EGTA was omitted from bath and pipette solutions and when Ca^{2+} was added to the bathing solution. Additionally, I_{IR} was inhibited by Cs^+ but was only sensitive to millimolar concentrations of Ba^{2+}.

These features collectively suggest that I_{IR} is neither a K^+-selective, inwardly-rectifying current like that described by Quayle *et al.* (1993) in arteriolar smooth muscle, nor a current involving both mono- and divalent cations (reversal potential, 0 mV) as observed by Inoue & Brading (1990) in guinea-pig bladder. Instead, I_{IR} appears to be a cation current carried by both Na^+ and K^+ and to exhibit properties similar to those of the inwardly-rectifying current (I_h) described in rabbit jejunal cells. In the jejunum, this current was also relatively insensitive to Ba^{2+}, it was modified by changes in extracellular $[Na^+]$ and $[K^+]$ and characterized by a reversal potential of −25 mV (Benham *et al.*, 1987). I_h-like currents with reversal potentials and other characteristics similar to those of I_{IR} have now been described in a variety of neurones (−50 mV to −20 mV; Pape, 1996), in salamander photoreceptors (−35 mV; Wollmuth & Hille, 1992) and in guinea-pig sino-atrial node cells (−31 mV; BoSmith *et al.*, 1993). The most notable differences between I_{IR} (present study) and I_h in the rabbit jejunum were the shorter activation time-constants in rat detrusor although these were well within the previously-reported range (Pape, 1996).

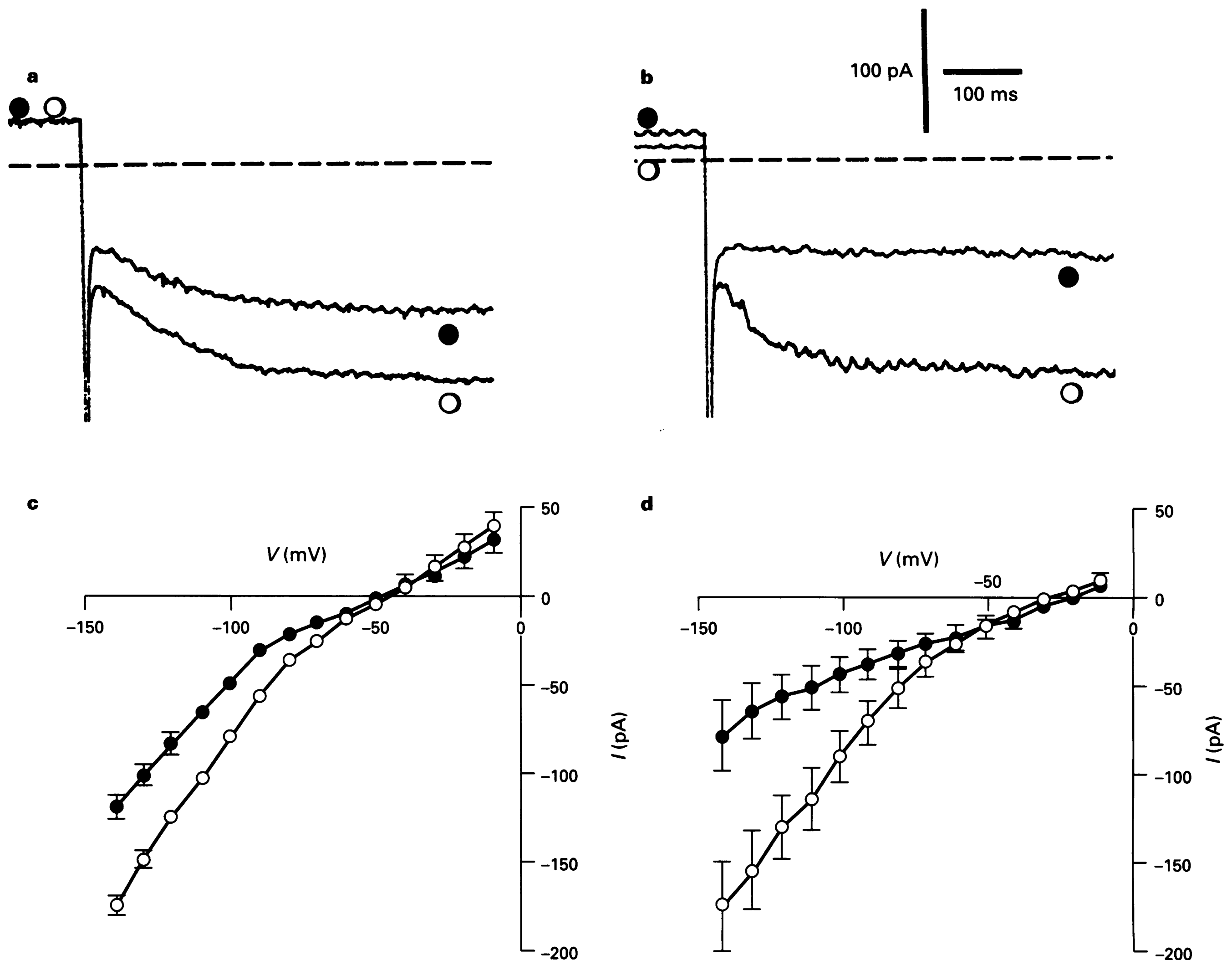

Figure 4 Effect of barium and caesium on inwardly-rectifying currents in rat detrusor cells. Under calcium-free conditions, both barium (1 mM, a) and caesium (1 mM, b) inhibited the inwardly-rectifying current which was induced by stepping from a holding potential of −10 mV to −140 mV. (c, d) Full current-voltage relationships for currents induced by stepping from −10 mV to the indicated series of test potentials under control conditions (○) or in the presence of 1 mM barium (c, ●) or 1 mM caesium (d, ●). Each point represents the mean ± s.e. mean, $n=4$.

Sensitivity of I_{IR} *to K-channel inhibitors*

At high concentrations (>10 mM), TEA inhibits most K-currents (Edwards & Weston, 1991). Interestingly, therefore, TEA (20 mM) had no effect on the magnitude of I_{IR} although it markedly inhibited the detrusor outward current, $I_{K(V)}$. Such TEA-insensitivity is a feature of I_h (Pape, 1996) and it is noteworthy that Wollmuth & Hille (1992) characterized I_h in salamander rods in the presence of 90 mM TEA.

Ciclazindol is a potent inhibitor of $I_{K(V)}$ in many smooth muscles (Edwards & Weston, 1993) including rat portal vein (Noack *et al.*, 1992b). However, I_{IR} is absent from this tissue (Edwards, unpublished) and it was thus of interest to determine the effects of ciclazindol on currents in the rat detrusor. As expected, $I_{K(V)}$ was reduced by ciclazindol but this agent also produced a marked inhibition of I_{IR}, an action which seemed to be associated with a shift in the activation voltage threshold of this current in a hyperpolarizing direction.

Terikalant is a potent and selective inhibitor of the inwardly-rectifying K-current (I_{K1}) in cardiac muscle (Escande *et al.*, 1992). Although I_{IR} in rat detrusor was inhibited by terikalant, a high concentration (100 μM) was required and this agent exerted even greater inhibitory effect on outward K-currents.

Inhibition of I_{IR} *by ZD7288*

I_{IR} was slowly inhibited by ZD7288 in a concentration-dependent manner as previously reported in sino-atrial node cells (BoSmith *et al.*, 1992). However, the potency of ZD7288 was lower in rat detrusor cells than in the cardiac cells which were sensitive to concentrations of ZD7288 as low as 0.1 μM. In addition to possible species and tissue differences, this may also reflect the relatively long exposure period of 35 min employed by BoSmith *et al.* (1993) in their studies of the sino-atrial node. In the present study, an increase in the inhibitory potency of ZD7288 was obtained when cells were exposed to this agent for incubation periods of up to 40 min. The basis for the slow action of ZD7288 is not known although it may reflect an intracellular site of action (BoSmith *et al.*, 1993).

At concentrations which produced a marked inhibition of I_{IR}, ZD7288 had relatively little inhibitory effect on outward K-currents in rat detrusor. This finding, together with the selective actions of this agent on I_h in cardiac muscle (BoSmith *et al.*, 1993), indicates that ZD7288 is a more selective inhibitor of I_h-like currents than other bradycardic agents, such as zatebradine (see Pape, 1996).

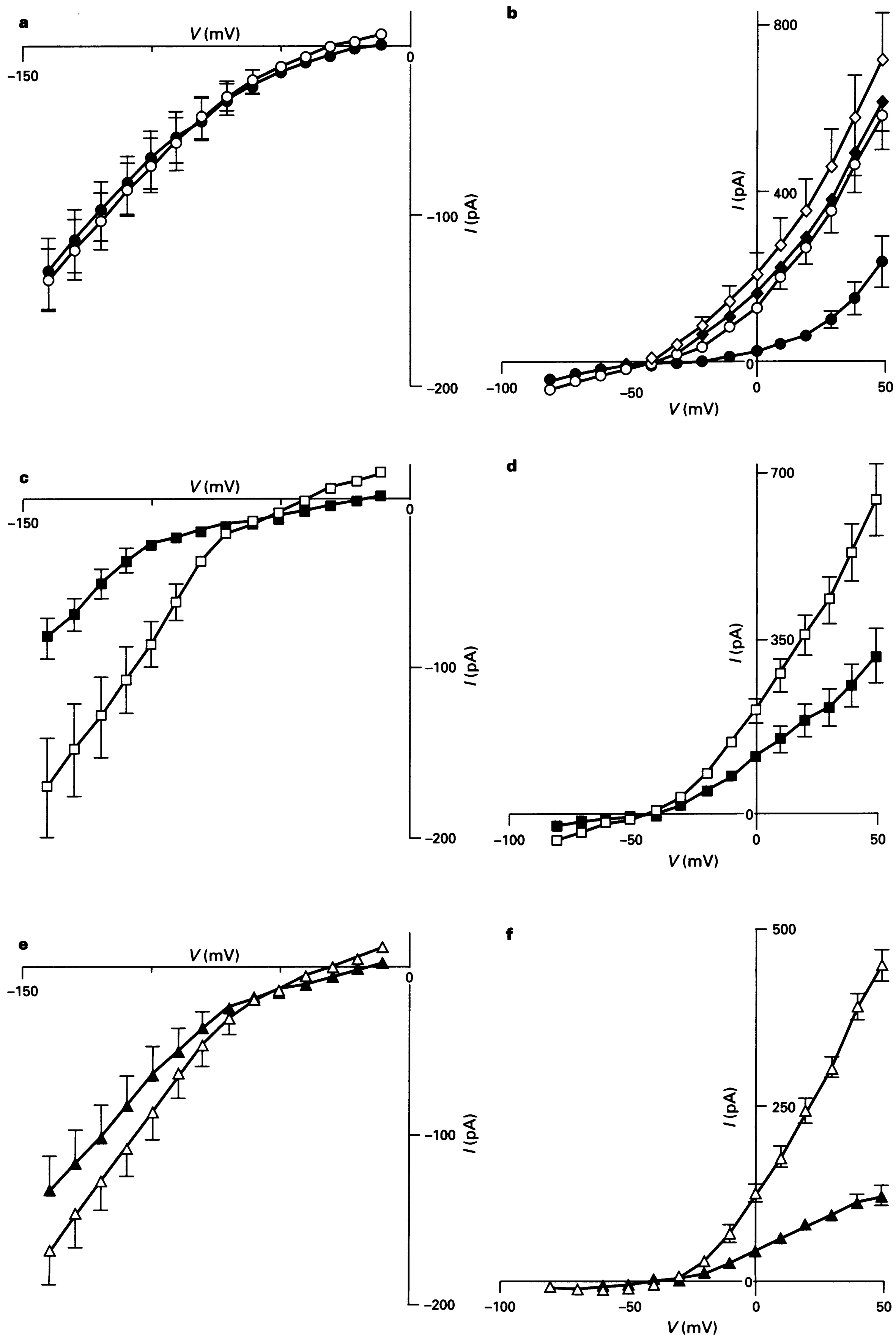

Figure 5 Effects of tetraethylammonium (TEA), ciclazindol and terikalant on current (*I*)-voltage (*V*) relationships determined in rat detrusor cells. (a–f) Each graph shows full *I-V* relationships for currents obtained on stepping from a holding potential of −100 mV (b, d, f) or −10 mV (a, c, e) under control conditions (○, □, △) or in the presence of 20 mM TEA (●, a, b), 100 μM ciclazindol, (■ c, d) or 100 μM terikalant (▲, e, f). The peak current generated under control conditions (◇) and in the presence of 20 mM TEA (◆) is also shown (b). Note the negative shift in the voltage-dependence of activation of the inward current in the presence of ciclazindol (c). All currents were measured at the end of the 500 ms test pulse. Each point represents the mean ± s.e. mean, $n=4-6$.

Role of I_{IR} *in whole tissues*

Bladder smooth muscle exhibits spontaneous contractions triggered by bursts of spike potentials (Brading, 1992). Assuming that I_{IR} exerts a pacemaker-like role, inhibitors of this current should cause quiescence analogous to the bradycardia observed in cardiac muscle (BoSmith *et al.*, 1993) and the decreased firing frequency seen in neurones (Pape, 1996). To test the possible physiological role of this current, detrusor strips were set up for mechanical recording and exposed to increasing concentrations of ZD7288 which produced an *increase* in the amplitude and frequency of phasic contractions. These effects occurred at concentrations which in single cells had no detectable effects on K-currents such as $I_{K(V)}$ or $I_{K(A)}$, inhibition of which would have resulted in enhanced mechanical activity in whole tissues. It is unlikely that the stimulatory effect of ZD7288 resulted from inhibition of $I_{K(ATP)}$ since glibenclamide had no effect on spontaneous mechanical activity. Furthermore, in preliminary experiments, ZD7288 did not inhibit $I_{BK(Ca)}$ (Green, unpublished observations). Thus, the possibility that ZD7288 enhances calcium currents or that it possesses actions unrelated to channel modulation cannot be excluded.

Conclusions

These studies have shown that the inwardly-rectifying current (I_{IR}) in rat bladder exhibits the properties of the hyperpolarization-activated cation current known as I_h (see Pape, 1996). Although inwardly-rectifying currents are present in smooth muscle (see Bolton & Beech, 1992), they are relatively uncommon and I_h-like currents are rare. Such a conductance does not appear to be a typical feature of bladder smooth muscle

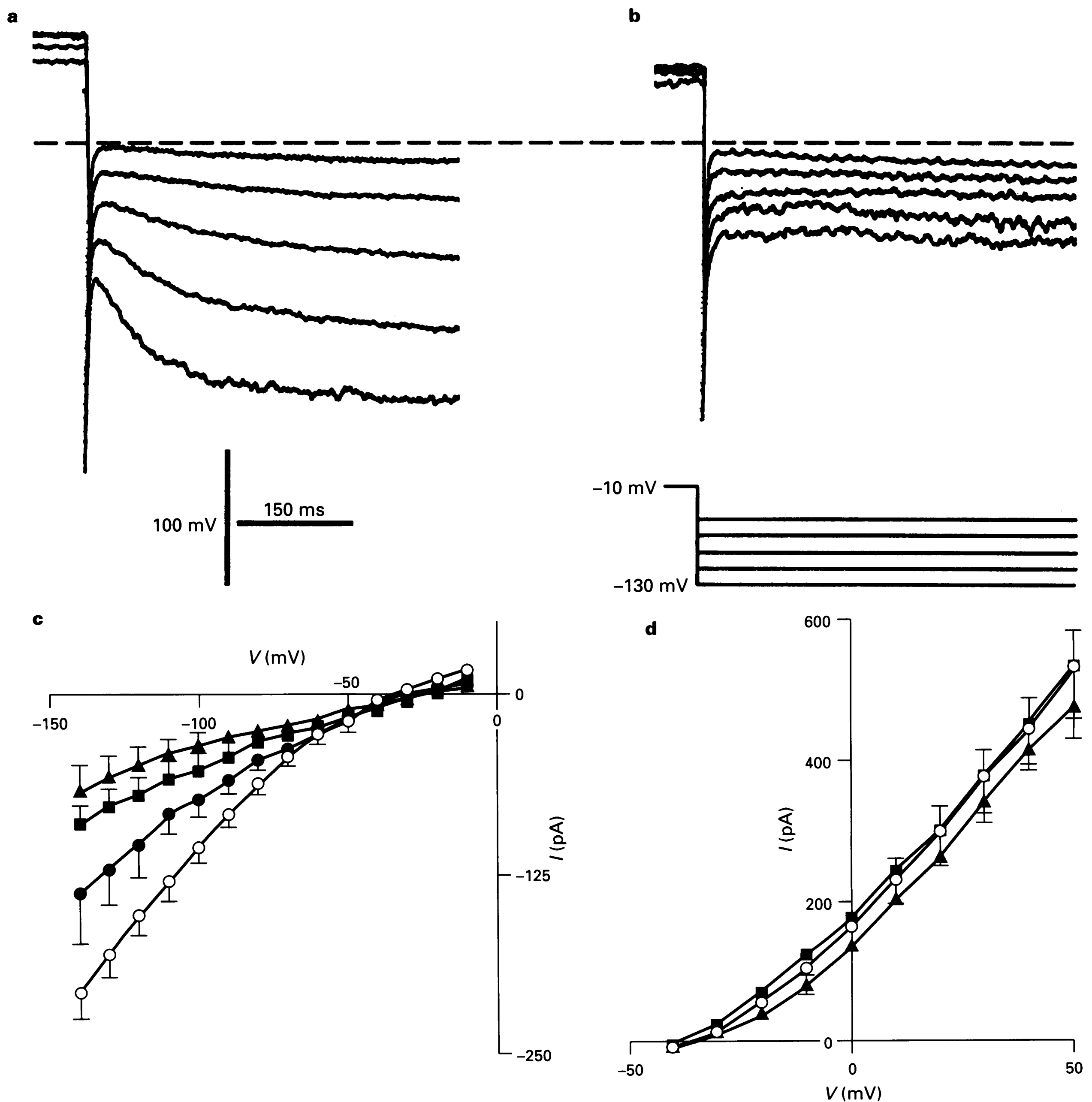

Figure 6 Effects of ZD7288 on whole-cell membrane currents in rat detrusor cells. (a, b) Inwardly-rectifying currents were generated by stepping to the test potentials indicated from a holding potential of −10 mV under control conditions (a) and after a 15 min exposure to 30 μM ZD7288 (b). Each trace is a computer-generated mean obtained from 4 cells from different animals. (c, d) Full current-voltage relationships for currents obtained on stepping from a holding potential of −10 mV (c) or −100 mV (d) under control conditions (○) or in the presence of 10 μM (●), 30 μM (■) and 100 μM (▲) ZD7288. All currents were measured at the end of the 500 ms test pulse. Each point represents the mean ± s.e. mean, n = 4–6.

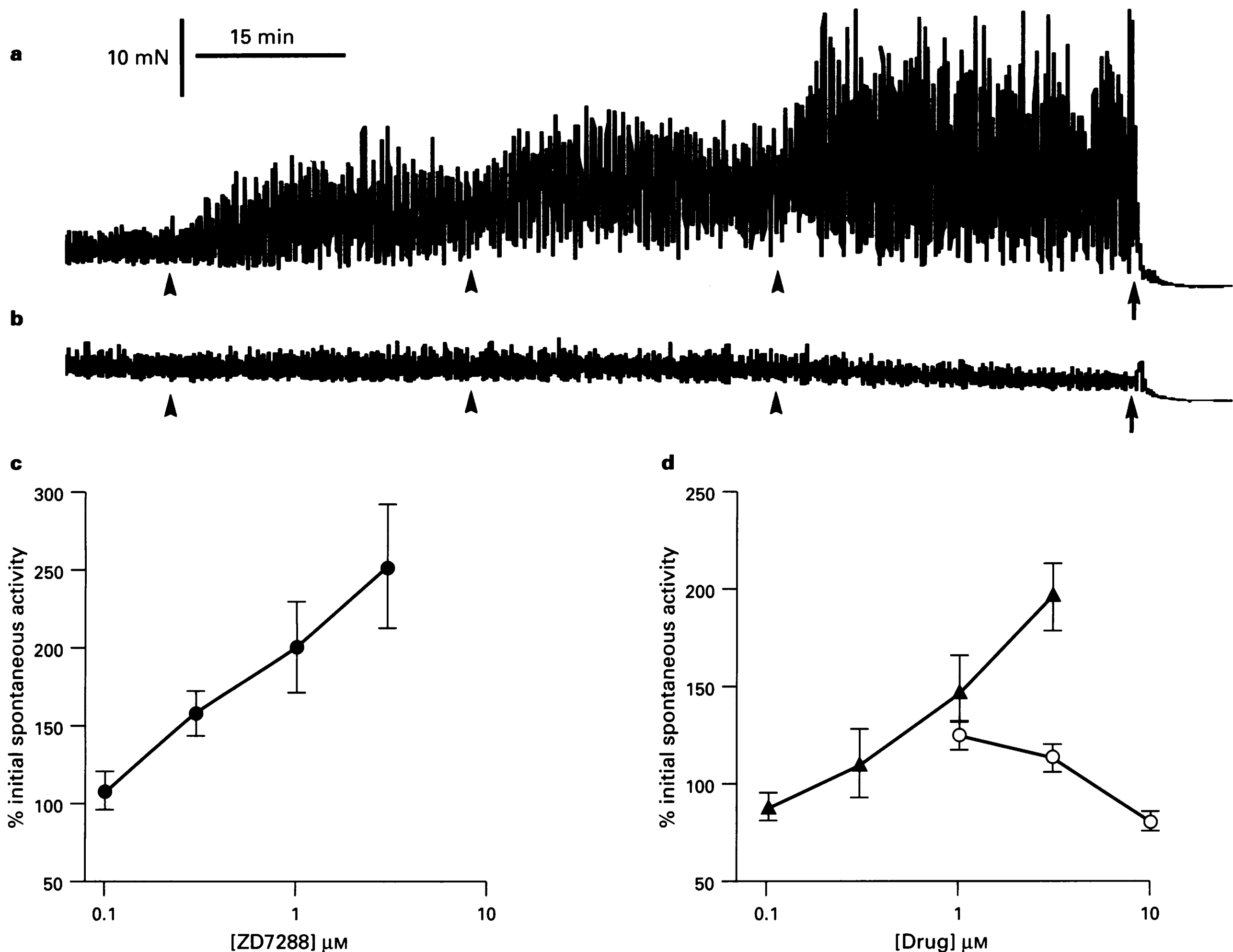

Figure 7 (a, b) Typical traces showing that ZD7288 (a) produced a concentration-dependent increase in mechanical activity in contrast to glibenclamide (b). In each trace, the cumulative addition of 10 μM, 30 μM and 100 μM ZD7288 (a) or 1 μM, 3 μM and 10 μM glibenclamide (b) is indicated by the arrowheads. At the end of each experiment tissues were exposed to 100 μM aminophylline (arrow) to define the baseline tension. (c, d) Comparison of the effects of ZD7288 (●, c) with those of ciclazindol (▲, d) and glibenclamide (○, d). Ordinate scale: total integrated mechanical activity (above baseline) was expressed as a percentage of the initial value which was defined as 100%. Each point in (c) and (d) represents the mean value ± s.e. mean, $n = 4$.

since it is absent from the human detrusor (Green *et al.*, 1995) and its presence was not detected in the guinea-pig (Inoue & Brading, 1990). Indeed, within smooth muscles, the presence of I_h has only been previously described in rabbit jejunum (Benham *et al.*, 1987).

Bladder smooth muscle is relaxed by openers of K_{ATP} such as levcromakalim and pinacidil, an action which is inhibited by the sulphonylurea, glibenclamide (Edwards *et al.*, 1992). K_{ATP} is now believed to comprise an inwardly-rectifying K-channel coupled to a sulphonylurea binding site (Inagaki *et al.*, 1995; 1996). It is thus surprising that no inwardly-rectifying *K-current* was detected in the rat bladder. Furthermore, preliminary studies indicate that $I_{K(ATP)}$ induced in this tissue by levcromakalim also exhibits no inwardly-rectifying properties (Green *et al.*, 1995). Experiments designed to clarify these anomalies are under way.

It was hoped that clues to the functional role of I_h in the rat detrusor would be obtained by use of ZD7288 which produced a marked inhibition of this current in isolated detrusor cells. However, the expected reduction in spontaneous activity resulting from the presumed inhibition of pacemaker potentials (see Pape, 1996) did not occur in the presence of this agent. Instead, mechanical activity actually increased and similar results were also obtained with ciclazindol which, like ZD7288, also inhibited I_h. In the case of ciclazindol, such an increase in mechanical activity can easily be explained by inhibition of $I_{K(V)}$, an action which might mask the effects of I_h inhibition, but such an explanation would not seem to be tenable for ZD7288. Moreover, in support of the view that ZD7288 had additional modulatory effects in the whole-tissue experiments, the time-course of action of ZD7288 on mechanical activity was faster than that observed in single-cell studies. Further studies on the basis of these effects of ZD7288 and on the role of I_h are in progress.

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