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Ionic currents and inhibitory effects of glibenclamide in seminal vesicle smooth muscle cells

H. Sadraei & [1]D.J. Beech

Department of Pharmacology, University of Leeds, Leeds, LS2 9JT

1 Whole-cell voltage-clamp recordings were made from smooth muscle cells isolated from guinea-pig seminal vesicle.

2 When the recording pipette solution contained 130 mM KCl and a low concentration of EGTA (0.2 mM), a dominant outward current was elicited by depolarization to positive of −30 mV from a holding potential of −50 mV. The current was non-inactivating, stimulated by intracellular Ca^{2+} and blocked by bath-applied 1 mM tetraethylammonium but not 1 mM 3,4 diaminopyridine.

3 If 10 mM EGTA was added to the KCl pipette solution and the holding potential was −50 mV, or more negative, the major current elicited by depolarization to positive of −30 mV was an A-type K^+-current. This current inactivated rapidly (within 100 ms) and was blocked by bath-applied 1 mM 3,4-diaminopyridine but not 10 mM tetraethylammonium.

4 An inward voltage-gated Ca channel current was observed on depolarization to positive of −30 mV with 1.5 mM Ca^{2+} or 10 mM Ba^{2+} in the bath solution and when Cs^+ replaced K^+ in the pipette. The Ba^{2+}-current was shown to be abolished by bath-applied 100 μM Cd^{2+} and inhibited by 90% by 1 μM nifedipine, and thus appeared to be carried by L-type Ca channels.

5 High concentrations of glibenclamide (10–500 μM) inhibited A-type K^+-current, Ba^{2+}-current and contraction of the whole tissue induced by noradrenaline or electrical field stimulation.

6 From these data we suggest that seminal vesicle smooth muscle cells express Ca^{2+}-dependent K channels, A-type K channels and L-type Ca channels which are inhibited by tetraethylammonium, 3,4-diaminopyridine and nifedipine, respectively. In addition, an unexpected relaxant effect of high concentrations of glibenclamide may be explained by inhibition of the Ca channels.

Keywords: Ion channel; smooth muscle; seminal vesicle; 3,4-diaminopyridine; nifedipine; glibenclamide

Introduction

It has been suggested that the seminal vesicle is important for the reproduction of mammals because vesiculectomy causes a serious decline in fertility (Clavert *et al.*, 1990). The mechanisms which determine the contractile state of the smooth muscle cells in the seminal vesicle wall, and the pharmacology of these mechanisms, has nevertheless received limited attention. Intracellular microelectrode studies on guinea-pig seminal vesicle smooth muscle cells indicate that membrane potential changes play an important role (Kajimoto *et al.*, 1971; Ohkawa, 1982). A variety of spontaneous depolarizations occur; some are small, ranging from 2 to 10 mV, and appear to be excitatory junction potentials, and others may be considered to be action potentials, being brief (<100 ms), about 40 mV in amplitude and sometimes superimposed on regular slow waves of depolarization. Ohkawa (1982) showed that a transient phase of contraction follows shortly after a period of spike activity.

Although ion channels and their pharmacology have been studied in a wide variety of smooth muscles, for example from small intestine (Ohya *et al.*, 1986), trachealis (Hisada *et al.*, 1990), portal vein (Beech & Bolton, 1989) and arcuate artery (Gordienko *et al.*, 1994), they have not been studied in seminal vesicle smooth muscle cells. In this study, we characterized the ion channel activities of single smooth muscle cells isolated from guinea-pig seminal vesicle by making whole-cell voltage-clamp recordings and by testing the sensitivities of ionic currents to a variety of inhibitors. In addition, we described an unexpected relaxant effect of glibenclamide (a sulphonylurea used in the treatment of non-insulin-dependent diabetes) on seminal vesicle smooth muscle and investigated if this effect might be explained by an action on ion channels. Some of these results were presented at a British Pharmacological Society meeting (Sadraei *et al.*, 1994).

Methods

Smooth muscle cell isolation and patch-clamp recording

Male guinea-pigs (350–400 g) were killed by cervical dislocation followed by exsanguination. Seminal vesicles were removed, and placed in bath solution pre-gassed with 100% O_2. Superficial connective tissue was removed and each seminal vesicle split open and the endothelial layer gently stripped off with a pair of forceps. The tissue was cut into 5–7 pieces (2 × 5 mm) and immersed in dispersion solution for 10 min at 37°C. This solution was then replaced with dispersion solution containing 0.2–0.35% collagenase (type 1, Worthington Biochemical Corporation) and 0.15–0.25% protease (type XXV, Sigma) and the incubation continued for a further 20 min. The tissue was then washed in dispersion solution to remove the enzymes and mechanically agitated. Resulting suspensions of cells were centrifuged (1000 rev min^{-1}, 5 min) and the pellets resuspended in fresh dispersion solution. Cell suspensions were kept at 4°C and recordings made from cells within 10 h. Most of the cells were expected to be from the inner circular layer of smooth muscle which is dominant in the seminal vesicle (Al-Zuhair *et al.*, 1975). Relaxed cells were spindle-shaped and had a length of 156 ± 13 μm and a width of 14 ± 1 μm (n = 44). Aliquots of cells were placed in the recording chamber about 10 min before recording and once the recording had started, solution perfused continuously through the 0.2 ml chamber by gravity flow at about 2 ml min^{-1}. The solutions in the bath were fully exchanged in <1 min. All recordings were carried out at room temperature (21–25°C).

[1] Author for correspondence.

Membrane currents were recorded by the whole-cell configuration of the patch-clamp technique (Hamill *et al.*, 1981). Patch pipettes were pulled from borosilicate glass capillaries (1.5 mm o.d., 0.85 mm i.d.; Clark Electromedical Instruments) and then fire-polished; they had resistances of 1 – 4 MΩ when filled with pipette solution. The patch-clamp amplifier had a 500 MΩ feedback resistor and was constructed in the Department; it was not equipped with series resistance compensation circuitary and series resistance (estimated to $\leqslant$10 MΩ) was thus not corrected for. Voltage-pulses were generated by a stimulator in the amplifier. Signals were filtered by a single-pole low-pass filter at 1 kHz and captured on-line via an analogue-to-digital convertor (Lab-PC, Data Acquisition Board, National Instruments) to an Epson AX2 PC. The software for data capture and analysis was the Voltage Clamp Analysis programme written by J. Dempster (University of Strathclyde). A non-linear curve fitting method was used to fit exponential curves to a selected region of the record. Leakage current was evaluated by measuring the mean inward current when the cell was hyperpolarized from the holding potential.

The dispersion solution contained (mM): NaCl 126, KCl 6.0, HEPES 10, $CaCl_2$ 0.05, and glucose 10, titrated to pH 7.4. The bath solution contained (mM): NaCl 135, KCl 5.0, $CaCl_2$ 1.5, $MgCl_2$ 1.2, HEPES 10, glucose 10, titrated to pH 7.4. For recording Ba^{2+}-current, 1.5 mM $CaCl_2$ was replaced by 10 mM $BaCl_2$, which was expected to block residual K^+-currents and enhance inward current amplitude. The pipette solution for K^+-current recording contained (mM): KCl 130, $MgCl_2$ 2, HEPES 10, Na_2ATP 3, EGTA 0.2 or 10. For Ca channel current recording the KCl was replaced by CsCl and the EGTA concentration was always 10 mM. All pipette solutions were titrated to pH 7.4, passed through a 0.2 μm pore filter (Acrodisc, Gelman Sciences) and stored at −20°C. Tetraethylammonium chloride (TEA), 3,4-diaminopyridine (3,4-DAP), nifedipine, glibenclamide, noradrenaline, $CdCl_2$, HEPES (N-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulphonic acid]) and EGTA (ethylene glycol-*bis*(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid) were from Sigma. Stock solutions for drugs were prepared directly in bath solution or in 100% dimethylsulphoxide (DMSO). The final bath concentrations of DMSO for the highest concentrations of drug used were: 0.5% for glibenclamide, 0.1% for levcromakalim and 3,4-DAP and <0.01% for nifedipine (see Figure legends for further details).

Contraction measurements in isolated seminal vesicles

Seminal vesicles were placed in McEwen's solution (in mM: NaCl 130, KCl 5.6, NaH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.2, glucose 11, and sucrose 13) and incubated at 37°C and bubbled with 5% CO_2 and 95% O_2. From a resting tension of 0.5 g, isometric contractions were recorded with a UF1 transducer and displayed on a Devices MX2 pen recorder. Electrical stimulation was delivered through parallel platinum wire electrodes (2 cm long 0.5 cm apart) in trains of rectangular pulses at 1 min intervals. Stimulation parameters were: train duration 5 s, pulse width 1 ms, frequency 10 Hz and current 1 A. Drugs were added directly to the organ bath in volumes usually not exceeding 5% of bath volume. Experiments were conducted in parallel with time-matched controls using tissue from the same animal adding vehicle instead of drug. Mean and s.e.mean values were calculated for each group of results and inter-

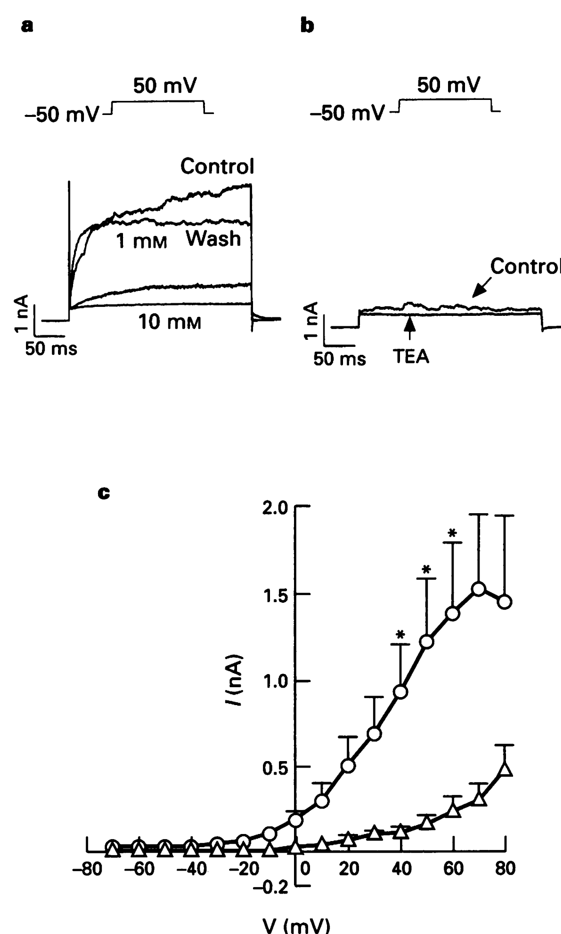

Figure 2 Ca^{2+}-dependence and TEA-sensitivity of sustained outward current. The KCl pipette solution was used. (a) Current elicited by depolarizing to +50 mV from a holding potential of −50 mV with 0.2 mM EGTA in the pipette solution. Typical records are shown for the control period, after bath-application of 1 mM and 10 mM TEA, and after wash-out of TEA. (b) The same protocol was used as in (a) but with 10 mM EGTA in the recording pipette. Records are shown for the control period and after bath-application of 10 mM TEA. (c) The amplitude of outward current at the end of 300 ms-test voltage steps which was inhibited by 1 mM TEA was measured and the mean amplitude of this current determined for recordings made with 0.2 mM (○) and 10 mM EGTA (△) in the recording pipette. The mean (±s.e.mean, n=5 for each) values are plotted against test voltages. *Indicates statistically significant differences.

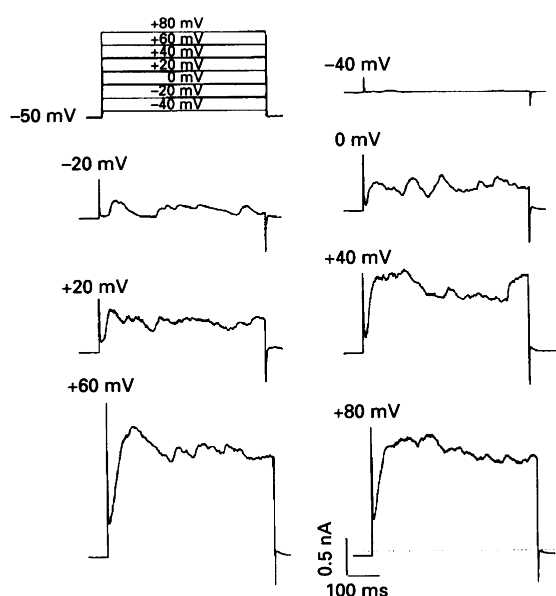

Figure 1 Outward currents recorded from a guinea-pig seminal vesicle smooth muscle cell with the KCl pipette solution containing a low concentration of EGTA (0.2 mM). A series of current records are shown which were elicited by depolarizing from a holding potential of −50 mV to test potentials ranging from −40 mV to +80 mV in 20 mV increments for 500 ms in each case. The broken line on the current trace for the +80 mV test step indicates zero current.

group comparison made with their time-matched controls using Student's unpaired t tests.

Results

Separation of two K-currents using tetraethylammonium and 3,4-diaminopyridine

The study was initiated by looking for ion channel activity induced by depolarization from a holding potential of −50 mV, the resting potential of these cells (Ohkawa, 1982). A KCl-based pipette solution was used and this contained a low concentration of EGTA (0.2 mM) to avoid excessive buffering of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The K^+- and Cl^--equilibrium potentials were calculated to be −82 mV and −2 mV respectively. Application of square depolarizing voltage steps to positive of −30 mV from a holding potential of −50 mV induced an outward current which was sustained for the duration of the 0.5 s test-pulse (Figure 1). The mean amplitude of this current at 0 mV was 320 ± 70 pA (n = 6). In about half of the cells, sporadic transient outward currents with a duration of 0.1–0.2 s were superimposed on the sustained current (Figure 1). The sustained current was reversibly inhibited by 81 ± 8% and 88 ± 7% by bath-applied 1 mM and 10 mM tetraethylammonium (TEA) (n = 6) respectively (Figure 2a) but was unaffected by 3,4-diaminopyridine (3,4-DAP); in the presence of 1 mM 3,4-DAP the sustained current was 3.2 ± 0.59 nA, compared with 3.46 ± 0.79 nA in its absence (n = 4; not shown). This current was also dependent on $[Ca^{2+}]_i$ (Figure 2). To investigate its Ca^{2+}-dependence, outward current amplitudes were compared for cells loaded from the recording pipette with 0.2 mM or 10 mM EGTA. The mean amplitude of current which was inhibited by 1 mM TEA at a test potential of +60 mV (holding potential −50 mV) was 1.39 ± 0.4 nA (n = 6) with 0.2 mM EGTA in the pipette, and 0.23 ± 0.09 nA (n = 6) with 10 mM EGTA in the pipette. We also investigated the effect of nifedipine, an inhibitor of Ca^{2+}-influx through voltage-gated Ca channels (see below). At a test potential of +20 mV the sustained outward current was inhibited by 68 ± 30% by nifedipine (1 μM, n = 5). Thus, the sustained outward current was inhibited if $[Ca^{2+}]_i$ was buffered to a low level and it may have been activated in part by Ca^{2+} moving into the cell via voltage-gated Ca channels.

When the KCl pipette solution included a high concentration of EGTA (10 mM) an additional type of outward current became particularly obvious (Figure 3). This current was activated by depolarizations to positive of −30 mV (more consistently from a holding potential of −60 mV rather than −50 mV) and it was distinguished by its rapid activation and inactivation kinetics, which gave it a transient appearance. The transient current was not affected by bath-applied 10 mM TEA (n = 6; Figure 3a) but was inhibited by 62 ± 9% by 1 mM 3,4-DAP (n = 5; Figure 3b). The slight effect of TEA seen in Figure 3a appeared to be due to inhibition of residual Ca^{2+}-dependent outward current because 10 mM TEA had no effect in addition to that of 1 mM TEA (not shown) and the TEA-sensitive current had a sporadic character. The partial and irreversible inhibition of sustained current induced by 3,4-DAP (Figure 3b) was not observed consistently and may have resulted from an increase in the intracellular concentration of EGTA during this experiment; with 10 mM EGTA in the

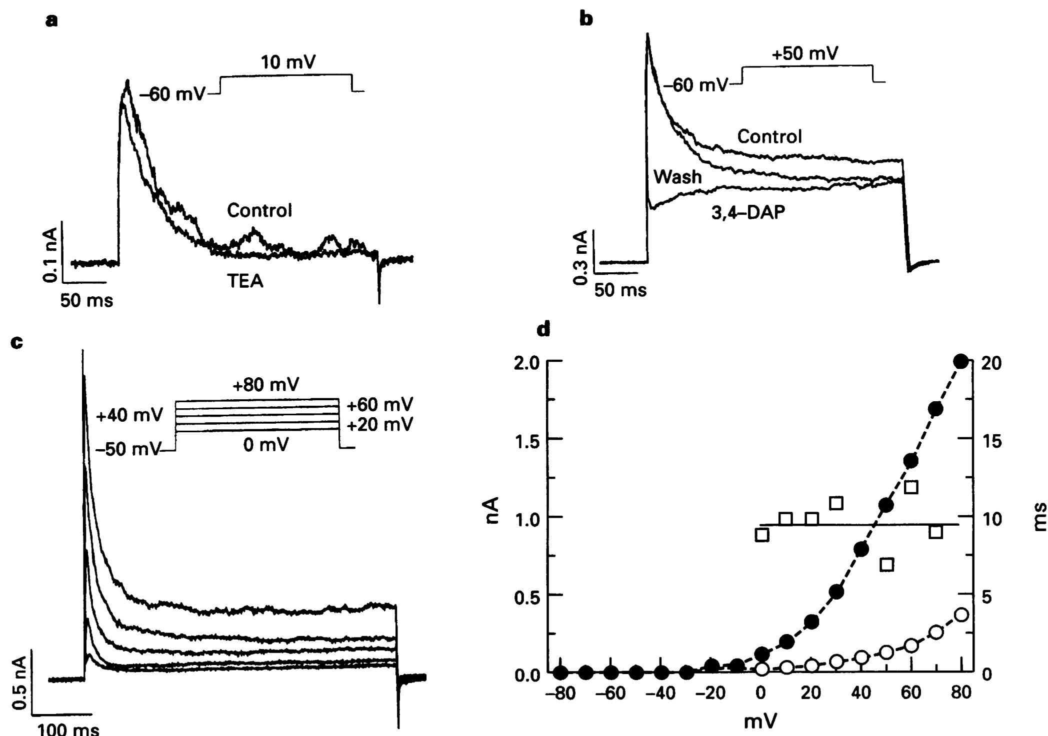

Figure 3 Transient voltage-dependent K-current recorded using a KCl pipette solution containing 10 mM EGTA. (a) Current elicited by depolarizing from a holding potential of −60 mV to +10 mV before (control) and after bath-application of 10 mM TEA. (b) Current elicited by depolarizing from a holding potential of −60 mV to +50 mV before (control), after bath-application of 1 mM 3,4-diaminopyridine (3,4-DAP), and after wash-out of 3,4-DAP. (c) A series of current records elicited by depolarizing from a holding potential of −50 mV to test potentials ranging from 0 mV to +80 mV in 20 mV increments. Tetraethylammonium (TEA, 1 mM) was in the bath solution. A large current reached a peak in 5 to 10 ms (shortly after the capacity current) and then decayed over 100 ms. A smaller sustained outward current was also evident, particularly at the more positive test potentials. (d) Current-voltage relationship for the peak amplitude of the transient outward current (●) and the sustained current at the end of the test voltage step (○); left-hand ordinate scale. Linear leak current was measured at voltages between −50 mV and −80 mV and subtracted. Also plotted are the first time constants for bi-exponential functions fitted to the decay of the currents shown in (c); (□), right-hand ordinate scale.

pipette the sustained current was 0.91±0.24 nA in the presence of 1 mM 3,4-DAP, and 0.89±0.21 nA in its absence ($n=5$; not shown). The transient current did not appear to be Ca^{2+}-dependent because it was large when there was 10 mM EGTA in the recording pipette and its peak amplitude was not inhibited by bath-applied 1 μM nifedipine (not shown). At a test potential of +60 mV the peak current amplitude was reached in 5±1 ms (mean±s.e.mean, $n=6$) and complete current decay occurred in 106±9 ms (Figure 3). The decay of the current elicited by stepping to 0, 10 or 20 mV could be described well by single exponential functions with time constants of 20–30 ms but at more positive test potentials a biexponential function was required to provide an adequate description. The first time constants of the biexponential functions used to describe the actual currents shown in Figure 3c, which were similar to those found for single exponential functions, are plotted in Figure 3d. These values indicate that the rate of inactivation was not voltage-dependent between 0 mV and 80 mV.

Nifedipine-sensitive Ca channel current

Smooth muscle cells express L-type voltage-gated Ca channels and sometimes T-type Ca channels (Kitamura *et al.*, 1989). In seminal vesicle smooth muscle cells, a net inward Ca channel current was evident on depolarization when CsCl replaced KCl in the pipette solution (Figure 4). Depolarizations to voltages positive of −30 mV (holding potential of −60 mV) elicited a Ca channel current which had a maximum amplitude of about 50 pA at a test potential of +30 mV with 1.5 mM Ca^{2+} in the bath solution, compared with 250 pA with 10 mM Ba^{2+} in the bath solution (Figure 4). Ba^{2+}-current elicited by a square depolarizing step to +20 mV reached its peak amplitude in 17±2 ms ($n=10$) and inactivation was slow; the small current decay during a 300 ms-test step to +30 mV (Figure 4b) could be described by a single exponential which had a mean time constant of 149±13 ms ($n=6$). Bath-applied Cd^{2+} (100 μM) abolished the Ba^{2+}-current at all voltages studied (Figure 4d), and the dihydropyridine Ca antagonist, nifedipine, inhibited Ba^{2+}-current elicited by stepping to +20 mV with an IC_{50} of 7 nM (Figure 4d,e). The residual Ba^{2+}-current in the presence of nifedipine decayed more rapidly than the control current (Figure 4d). The mean time constant of single exponential functions fitted to the current decay at +30 mV was reduced by 1 μM nifedipine from 199±47 ms to 30±4 ms. The effect of nifedipine was also voltage-dependent as 10 nM nifedipine inhibited the Ba^{2+}-current by 72±3% at −10 mV and by 15±5% at +50 mV ($n=5$, not shown).

Glibenclamide-induced relaxation and inhibition of ionic currents

During a study of the pharmacology of tension development in the seminal vesicle it was found that glibenclamide, an inhibitor of ATP-sensitive K channels (reviewed by Edwards & Weston, 1993), was a relaxant of contractions induced by noradrenaline or electrical field stimulation (Sadraei, 1994). Figure 5 shows averaged data where contractions were evoked by electrical field stimulation or exogenously applied noradrenaline. Contractions were measured in the presence of increasing concentrations of glibenclamide and compared with time-matched vehicle (DMSO) controls. Glibenclamide in-

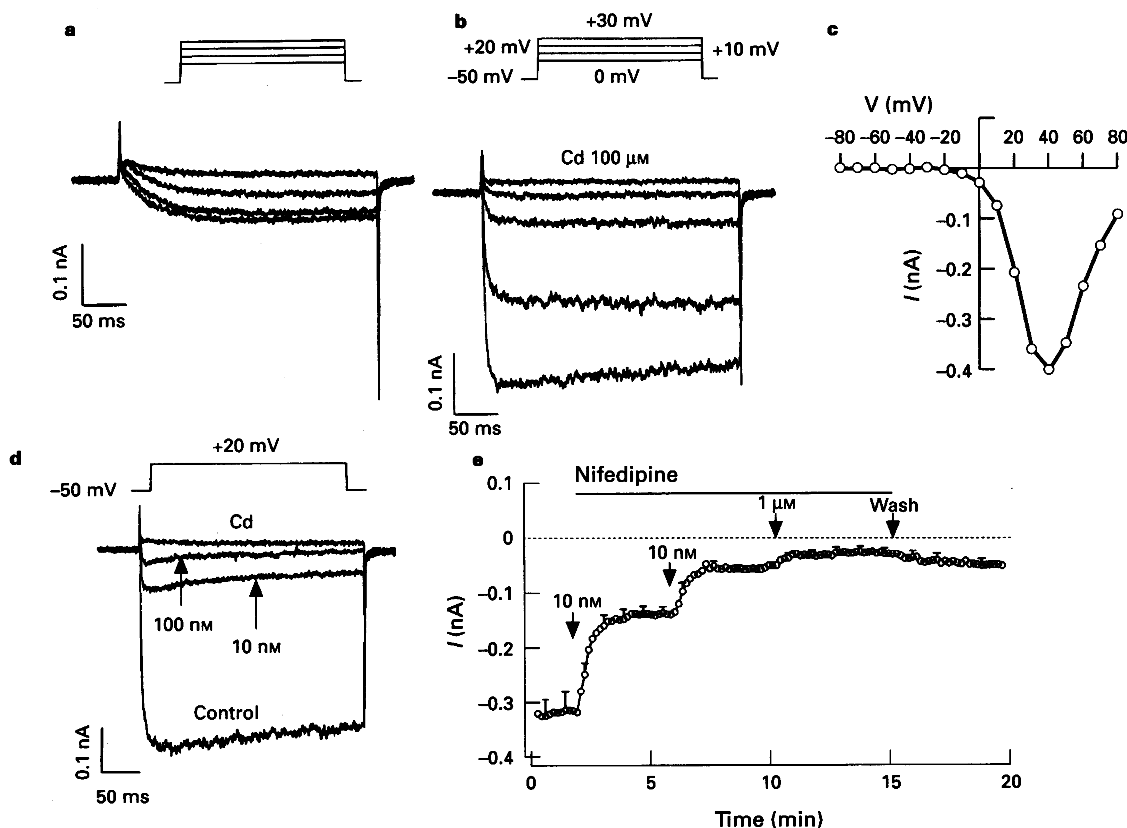

Figure 4 Ca channel currents recorded with the CsCl pipette solution. Currents (in increasing amplitude) were elicited by depolarizing test steps to 0, 10, 20 and 30 mV for 300 ms from a holding potential of −50 mV with 1.5 mM Ca^{2+} (a) and 10 mM Ba^{2+} in the bath solution (b). A current record after the addition of 0.1 mM Cd^{2+} is also superimposed for the 0 mV test voltage step. (c) For a single experiment, peak Ba^{2+}-current amplitude is plotted against test potential after subtraction of Cd^{2+}-insensitive current. (d) Current elicited by depolarizing to +20 mV for 300 ms from a holding potential of −50 mV with 10 mM Ba^{2+} in the bath solution. Control current record and ones in the presence of 10 nM nifedipine, 100 nM nifedipine and 100 μM Cd^{2+} are shown. (e) Plotted against time are the mean peak Ba^{2+}-current amplitudes elicited as in (d) for 5 different cells. Upward standard error bars are shown for every sixth point. Nifedipine was bath-applied as indicated.

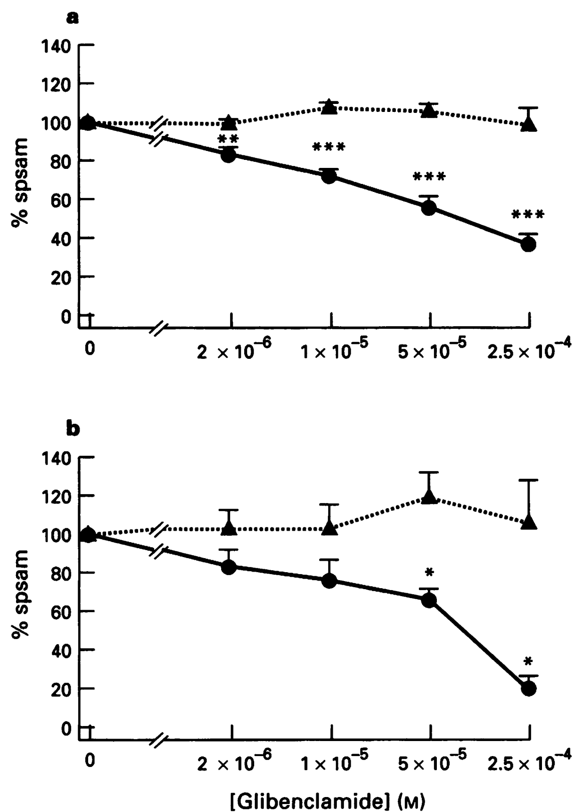

Figure 5 Effect of glibenclamide on whole tissue contraction. Mean amplitudes of contractions induced by electric-field stimulation (a) or by exogenously applied 50 μM noradrenaline (b) are plotted as a percentage of the control contraction in the presence of increasing concentrations of glibenclamide (●) and for the time-matched vehicle (DMSO) controls (▲) ($n=6$ for each). Statistically significant differences from the time-matched control values are indicated: *$P<0.05$; **$P<0.01$; ***$P<0.01$.

hibited contractions, most noticeably at concentrations $\geqslant 10$ μM. In an attempt to elucidate the mechanisms underlying the relaxant action of glibenclamide its effects on ionic currents were tested. The most marked effects of glibenclamide were on the transient voltage-dependent outward current and Ba^{2+}-current. Glibenclamide (500 μM) reduced the transient outward current by $87 \pm 4\%$ ($n=4$, test potential $+60$ mV; Figure 6a,c) and glibenclamide (100 μM) reduced the Ba^{2+}-current by $41 \pm 4\%$ ($n=5$, test potential $+30$ mV; Figure 6b,c). Thus, glibenclamide inhibited the transient outward current, the Ba^{2+}-current and contraction of the whole tissue at $\geqslant 10$ μM. Figure 6c shows, for comparison, the concentration-dependence of the inhibitory effect of glibenclamide on ATP-sensitive K channel activity induced by intracellular guanosine diphosphate in portal vein smooth muscle cells, showing a 4000 times greater sensitivity.

Discussion

Three types of voltage-dependent ionic current were observed in smooth muscle cells freshly isolated from the guinea-pig seminal vesicle. All of them were activated by depolarization to -30 mV or more positive. The first current was an outwardly rectifying TEA-sensitive Ca^{2+}-dependent current and the second a 3,4-diaminopyridine-sensitive A-type current; both appeared to be carried mostly by K^+ ions. The third current was carried by voltage-gated Ca channels which were potently inhibited by nifedipine. We also investigated a relaxant action of high concentrations of glibenclamide ($\geqslant 10$ μM) and suggest that this might occur because voltage-gated Ca channels are inhibited.

The largest ionic current under quasi-physiolgical conditions was a Ca^{2+}-dependent outward current. This current usually had a sustained, smooth appearance but sometimes sporadic transient outward currents were superimposed. It seems likely that it was the large conductance Ca^{2+}-dependent K channel which carried the sustained and sporadic transient outward currents. This suggestion is based on the sensitivities of the current to 1 mM external TEA^+ and high concentrations of intracellular EGTA, and on the insensitivity to 3,4-DAP. These properties compare best with those of the large conductance Ca^{2+}-dependent K channel studied in many other smooth muscle types (reviewed by Bolton & Beech, 1992). The inhibition of sustained current by nifedipine may suggest that Ca^{2+}-influx through L-type Ca channels contributed to the rise in $[Ca^{2+}]_i$ which activated the current, although the possibility that the K channels were inhibited directly by nifedipine cannot be excluded (Terada *et al.*, 1987). The sporadic outward currents were clearly defined in only a few cells and were, therefore, not studied in detail. A similarity to STOCs (Benham & Bolton, 1986) is evident, however, and so they may have resulted from brief, spontaneous releases of Ca^{2+} from intracellular stores. The role of Ca^{2+}-dependent K^+-current in seminal vesicle smooth mucsle cells may be to aid in the repolarization phase of the action potential and/or to provide an inhibitory influence when Ca^{2+} is released from intracellular stores following activation of receptors coupled to phospholipase C by excitatory transmitter substances (e.g. noradrenaline or acetylcholine).

The transient voltage-dependent outward current closely resembled the A-currents of other cell types (reviewed by Rogawski, 1985; Rudy, 1988) and some smooth muscles (reviewed by Bolton & Beech, 1992). All of the characteristics we investigated aligned well with those of the A-current; rapid activation and inactivation kinetics, lack of requirement for $[Ca^{2+}]_i$, and block by 3,4-DAP but not TEA. An additional important feature of A-type K channels is that they become available only for activation at holding potentials hyperpolarized of the resting membrane potential. Although we were unable to construct a steady-state availability curve because the recordings became unstable at potentials negative of -60 mV, the transient current was observed in only about 10% of cells when the holding potential was -50 mV but at least 95% of cells when the holding potential was -60 mV. This observation may be explained if the channels became more available negative of -50 mV, which is the average resting potential of seminal vesicle smooth muscle cells. Ohkawa (1982) observed action potentials with a small after-hyperpolarization and membrane potentials ranging from -30 to -80 mV have been reported (Kajimoto *et al.*, 1971). It is, therefore, conceivable that the A-current does play a functional role in the seminal vesicle, perhaps to regulate action potential frequency.

The Ca channel current in seminal vesicle smooth muscle cells appeared to be carried exclusively by L-type Ca channels. It was very sensitive to inhibition by the dihydropyridine Ca antagonist, nifedipine and its activation and inactivation kinetics were similar to those seen in other smooth muscles where the L-type channel is dominant (reviewed by Kitamura *et al.*, 1989). As might be expected from previous studies on cardiac myocytes (Lee & Tsien, 1983) and vascular smooth muscle cells (Hering *et al.*, 1988) the residual current in the presence of nifedipine inactivated more rapidly than the control current. However, the greater block at more negative voltages is the opposite of that commonly observed with dihydropyridine Ca antagonists in other studies (for example, see Hering *et al.*, 1989). It could be that two types of Ca channel are present in seminal vesicle cells, as has been reported by Neven *et al.* (1993) in cultured rat aortic myocytes. In the aortic myocytes, two types of high voltage-activated Ca-current were observed with different sensitivities to blockade by dihydropyridine Ca antagonists, the more sensitive one activating in a more negative voltage range. In the seminal vesicle smooth muscle cells there was no evidence for T-type Ca channel activity but be-

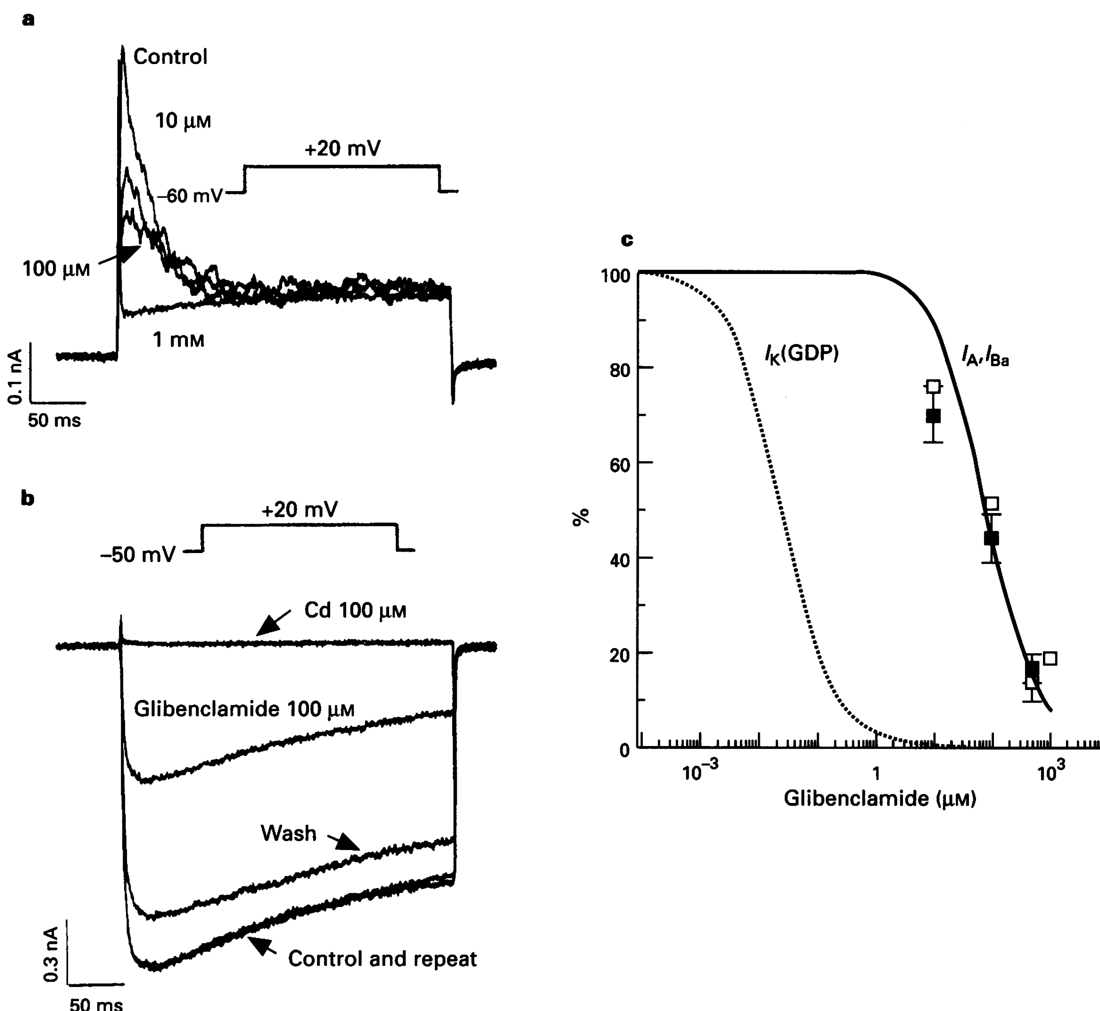

Figure 6 Effects of glibenclamide on ionic currents. (a) Transient voltage-dependent K-current recorded with the 10 mM EGTA/KCl pipette solution. Current was elicited by depolarizing to +20 mV for 300 ms from a holding potential of −60 mV and records are shown for the control period and after bath-application of 0.01, 0.1 and 1 mM glibenclamide. (b) Ca channel currents recorded with 10 mM extracellular Ba^{2+} and using the CsCl pipette solution. Current was elicited by depolarizing to +20 mV for 300 ms from a holding potential of −50 mV and records are shown for the control period (two records are shown to demonstrate the stability of the recording), after bath-application of 0.1 mM glibenclamide, 5 min after wash-out of glibenclamide and in the presence of 0.1 mM Cd^{2+}. (c) Concentration-inhibition curves for the effects of glibenclamide on the transient K^+-current (I_A; □, n=2–4) and the Ba-current (I_{Ba}; ■, n=6). For the transient K^+-current each point is the mean value for two experiments except for the value for 500 μM glibenclamide which is a mean for four experiments. When DMSO was applied before glibenclamide (as a vehicle control) it was found to have no effect on K^+-current but there was a small transient inhibition of the Ba^{2+}-current in some cells; in all cases, sustained effects of glibenclamide were measured. The continuous smooth curves are Hill equations with slope factors of 1. The continuous curve has a mid-point at 80 μM, and the dotted curve has a mid-point at 25 nM. The latter is shown for comparison with the transient K^+-current and Ba^{2+}-current data and is the concentration-inhibition relationship for the effect of glibenclamide on guanosine diphosphate-induced K-current in portal vein smooth muscle cells (from Beech *et al.*, 1993).

cause we could not clamp cells at potential more negative than −60 mV we cannot say that T-type channels would not have become available for activation after severe hyperpolarization. Nifedipine (10 nM to 1 μM) inhibited contractions induced by electrical field stimulation, α-adrenoceptor activation or muscarinic receptor activation (Sadraei, 1994), suggesting that L-type Ca channels are a major Ca^{2+}-influx pathway in seminal vesicle smooth muscle.

The observed relaxant effect of glibenclamide on the seminal vesicle was surprising because as a K channel inhibitor it would be expected to cause contraction, which low concentrations (<1 μM) appear to do in some vascular beds (for example, see Jackson, 1993). It is important to note, however, that the effects of glibenclamide we observed occurred at much higher concentrations than those needed to inhibit ATP-sensitive K channels in other smooth muscles (Figure 6; Beech *et al.*, 1993; Xu & Lee, 1994). We have not observed effects of low concentrations of glibenclamide, perhaps because we have not been able to induce K^+-current with levcromakalim (an activator of ATP-sensitive K channels; reviewed by Edwards & Weston, 1993) under our experimental conditions in single seminal vesicle smooth muscle cells (data not shown).

Our observations with high concentrations of glibenclamide add to a developing picture where >10 μM glibenclamide is not selective for the ATP-sensitive K channel but inhibits a variety of ion channel and other protein activities. We have found inhibition of A-type K^+-current and Ca channel current. We also observed partial inhibition of Ca^{2+}-dependent K^+-current but have not excluded the possibility that this effect resulted from Ca channel inhibition (data not shown). Bian & Hermsmeyer (1994) have recently reported that ⩾10 μM glibenclamide also inhibits Ca channel currents in rat cultured aortic smooth muscle cells. Other studies have shown inhibition by glibenclamide of delayed rectifier K^+-current at ⩾10 μM (Reeve *et al.*, 1992; Crépel *et al.*, 1993; Beech *et al.*, 1993), CFTR-dependent Cl^--current with an IC_{50} of 20 μM (Sheppard & Welsh, 1992), cyclic AMP-dependent protein kinase in rat liver cytosol with an IC_{50} of 0.2 mM (Okuno *et al.*,

1988) and Ca^{2+}-uptake into intracellular stores in smooth muscle at ≥5 μM (Chopra *et al.*, 1992). Drugs which stimulate ATP-sensitive K channels also have effects on other ion channels. Levcromakalim (or cromakalim) inhibits Ca channel current at >10 μM (Okabe *et al.*, 1990; Sadraei, 1994), CFTR-dependent Cl^--current with an IC_{50} of 50 μM (Sheppard & Welsh, 1992) and K-current carried by Kv3.1 stably-expressed in cell-lines with an IC_{50} of 0.2 mM (Grissmer *et al.*, 1994). P1075, a K channel opening drug derived from pinacidil, causes a pronounced inhibition of volume-regulated Cl^- current in the A10 cell-line at 0.3 μM (Holevinsky *et al.*, 1994). Whether or not common mechanisms link any of the various effects of glibenclamide or K channel opener drugs remains to be determined.

From this study it seems likely that large conductance Ca^{2+}-dependent K channels, A-type K channels and L-type Ca channels could underlie the electrical activity seen in the smooth muscle of the seminal vesicle. This profile of ionic currents is similar to that seen in smooth muscle cells from the guinea-pig ureter (Lang, 1989). The ionic currents carried by these channels have pharmacological profiles which are similar to those already described for other smooth muscles but we have found, in addition, that the ionic currents carried by A-type K channels and L-type Ca channels are inhibited by glibenclamide.

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