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ROLE OF K^+ CHANNELS IN SPONTANEOUS ELECTRICAL AND MECHANICAL ACTIVITY OF SMOOTH MUSCLE IN THE GUINEA-PIG MESOTUBARIUM

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SUMMARY

1. The spontaneous electrical and mechanical activity and the efflux rate of $^{86}Rb^+$ in the guinea-pig mesotubarium were studied in the presence of agents interacting with K^+ channels.

2. Tetraethylammonium (TEA, 10 mM) increased the amplitude of the action potentials while having no consistent effect on the frequency or amplitude of spontaneous contractions.

3. 4-Aminopyridine (4-AP, 1–5 mM) caused a graded increase in the duration of the contractions and of the electrical slow waves, and a decrease in the duration of the relaxed period between contractions. At 4 mM-4-AP or more the cell was unable to repolarize from the slow wave and the membrane depolarized to −26 mV from the normal resting potential of −63 mV. The rate of $^{86}Rb^+$ efflux in the presence of 5 mM-4-AP was higher than that at 60 mM-K^+, where the membrane potential is −24 mV.

4. 4-AP (5 mM) evoked a contracture in Ca^{2+}-free solution, containing 1 mM-EGTA, both at the normal $[K^+]_o$ of 5·9 mM and at 60 mM-K^+, suggesting release of intracellular Ca^{2+}.

5. Apamin (0·1–1 μM) and charybdotoxin (1–10 nM), blockers of Ca^{2+}-dependent K^+ channels, were without effects on the spontaneous electrical and mechanical activity.

6. The K^+ channel opener pinacidil (10 μM) inhibited the spontaneous contractions and hyperpolarized the membrane by about 7 mV. The permeability to $^{86}Rb^+$ was increased by a factor of 1·4.

7. It is concluded that different K^+ channels are involved in the generation of spikes and slow waves: one sensitive to TEA and responsible for repolarization of the individual action potential, and another sensitive to 4-AP and responsible for repolarization of the slow wave. The duration of the relaxed period can be influenced by activation of K^+ channels sensitive to pinacidil.

INTRODUCTION

The guinea-pig mesotubarium shows regular spontaneous contractions with a frequency of about 4 h^{-1} and a duration of 5–10 min, alternating with relaxed periods of a duration of several minutes. The contractions are caused by trains of action

potentials on top of slow waves (Hellstrand & Lydrup, 1988). The frequency of spontaneous contractions can be gradedly increased by a slight depolarization of the membrane by stepwise increases in the extracellular K^+ concentration ($[K^+]_o$) from 5·9 to 20 mM. In a $[K^+]_o$ of 25 mM or more a sustained contracture is obtained. When $[Ca^{2+}]_o$ is increased from 2·5 to 10 mM at a $[K^+]_o$ of 5·9 mM, the duration of the relaxed period is increased and the frequency of contractions reduced. The permeability to K^+, estimated by the use of $^{86}Rb^+$ efflux, is increased in parallel with a hyperpolarization of the cell membrane from −63 to −74 mV (Lydrup & Hellstrand, 1990). Both $[Ca^{2+}]_i$ and the efflux rate of $^{86}Rb^+$ decrease during the relaxed period between contractions (Hellstrand & Lydrup, 1988; Himpens, Lydrup, Hellstrand & Casteels, 1990). This suggests a link between $[Ca^{2+}]_i$ and membrane ionic flux, and one hypothesis that could readily explain this relationship is that the increase in $[Ca^{2+}]_i$ during contraction activates Ca^{2+}-dependent K^+ channels, which are then inactivated during the relaxed period as intracellular Ca^{2+} is transported out of the cytoplasm via the cell membrane or taken up into intracellular organelles.

As the repetitive spontaneous activity in the mesotubarium seems to have a relationship to variations in the K^+ permeability, agents interfering with K^+ channels are expected to alter the electrical and mechanical activity. The K^+ channel antagonist tetraethylammonium (TEA) causes depolarization and induces action potentials in the normally quiescent smooth muscle cells of rabbit ear artery (Droogmans, Raeymakers & Casteels, 1977). In the spontaneously active smooth muscle of guinea-pig stomach TEA causes increased amplitude of the action potentials (Ito, Kuriyama & Sakamoto, 1970).

4-Aminopyridine (4-AP) blocks the A-current, a current activated by hyperpolarization, in neurones. Further, 4-AP enhances the mechanical activity in denervated rat portal veins (Leander, Arner & Johansson, 1977) and induces rhythmic contractions in canine tracheal smooth muscle (Imaizumi & Watanabe, 1983). In rat myometrium 4-AP affects the fast component of the outward current, while TEA affects both the fast and the slow components (Mironneau & Savineau, 1980).

Specific toxins are useful to characterize K^+ channels (for review see Moczydlowski, Lucchesi & Ravindran, 1988). Charybdotoxin blocks the large-conductance Ca^{2+}-activated K^+ channel in cultured rat skeletal muscle (Miller, Moczydlowski, Latorre & Phillips, 1985), while the small-conductance Ca^{2+}-activated K^+ channel in the same preparation is blocked by apamin (Blatz & Magleby, 1986). K^+ channels sensitive to these toxins have been identified in smooth muscle cells (Banks, Brown, Burgess, Burnstock, Claret, Cooks & Jenkinson, 1979; Beech & Bolton, 1989*b*).

K^+ channel openers such as pinacidil and cromakalim (BRL 34915) relax different smooth muscle preparations and have been shown to cause hyperpolarization (Hamilton, Weir & Weston, 1986; Weston, Southerton, Bray, Newgreen & Taylor, 1988). These substances have recently been suggested to act on ATP-regulated K^+ channels in smooth muscle cells (Standen, Quayle, Davies, Brayden, Huang & Nelson, 1989). In the present work 4-AP, TEA, apamin, charybdotoxin and pinacidil were used to characterize the influence of different K^+ channels in the generation of spontaneous activity in the mesotubarium. Some of the present results have been reported in preliminary form (Lydrup & Hellstrand, 1989).

METHODS

Virgin female guinea-pigs about 6 months old were used. They were kept two in each cage with food and water *ad libitum*. The lighting conditions were controlled (light:dark = 12:12 h, light on at 6 a.m.). The animals were killed by a blow on the neck. The piece of the mesotubarium originating from the distal part of the uterine horn and inserting near the ovary was used.

The preparations were mounted at 37 °C, adjusted to a preload of about 5 mN and allowed to equilibrate for about 1 h in a Krebs solution containing (in mM): NaCl, 122; KCl, 4·7; $MgCl_2$, 1·2; $CaCl_2$, 2·5; $NaHCO_3$, 15·5; KH_2PO_4, 1·2; glucose, 11·5; equilibrated with 96% O_2–4% CO_2 to give pH 7·3–7·4. High-K^+ solutions were prepared by adding the required amount of 3 M-KCl to the normal solution without correcting for the change in osmolarity. Ca^{2+}-free solution was obtained by omitting $CaCl_2$ and adding 1 mM-ethyleneglycol-bis-(β-aminoethyl ether)-*N,N,N',N'*-tetracetic acid (EGTA).

Isometric experiments were carried out in organ baths of either 50 or 1 ml, and force was measured by Grass FT 03 force transducers. The smaller bath was also used for the $^{86}Rb^+$ efflux measurements. For electrophysiological experiments a chamber of 2·6 ml volume was used (for description see Hellstrand & Lydrup, 1988). Conventional glass microelectrodes with resistance of 30–50 MΩ when filled with 3 M-KCl were used. The criteria for acceptable intracellular recordings were a sharp deflection from the reference level when entering a cell and a distinct change in voltage when leaving the cell. Maintenance of a steady recording for 3 min was required before measurements were begun.

For measurements of $^{86}Rb^+$ efflux the preparations were mounted in a temperature-controlled organ bath of 1 ml volume and allowed to equilibrate for 1 h before loading for 2 h with $^{86}Rb^+$ at 40 μCi ml^{-1} in normal Krebs solution. Inactive solution was then pumped through the chamber for 30 min to remove excess extracellular $^{86}Rb^+$ before collection of samples was started. After the experiment the muscle was dissolved in toluene (Soluene, Packard-Becker B.V., Groningen, The Netherlands). The $^{86}Rb^+$ content in each sample and the amount remaining in the muscle at the end of the experiment were counted in a liquid scintillation counter. The efflux was expressed as fractional release, i.e. the amount of $^{86}Rb^+$ lost during each 2 min period as a fraction of the total amount in the preparation at the start of the period. Changes in $^{86}Rb^+$ permeabilities under conditions when the membrane potential also changed were calculated as described by Jones (1980).

Chemicals used were EGTA, 4-aminopyridine, apamin (Sigma Chemical Company, St Louis, MO, USA), tetraethylammonium chloride (BDH Chemicals Ltd, Poole), propranolol, felodipine (AB Hässle, Mölndal, Sweden), phenoxybenzamine hydrochloride (Smith Kline & French Laboratories Ltd, Welwyn Garden City), atropine sulphate (Boehringer, Ingelheim, Germany), pinacidil (Lövens, Ballerup, Denmark), charybdotoxin (Latoxan, Rosan, France) and $^{86}RbCl$ (New England Nuclear, Du Pont de Nemours, Dreiech, Germany). A stock solution of felodipine (10^{-2} M) was prepared using polyethyelene glycol 400 (Merck, Darmstadt, Germany) and Cremophor RH 410 (BASF, Ludwigshafen, Germany) in proportions 9:1 as a solvent.

Statistical methods

Values are given as means ± S.E.M. with the number of observations in parentheses. For calculating mean values of membrane potential, several cells were used for making an average value from each animal and these average values were then used for calculating a mean value and S.E. of the mean based on the number of animals.

RESULTS

Tetraethylammonium

TEA (10 mM) was added to twelve muscle strips in organ baths. There was no consistent mechanical effect even if an increased frequency of contractions, an increased or decreased duration of contractions, or a decreased duration of the relaxed period could be seen in some preparations. In two muscle strips a marked

increase in the duration of the contraction at the expense of a shorter relaxed period was observed. One example of the subtle mechanical effect of TEA is shown in Fig. 1*A*. Addition of an excessive concentration (100 mM) of TEA increased the duration of contractions severalfold but the muscle was still able to relax.

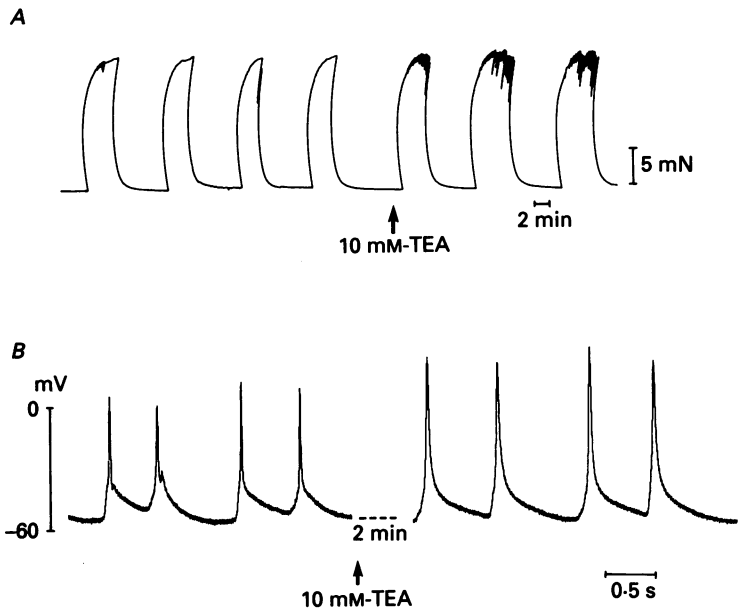

Fig. 1. *A*, isometric recording showing the effect of 10 mM-TEA on the spontaneous contractions of one mesotubarium strip. *B*, microelectrode recording showing the electrical effect of addition of 10 mM-TEA.

In spite of the absence of major effects of TEA on the mechanical activity, the action potentials in the presence of 10 mM-TEA always showed an increased amplitude (Fig. 1*B*). In three muscle strips the effect of 10 mM-TEA on the resting membrane potential was investigated. In normal Krebs solution, before the addition of TEA, the membrane potential was $-64{\cdot}3 \pm 0{\cdot}4$ mV and after the addition of TEA $-65{\cdot}3 \pm 1{\cdot}5$ mV.

4-Aminopyridine

4-Aminopyridine (1–4 mM) added to normal Krebs solution increased the duration of the spontaneous contraction period in a concentration-dependent manner and decreased the duration of the relaxed period between contractions. At a concentration of 4 mM-4-AP or more no spontaneous relaxation occurred (Fig. 2). After wash-out of 4-AP the muscle relaxed and normal spontaneous contractile activity was resumed. The effect of 4-AP was not influenced by addition of a combination of propranolol (10^{-5} M), phenoxybenzamine (10^{-5} M) and atropine (10^{-5} M) to block possible effects of endogenous transmitter release.

4-Aminopyridine (5 mM) was able to evoke contraction in the presence of the dihydropyridine Ca^{2+} antagonist felodipine or in the absence of extracellular Ca^{2+} as

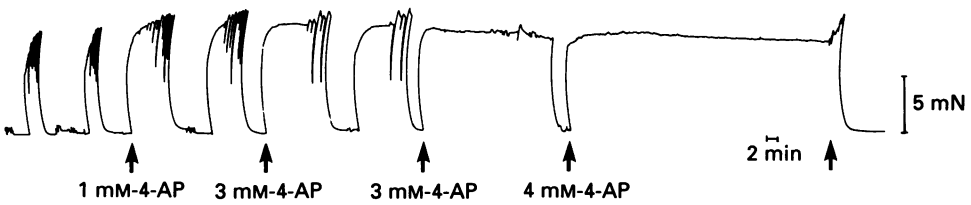

Fig. 2. Isometric recordings showing the effect on the spontaneous contractions of one mesotubarium preparation of addition of 1–4 mM-4-AP.

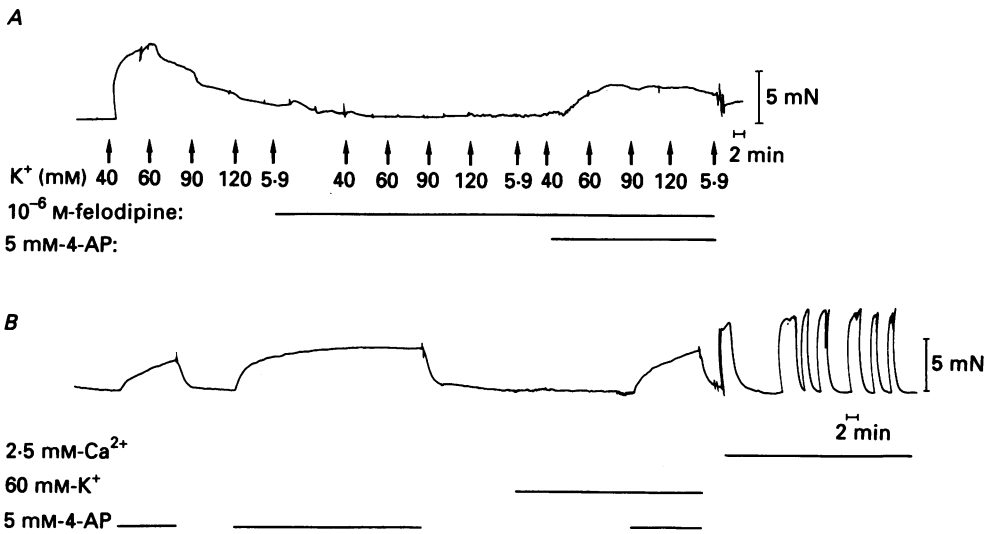

Fig. 3. *A*, isometric recording showing three successive series of depolarizations by cumulative addition of K^+: in the absence of drugs, in the presence of 10^{-6} M-felodipine, and in the presence of both 10^{-6} M-felodipine and 5 mM-4-AP. *B*, the effect of adding 5 mM-4-AP to a mesotubarium strip in a Ca^{2+}-free solution containing 1 mM-EGTA. Ca^{2+} was added at the end of the record. $[K^+]_o$ was either 5·9 or 60 mM as indicated.

illustrated in Fig. 3*A* and *B*. In Fig. 3*A* three successive series of depolarizations of one mesotubarium strip are shown. In the absence of drugs, force decreases with increasing degree of depolarization, a phenomenon which has been described previously (Lydrup & Hellstrand, 1990). In the presence of 1 μM-felodipine, no force is generated at any $[K^+]_o$. Addition of 5 mM-4-AP in the presence of 1 μM-felodipine causes development of force, which is better maintained at higher degrees of depolarization than that generated in the absence of drugs.

In Ca^{2+}-free medium, containing 1 mM-EGTA to chelate trace amounts of Ca^{2+}, addition of 5 mM-4-AP evoked a contracture both at the normal $[K^+]_o$ of 5·9 mM and at 60 mM-K^+ (Fig. 3*B*). After development of a contracture at a $[K^+]_o$ of 60 or 90 mM in the presence of 2·5 mM-extracellular Ca^{2+}, addition of 5 mM-4-AP caused a further increase in force (record not shown).

The effects of 4-AP on membrane activity recorded from one single cell are shown in Fig. 4. Panel *Aa* shows spontaneous action potentials before addition of 4-AP.

Panel *Ab*, obtained 1·5 min after addition of 4 mM-4-AP, shows the onset of a sustained depolarization which is maintained as long as 4-AP is present. Six minutes after start of wash-out of 4-AP (panel *Ac*) short periods of repolarization reappear and 10 min after wash-out (panel *Ad*) trains of action potentials are present.

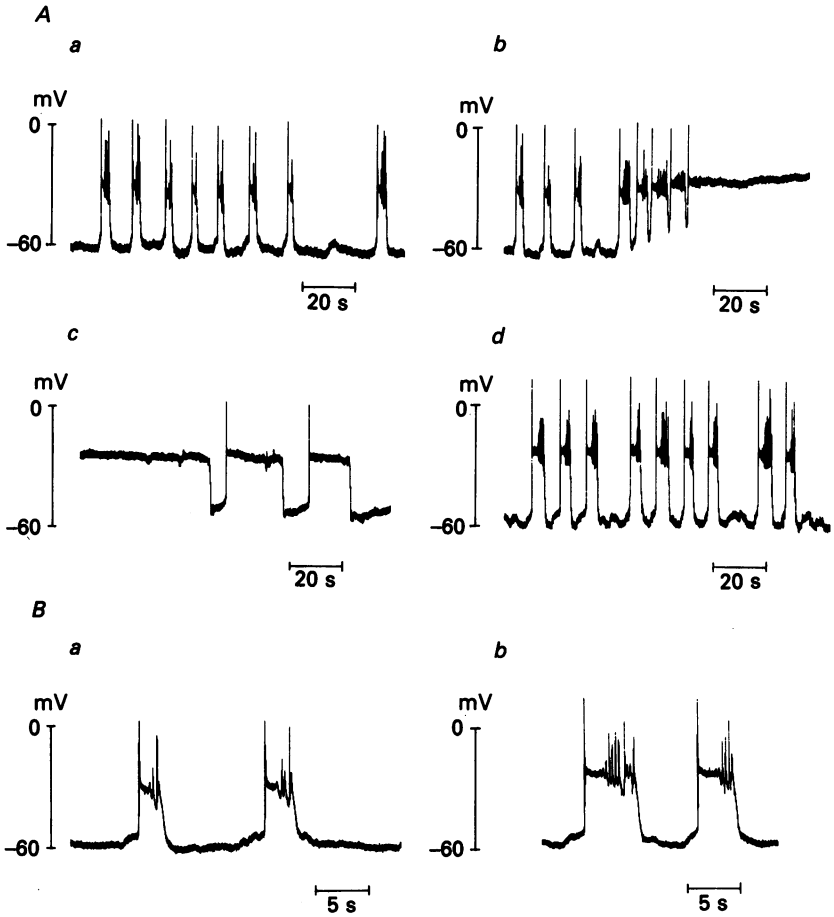

Fig. 4. Electrical recording from one cell showing the onset of action of 4 mM-4-AP and the return to normal activity after wash-out of 4-AP. *Aa*, normal solution before addition of 4-AP; *Ab*, 1·5 min after addition of 4 mM-4-AP; *Ac*, 6 min after start of wash-out of 4-AP; *Ad*, 10 min after start of wash-out of 4-AP. *Ba*, *Aa* on an expanded time scale; *Bb*, *Ad* on an expanded time scale.

However, recording on a faster time scale shows that the duration of the slow waves is longer 10 min after wash-out of 4-AP (panel *Bb*) than in the control period before addition of the drug (panel *Ba*). Likewise, the membrane potential during the slow wave, after repolarization from the first spike, is less negative (arrows in panels *Ba* and *Bb*).

The membrane potential during the sustained depolarization in the presence of 5 mM-4-AP was $-26 \pm 1{\cdot}0$ mV ($n = 5$). In a lower concentration of 4-AP (1 mM), not

giving rise to a sustained depolarization, the membrane potential during the slow wave, after the first spike (cf. arrows in Fig. 3*B*), was less negative than in normal solution: $-29{\cdot}2 \pm 1{\cdot}0$ mV ($n = 5$) *vs.* $-35{\cdot}8 \pm 0{\cdot}8$ mV ($n = 8$).

Fractional release of $^{86}Rb^+$ was increased during the contraction evoked by addition of 5 mM-4-AP (Fig. 5). The mean value of efflux rate in the presence of

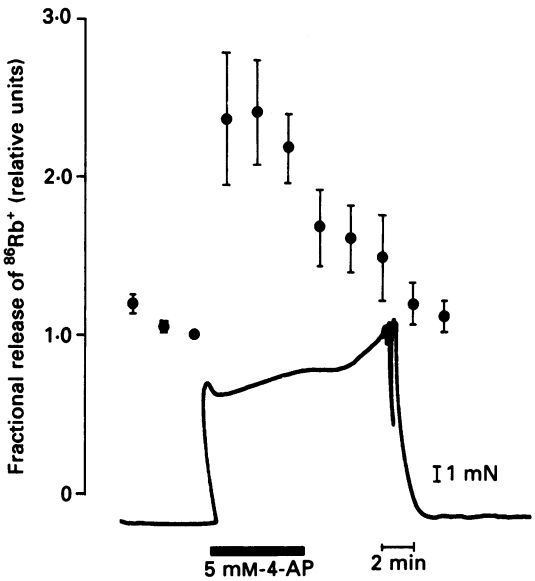

Fig. 5. Fractional release of $^{86}Rb^+$ and the effect of 5 mM-4-AP. Schematic drawing indicates the mechanical effect of 4-AP. Data obtained during the contraction, both in the presence of 4-AP and during the contraction after wash-out of 4-AP, is from six experiments, and data during the relaxed period are from four experiments.

4-AP was $2{\cdot}3 \pm 0{\cdot}1$ ($n = 6$) times the value in the relaxed muscle prior to addition of 4-AP. For calculation of the relative change in permeability caused by the addition of 4-AP, correction for the change in the membrane potential is necessary, since the flux rate is dependent on the electrochemical gradient at a given value of permeability. Application of the calculation described by Jones (1980) with a membrane potential of -26 mV in the presence of 5 mM-4-AP, *vs.* -63 mV in the relaxed muscle in normal solution, gives a relative permeability of 0·99 in the presence of 4-AP. In Table 1 fractional release of $^{86}Rb^+$, permeability and force during depolarization by increasing $[K^+]_o$ in the presence and absence of 4-AP are shown. The permeability and the amplitude of contraction at all $[K^+]_o$ are higher in the presence than in the absence of 4-AP. With increasing degree of depolarization the permeability decreases. In the absence of 4-AP this correlates with a decrease in force whereas in its presence force is nearly the same at all $[K^+]_o$.

Apamin

Addition of 0·1–1 μM-apamin had no pronounced effect on the contractile activity of the mesotubarium in normal solution. All preparations were able to relax and to contract in the presence of apamin. Minor changes in the spontaneous activity were

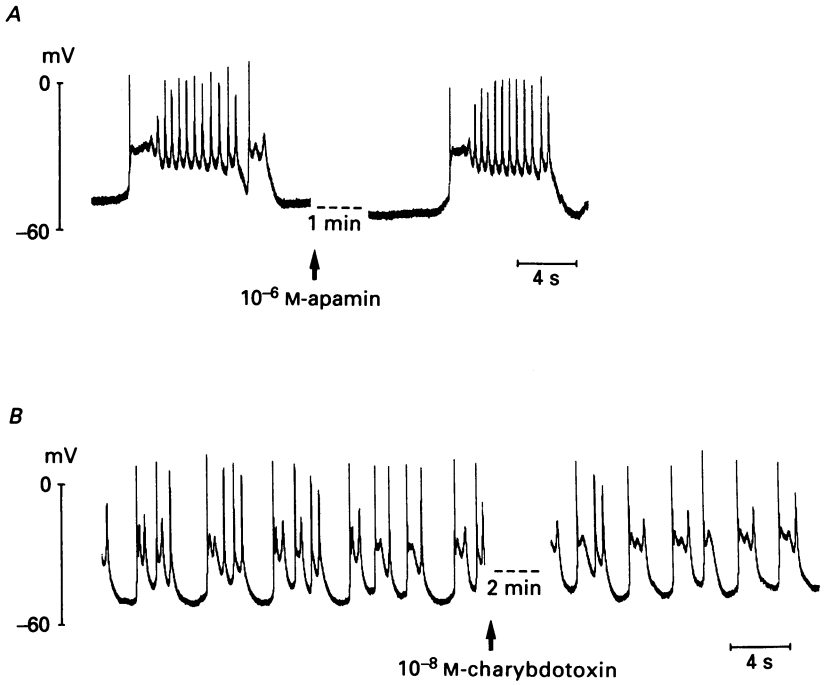

Fig. 6. *A*, electrical recording from one cell before and 2 min after addition of 10^{-6} M-apamin. *B*, electrical recording from one cell (from another animal) before and 1 min after addition of 10^{-8} M-charybdotoxin.

TABLE 1. Fractional release of $^{86}Rb^+$, permeability and force of the guinea-pig mesotubarium during a series of depolarizations with 60, 90 and 120 mM-K^+ in the absence and presence of 5 mM-4-AP

	−4-AP ($n = 6$)			+4-AP (5 mM; $n = 2$)		
$[K^+]_o$ (mM)	60	90	120	60	90	120
Force	0·58 ± 0·15	0·40 ± 0·13	0·32 ± 0·09	0·89	0·80	0·89
$^{86}Rb^+$ efflux rate	1·52 ± 0·15	1·50 ± 0·11	1·59 ± 0·11	2·93	2·67	2·61
Permeability	0·61	0·54	0·50	1·17	0·96	0·81

Fractional release and permeability are expressed relative to those in the relaxed muscle strip in normal solution before addition of 4-AP, while force is expressed in units of force at 40 mM-K^+ without 4-AP. For these calculations membrane potentials of −24, −19 and −12 mV in 60, 90 and 120 mM-K^+, respectively, were used (Lydrup & Hellstrand, 1990).

found in a few preparations after the addition of 1 μM-apamin; these consisted of increased or decreased frequency of contractions and shorter duration of contractions. However, the spontaneous activity of the mesotubarium is very sensitive to changes in the environment, and interventions such as exchange of solution easily influence the activity.

There was no effect on slow waves or action potentials by addition of 1 μM-apamin (Fig. 6*A*). In the presence of 1 μM-apamin the resting membrane potential was $-62{\cdot}4 \pm 0{\cdot}8$ mV ($n = 6$) and in the absence of apamin $-62{\cdot}8 \pm 1{\cdot}0$ mV in the same preparations.

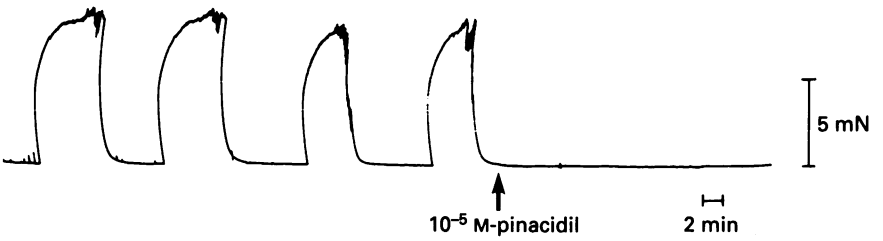

Fig. 7. Isometric recording showing the mechanical effect of 10^{-5} M-pinacidil added to normal Krebs solution during the relaxed period between spontaneous contractions.

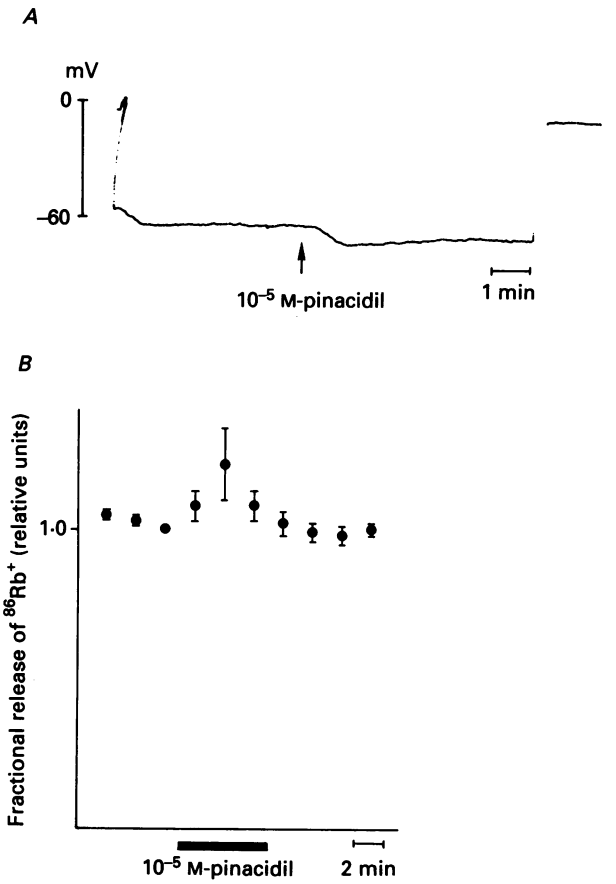

Fig. 8. The effect of 10^{-5} M-pinacidil on membrane potential (*A*) and fractional release of $^{86}Rb^+$ ($n = 5$) (*B*). Values normalized to the 2 min period prior to addition of pinacidil.

Charybdotoxin

No effect on the frequency or general appearance of the spontaneous contractions was observed after addition of 1 or 10 nM-charybdotoxin. The slow waves and action potentials were of the same size and amplitude as before addition of the toxin

(Fig. 6*B*). In the presence of 10 nM-charybdotoxin the resting membrane potential was $-60{\cdot}3 \pm 0{\cdot}7$ mV ($n = 3$) and in the absence of charybdotoxin -60 and -62 mV in two of these preparations.

Pinacidil

Addition of 10 μM-pinacidil abolished the spontaneous contractions in normal solution (Fig. 7). In ten muscle strips the duration of relaxation after addition of 10 μM-pinacidil varied between 16 and 86 min with a mean duration of 42 ± 7 min. In six of these strips no spontaneous contractions had appeared when the experiments were terminated, whereas in four strips spontaneous contractions appeared after 43 ± 15 min in the continued presence of pinacidil. In parallel with the relaxing effect, pinacidil caused a hyperpolarization of $7{\cdot}0 \pm 0{\cdot}6$ mV ($n = 8$) (Fig. 8*A*). The relaxing effect was accompanied by a transient increase in fractional release of $^{86}Rb^+$ by a factor of 1·22 (Fig. 8*B*). When taking the hyperpolarization of 7 mV into account this corresponds to an increased permeability by a factor of 1·44.

4-Aminopyridine (5 mM) added to the relaxed muscle in the presence of 10 μM-pinacidil caused a sustained contracture in the same manner as in normal solution without pinacidil. The effect of 10 mM-TEA in the presence of pinacidil was variable, as in the absence of pinacidil. No effect was observed after addition of 1 μM-apamin or 10 nM-charybdotoxin to a muscle relaxed by 10 μM-pinacidil.

DISCUSSION

The different effects of TEA, 4-AP and pinacidil on the spontaneous activity of the mesotubarium suggest that several K^+ channels are involved in the generation of slow waves and spikes. The effect of TEA on the single spike in the mesotubarium is consistent with its effect in guinea-pig stomach (Ito *et al.* 1970) and taenia coli (Suzuki, Nishiyama & Inomata, 1963) smooth muscle. The effect of TEA seems to be more marked with respect to the amplitude of action potentials than with respect to the pattern of spontaneous activity. The amplitude of contractions in the mesotubarium was usually not increased by TEA, unlike the case in for example guinea-pig stomach smooth muscle (Ito *et al.* 1970). This may be due to the specific pattern of activity in the mesotubarium, involving trains of repetitive action potentials lasting several minutes, with little variation in maximal force between long and short trains. Thus the contractions may represent maximal tetanic activation, and an additional inflow of Ca^{2+} due to spikes of higher amplitude would not appreciably increase the amplitude of contraction. This is also suggested by the small influence of increased $[Ca^{2+}]_o$ on the amplitude of spontaneous contractions, although it considerably increases $[Ca^{2+}]_i$ (Himpens *et al.* 1990; Lydrup & Hellstrand, 1990). The inconsistent effects of TEA on frequency and duration of contractions in the mesotubarium may reflect the general lability of spontaneous activity in this preparation. Differences in the response with hormonal phase cannot be excluded since the animals in the present study were not taken during a defined phase of the oestrus cycle. However, it has previously been found that neither the pattern of mechanical activity nor the resting membrane potential differ between the phases of pro-oestrus, oestrus and di-oestrus (Hellstrand & Lydrup, 1988).

The strong effect of 4-AP on the spontaneous activity of the mesotubarium is compatible with the finding of a K^+ current highly sensitive to 4-AP in the rabbit pulmonary artery (Okabe, Kitamura & Kuriyama, 1987). In rat myometrium, 4-AP has a weaker effect than TEA on the outward current, affecting only the fast component, while TEA affects both the slow and the fast components (Mironneau & Savineau, 1980). In single cells from rabbit portal vein the outward current activated upon depolarization from −70 mV consists of two components (Beech & Bolton, 1989*b*). One component is inhibited by 4-AP but insensitive to TEA, charybdotoxin and apamin, and activated at a more negative threshold than the second component, which is sensitive to TEA and charybdotoxin but insensitive to 4-AP and apamin. One additional 4-AP-sensitive current has been revealed in rabbit portal vein and guinea-pig ureter on depolarization from holding potentials more negative than the resting potential (Beech & Bolton, 1989*a*; Lang, 1989). This latter channel was compared to the A-current in neurones which is thought to be responsible for regulating repetitive firing (Connor, 1978). The fact that effects of 4-AP in the mesotubarium were seen in a more negative voltage range than those of TEA is compatible with these findings.

Apamin is a blocker of small-conductance Ca^{2+}-dependent K^+ channels in rat skeletal muscle (Blatz & Magleby, 1986), and has been shown to inhibit hyperpolarization induced by ATP or adrenaline in guinea-pig taenia coli (Maas & Den Hertog, 1979), to cause a small depolarization (3 mV), and to induce spike activity (Maas, Den Hertog, Ras & Van den Akker, 1980). Apamin was found to have no effect on either the electrical or mechanical activity of the mesotubarium. It should be noted that none of the current components in isolated smooth muscle cells of rabbit portal vein was sensitive to apamin (Beech & Bolton, 1989*a*, *b*), consistent with the absence of an effect of apamin on the electrical activity of the intact mesotubarium. We have also tested apamin on the rat portal vein. In a concentration of 1 μM it was without effect on the mechanical spontaneous activity (author's unpublished observations).

Charybdotoxin blocks Ca^{2+}-dependent K^+ channels of a large conductance in rat skeletal muscle cells (Miller *et al.* 1985). Like apamin, charybdotoxin was without effects on the spontaneous mechanical and electrical activity of the mesotubarium. Three different blockers of Ca^{2+}-dependent K^+ channels, TEA, apamin and charybdotoxin, have thus been shown to be without major effects on the spontaneous contractile activity of the mesotubarium. Ca^{2+}-dependent K^+ channels have been universally found in a wide variety of cells (e.g. Marty, 1981; Benham, Bolton, Lang & Takewaki, 1986) and in the mesotubarium indirect evidence for the existence of this type of channel has been obtained (Lydrup & Hellstrand, 1990). However, the present experiments do not give evidence supporting that activation/inactivation of this type of channel is responsible for the long relaxed period characteristic of the spontaneous contractile activity in the mesotubarium, unless Ca^{2+}-dependent K^+ channels in the mesotubarium are insensitive to the drugs used. Instead, a current sensitive to 4-AP seems to be involved in the maintenance of the relaxed period between contractions. A continual decrease in K^+ permeability during the relaxed period is suggested by the decrease in $^{86}Rb^+$ efflux rate (Hellstrand & Lydrup, 1988).

Several of the present findings suggest that 4-AP, in addition to its effect on

membrane activity, causes release of Ca^{2+} from intracellular stores: addition of 4-AP to a Ca^{2+}-free medium containing EGTA evokes force development, the amplitude of a contracture elicited by high-K^+ solution could be further increased by addition of 4-AP, and force is generated in the presence of felodipine after depolarization by high-K^+ solution containing 4-AP. Interestingly, Savage (1989) has recently shown that contractions evoked by 4-AP in frog rectus abdominis muscle are relatively resistant to depletion of extracellular Ca^{2+} but blocked by verapamil and Mn^{2+}. Addition of 5 mM-4-AP to the normal solution causes a sustained depolarization to about the same membrane potential as that at 60 mM-K^+. However, the ensuing contraction has a greater amplitude when 4-AP is present, which also suggests Ca^{2+} release from intracellular stores. The $^{86}Rb^+$ efflux rate in the presence of 5 mM-4-AP was found to be higher than that at 60 mM-K^+. This could be explained by the presumably higher intracellular Ca^{2+} concentration in 4-AP than at 60 mM-K^+, which would increase the current through Ca^{2+}-dependent K^+ channels. This could be one clue to the role of such channels in the mesotubarium (cf. above). We have previously shown that a substantial component of the $^{86}Rb^+$ efflux in the depolarized mesotubarium is dependent on Ca^{2+} (Lydrup & Hellstrand, 1990). An increase in the Ca^{2+}-dependent $^{86}Rb^+$ efflux in the presence of 4-AP could conceivably mask a blocking of the 4-AP-sensitive current component. The $^{86}Rb^+$ efflux rate in the depolarized muscle was higher in the presence than in the absence of 4-AP. In 60 mM-K^+ and above no spikes are present (Lydrup & Hellstrand, 1990) and it was verified in two control experiments that the addition of 4-AP does not substantially alter the membrane potential in 60 mM-K^+. Thus the permeability to $^{86}Rb^+$ is increased after the addition of 4-AP, which presumably is due to higher $[Ca^{2+}]_i$ as inferred from the larger amplitude of contraction.

Besides the two kinds of K^+ channels that might be involved in the repolarization of the spike and the slow wave, respectively, a third type of channel, sensitive to pinacidil, seems to be able to increase the duration of the relaxed period. The existence of ATP-dependent K^+ channels has been suggested to be the basis for generation of cyclic activity in pancreatic islet cells (Cook & Hales, 1984) and their presence in smooth muscle cells has now also been demonstrated (Standen *et al.* 1989). Pinacidil has been suggested to open ATP-dependent K^+ channels in rat and rabbit mesenteric arteries (Standen *et al.* 1989) and in guinea-pig cardiac myocytes (Escande, Thuringer, Courteix, Laville & Cavero, 1989). Pinacidil inhibits the generation of spontaneous contractions in the mesotubarium and causes hyperpolarization and it is thus probable that ATP-dependent K^+ channels exist in the mesotubarium and that activation of these channels is the basis for the relaxing effect of pinacidil. The hyperpolarization induced by pinacidil in the mesotubarium is small compared to that reported in for example the rat portal vein (Hamilton, Weir & Weston, 1986). A hyperpolarization of the same small magnitude has also been reported from the rat myometrium by Hollingsworth, Amédée, Edwards, Mironneau, Savineau, Small & Weston (1987). The ability of some strips to contract in the presence of pinacidil could be related to the rather weak hyperpolarization observed in the mesotubarium.

The type of spontaneous activity found in the mesotubarium, involving trains of slow waves and spikes separated by relatively long quiescent periods, may represent

a general pattern found in many kinds of smooth muscle but quantitatively expressed in a wide variety of ways. The particularly clear separation in the mesotubarium of the different kinds of cyclic phenomena in the spontaneous activity offers opportunity to distinguish the functional role of different membrane channels. In this work it is shown that different K^+ channels, sensitive to TEA and 4-AP, affect the action potential and the slow wave, respectively, and that activation of ATP-dependent K^+ channels by pinacidil causes hyperpolarization and sustained relaxation in the mesotubarium. The absence of effect of different blockers of Ca^{2+}-dependent K^+ channels (TEA, apamin and charybdotoxin) suggests that this latter type of channel is not involved in the generation of spontaneous contractile activity of the mesotubarium, even though indirect evidence for their existence in the mesotubarium has been obtained based on effects of increased $[Ca^{2+}]_i$ on $^{86}Rb^+$ efflux (Lydrup & Hellstrand, 1990; present study). Instead, activation of a K^+ channel sensitive to 4-AP seems to be responsible for the termination of the contraction. Inactivation of this channel could be involved in the determination of the duration of the relaxed period between contractions. Probably other mechanisms are also involved, such as cyclic activation of the Na^+–K^+ pump. This could be mediated via changes in $[Na^+]_i$, possibly through the Na^+–Ca^{2+} exchange mechanism. Together with an inactivation of a K^+ current these changes could lead to the initiation of a new contraction.

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