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INWARD RECTIFICATION IN FRESHLY ISOLATED SINGLE SMOOTH MUSCLE CELLS OF THE RABBIT JEJUNUM

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SUMMARY

1. Single smooth muscle cells were obtained by collagenase digestion of longitudinal muscle of rabbit jejunum. Membrane potential or current under voltage clamp was recorded by patch-clamp technique in the whole-cell recording mode at 22–25 °C.

2. At a membrane potential of −50 mV small hyperpolarizing current pulses produced electrotonic potentials which asymptotically approached a steady-state size and did not 'sag'. Stronger hyperpolarization resulted in a 'sag' of the electrotonic potential. Under voltage clamp an inward current, i, was activated in the range −60 to −110 mV.

3. This current had an equilibrium potential of $-24{\cdot}5 \pm 3{\cdot}5$ mV which was shifted negatively by reducing the sodium concentration, and positively by raising the potassium concentration of the bathing solution.

4. This current was blocked by caesium (1 mM) but less affected by barium ions up to 10 mM.

5. The time course of i upon stepping into its activation range was accelerated by increasing negativity and following an initial short delay could be described by a single exponential with a time constant in the range 60 s (−60 mV) to 1 s (−130 mV).

6. It is concluded that these jejunal cells possess a current to which sodium and potassium ions make a contribution which is responsible for the inward rectification they show upon hyperpolarization. This current is activated in a range which would allow it to contribute to the slow potential changes shown by longitudinal jejunal muscle.

INTRODUCTION

Electrotonic potentials recorded in response to hyperpolarizing currents applied extracellularly to various smooth muscle preparations do not asymptotically approach some steady-state value but reach a peak and then decline in size with time (guinea-pig taenia caeci, Tomita, 1966; Bülbring & Tomita, 1967; longitudinal muscle of the guinea-pig ileum, Bolton, 1972; mouse vas deferens, M. E. Holman, unpublished observations; single cells of the toad stomach, Sims, Singer & Walsh, 1985). The decline of the electrotonic potential after reaching a peak size ('sag') suggests that at hyperpolarized potentials a time-dependent current is switched on (or off) which gives rise to the rectification which is exhibited. The repolarization

phase of the electrotonic potential was often followed by a 'rebound' depolarization which overshoots the resting membrane potential (Bolton, 1972; Sims *et al.* 1985).

Stimulation at high frequency of the non-adrenergic, non-cholinergic inhibitory motor neurones that innervate the guinea-pig small intestine (Holman & Weinrich, 1975), taenia caeci (Bennett, 1966) or the avian gizzard (Bennett, 1969) produces a series of partially fused inhibitory junction potentials but this hyperpolarization is not invariably well maintained. Partial repolarization which occurs during repeated nerve stimulation is often followed by a rebound depolarization which can trigger a burst of action potentials and contraction (rebound excitation, Bennett, 1966; Campbell, 1966; Daniel, Helmy-Elkholy, Jager & Kannan, 1983). This partial repolarization during nerve stimulation and rebound depolarization has in part been explained by the run-down of the store of inhibitory transmitter (Holman & Weinrich, 1975) or by the activation of slow, excitatory substance P receptors during repetitive stimulation (Neil, Bywater & Taylor, 1983) but could equally well be due to the same process causing 'sag' of the hyperpolarizing electrotonic potential.

Hyperpolarizing electrotonic potentials in a number of electrophysiological preparations show similar inward rectification to current injection. In frog muscle cells (Standen & Stanfield, 1978), starfish egg cells (Hagiwara & Takahashi, 1974) or olfactory cortex neurones (Constanti & Galvan, 1983) the inward rectification their membrane shows is known to be due to the activation of a potassium-specific conductance. However, the inward rectification recorded in cardiac sino-atrial node and Purkinje cells (DiFrancesco, 1985) or mouse sensory neurones (Mayer & Westbrook, 1983) has been postulated to result from the activation of a conductance passing both sodium and potassium ions.

The development of whole-cell voltage-clamp recordings from single smooth muscle cells (Walsh & Singer, 1981; Bolton, Lang, Takewaki & Benham, 1985) now provides a means of investigating the conductance change underlying the inward rectification recorded in many smooth muscle preparations without any of the problems which beset the application of voltage-clamp techniques to strips of whole smooth muscle preparations (Bolton, Tomita & Vassort, 1981). We report here that the inward current activated upon hyperpolarization of jejunal cells of the rabbit represents the activation of a cation-specific conductance change perhaps similar to that in cardiac cells and sensory neurones.

METHODS

Single smooth muscle cells were separated from the longitudinal muscle of rabbit jejunum by three 10 min incubations in a calcium-free saline containing collagenase and trypsin inhibitor (Benham, Bolton, Lang & Takewaki, 1985, 1986). Experiments were performed in the 'whole-cell' recording mode using low-resistance patch pipettes (2–5 MΩ) and a List EPC-5 patch-clamp apparatus (Hamill, Marty, Neher, Sakmann & Sigworth, 1981).

The bath solution contained (mM): NaCl, 126; KCl, 6; sodium HEPES, 10; NaH_2PO_4, 1·2; $MgCl_2$, 1·2; $CaCl_2$, 1·5–2.5; and glucose, 11 mM. The solution was buffered to pH 7·2. The pipette contained the same saline except NaCl was 6 mM, KCl 126 mM, and 0·7–10 mM-EGTA was added. All experiments were carried out at room temperature (20–25 °C). Data were recorded on FM tape and analysed using a B.B.C. microcomputer after digitization with a Cambridge Electronic Design 1401 intelligent laboratory analog-to-digital interface. Data for construction of the I–V or steady-state activation plots were obtained by either replaying the data from the FM tape onto a Gould chart recorder or from a direct measurement of plots of the data after digitization.

A recursive method of minimizing the squares of the deviations about a mathematic function (see Results) was used to fit the time courses of the inward current, i, and steady-state activation of i against potential. When fitting a curve to the data the criterion for best fit was that of minimizing an unweighted sum of the squares of the deviations. If S is this sum and $b_1, b_2, \ldots b_r$ are the variables which have to be minimized then at the minimum all the $\delta S/\delta b_i$ are zero. More than one algorithm was used for performing the fit but it was always possible to check the result by testing how close to zero these derivatives were. Occasionally the computer found local 'minima' which were not true minima. However a visual check detected this without any ambiguity and another minimum was found using different starting values. The algorithms used had to be iterative and the method of Broyden, Fletcher & Shanno (see Nash, 1979) was used for fitting any specified function. A computer program in BASIC using this method is listed in Nash (1979). For fitting the function $y = b_1 - b_2\, e^{b_3 x}$, to a set of points (x_i, y_i), which was used extensively, we developed a special algorithm which reduced the number of variables from three to one and was much faster. This uses the fact that $b_1 + b_2\, e^{b_3 x}$ depends linearly on b_1 and b_2, so that given b_3, b_1 and b_2 can be found analytically. Hence this algorithm only tries values of b_3. It finds b_3 such that S is least. The two algorithms were found to give identical results, as expected since they both use the same criterion of best fit.

RESULTS

Single jejunal cells had membrane potentials between −30 and −60 mV. Depolarized cells showed spontaneous oscillations and sometimes action potentials (Fig. 1*A* and Bolton *et al.* 1985). Steady membrane potentials, approximately −50 mV, could be obtained by passing through the pipette a small, inward, hyperpolarizing current. Injections of further current in the form of rectangular depolarizing or hyperpolarizing pulses produced electrotonic potentials whose rising and falling phases could be described by a single exponential (Bolton *et al.* 1985). Larger depolarizing electrotonic potentials triggered one or more action potentials (Fig. 1*B* and Bolton *et al.* 1985) while electrotonic potentials in response to a large hyperpolarizing current pulse showed a distinct 'sag' particularly at potentials negative of −70 mV (Fig. 1*A* and *C*). These sagging electrotonic potentials were often followed by a rebound depolarization (Fig. 1*A* and *C*) similar to that recorded in intact smooth muscle preparations such as the guinea-pig ileum (Bolton, 1972) and taenia caeci (Bennett, 1966). This sagging produced a pronounced inward rectification in the steady-state current–voltage relationship.

Under voltage clamp, jejunal cells were usually held between −40 and −60 mV i.e. potentials positive to the range at which these hyperpolarizing electrotonic potentials sagged. Hyperpolarizing command steps in potential smaller than 10–20 mV from a holding potential of −50 mV triggered first a capacitive current, followed by a steady current of 5–20 pA (Fig. 2*A*). Larger hyperpolarizing steps in potential evoked a larger instantaneous current which then increased with time over 10–15 s. This time-dependent inward current (i) was larger and developed more quickly as larger hyperpolarizing command steps were used (Fig. 2*A*). Upon repolarization to the holding potential, inward tail currents could also be recorded. These were usually maximal after command steps to −110 or −120 mV suggesting that i was fully activated in this range (see below).

The current activated upon hyperpolarization, i, did not inactivate even during command pulses lasting 15 s. The current was, however, deactivated upon repolarization to potentials near or positive to the resting potential. In Fig. 2*Ab* the cell was held at −120 mV. Depolarizing steps in potential caused an outward current which

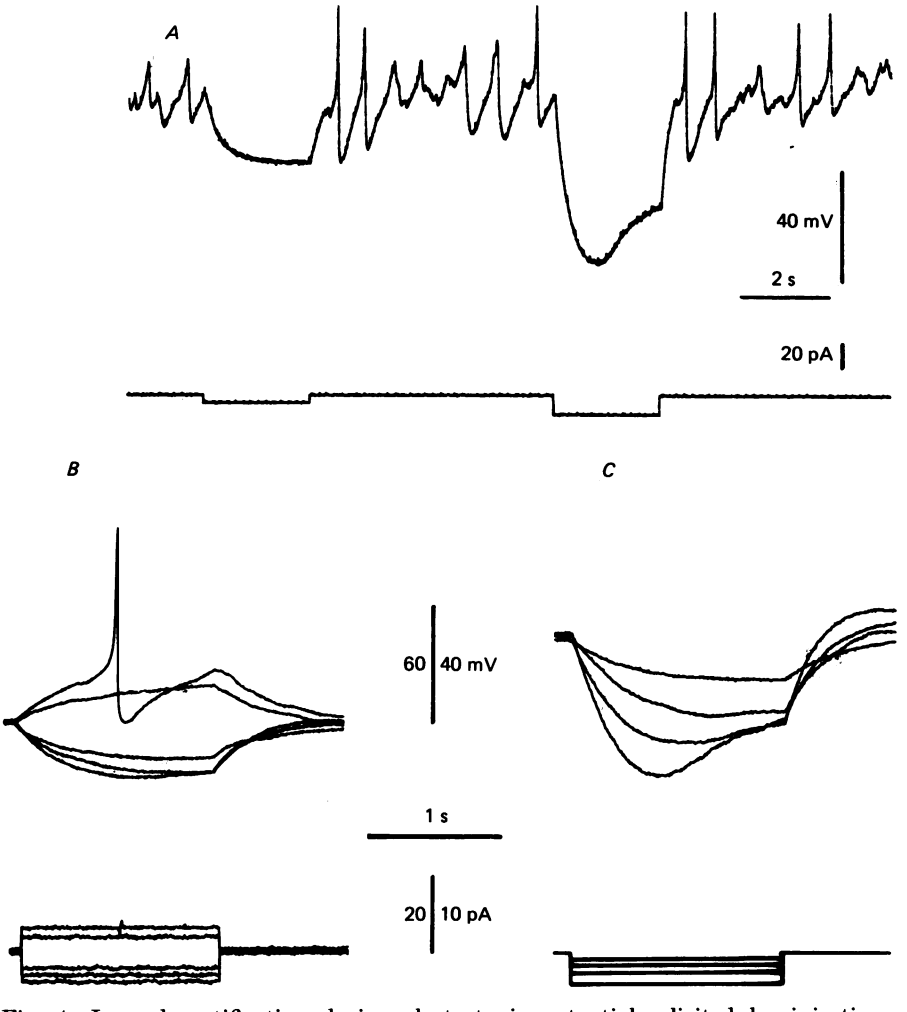

Fig. 1. Inward rectification during electrotonic potentials elicited by injections of rectangular hyperpolarizing currents into single cells of the rabbit jejunum. *A*, cell showing spontaneous action potentials in the absence of constant hyperpolarizing current. *B* and *C*, cells were hyperpolarized to −50 to −60 mV by passing a constant hyperpolarizing current; depolarizing and hyperpolarizing rectangular currents were then applied. Note action potential discharge when depolarizing pulses were sufficiently large (*B*) while hyperpolarizing electrotonic potentials sagged during injections of large hyperpolarizing pulses (*C*).

increased with time reflecting deactivation of i. This deactivation was very slow at potentials negative of −80 mV. In this cell the instantaneous membrane (or chord) conductance, measured from the instantaneous current deflexion upon polarization, was 0·8 nS at −50 mV and 1·7 nS at −110 mV; the difference represents the appearance of an additional membrane conductance (g) upon hyperpolarization negative to −60 mV (see also Fig. 2*B*).

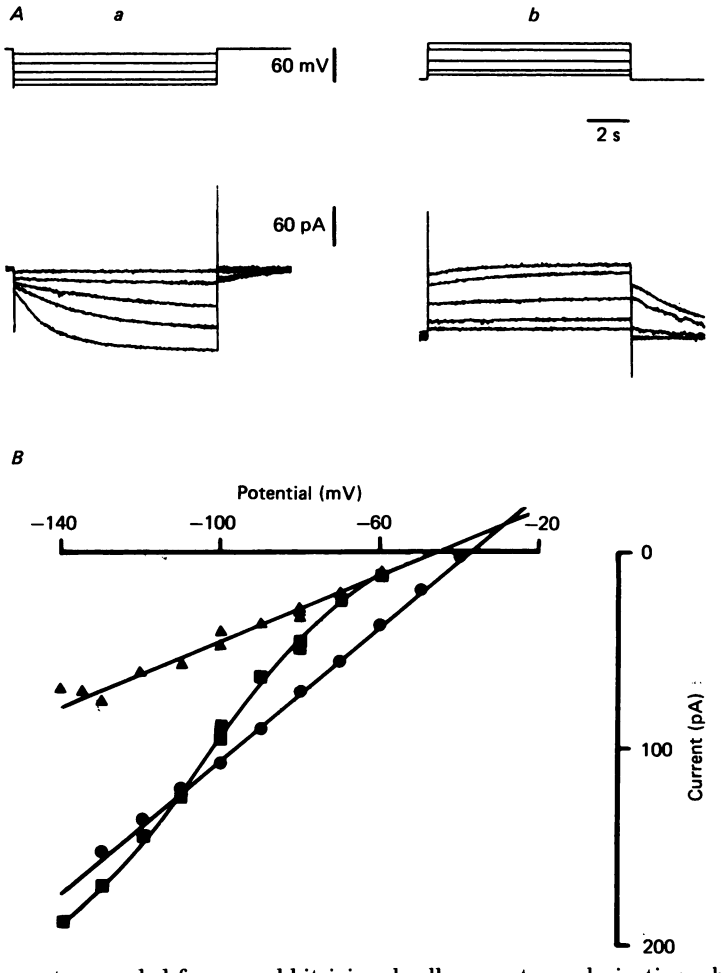

Fig. 2. Currents recorded from a rabbit jejunal cell upon step polarization when held at −60 (*Aa*) and −120 mV (*Ab*). *Aa*, hyperpolarizing command pulses from −60 mV elicited a time-dependent increase in inward current (*i*) whose amplitude and rate of onset increased with further hyperpolarization. *Ab*, depolarizing command pulses from a potential where *i* was fully activated (−120 mV) produced a time-dependent increase in outward current reflecting deactivation of *i*. *B*, instantaneous (chord) current–voltage relationship upon stepping from −50 (▲) and −110 mV (●) in another experiment. Note that the chord conductance plots are linear at both potentials (lines drawn by eye). The intersection of the extrapolated chord conductances gives an indication of the reversal potential of *i* (−30 mV). Plot of the 'steady-state' current at the end of a 10 s pulse against potential after stepping from a holding potential of −50 mV (■).

Reversal potential of i

Experiments were done in which cells held at −50 or −110 mV were stepped in a hyperpolarizing or depolarizing direction (Fig. 2*A*) respectively and the instantaneous current flowing at the start of the step was measured. The intersection of the instantaneous current–voltage relationships recorded from holding potentials where *g* was not activated (−50 mV; Fig. 2*B*) and where *g* was fully activated (−110 mV)

provided a method of estimating the reversal potential of i (Mayer & Westbrook, 1983). In Fig. 2*B* the instantaneous current–voltage plots were linear if i was not activated (but often not linear due to inward rectification if it was activated) and the extrapolated lines intersected at a potential of about −30 mV. In eleven similar experiments the mean value for this intersection was −24·5±3·5 mV (mean±S.E. of

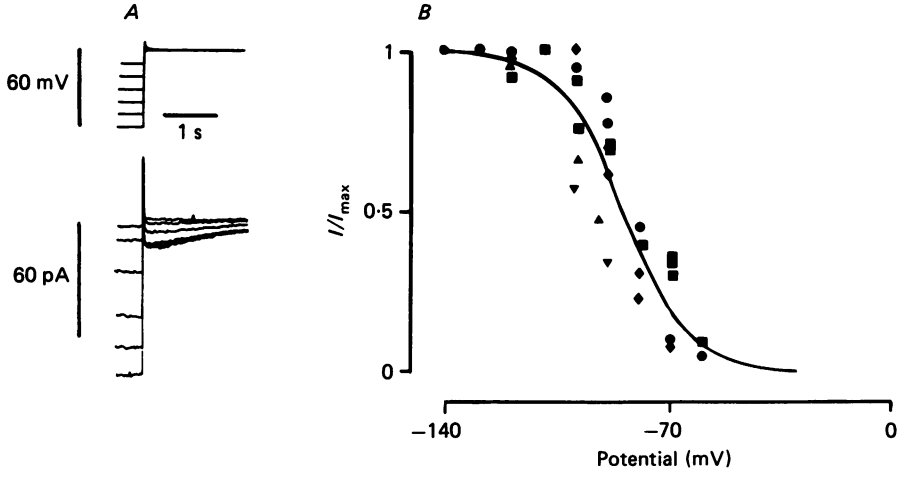

Fig. 3. Effect of potential on the activation of i. *A*, superimposed tail currents upon returning to −50 mV following a hyperpolarizing command pulse (9 s) to different potentials. Tail currents grew in amplitude with increasing hyperpolarization reaching a maximum at potentials negative to −100 mV. *B*, 'steady-state' activation curve of i for five different experiments. Points were generated by expressing the initial tail current (I) upon repolarization from some potential as a fraction of the maximal tail current observed (I_{max}) and plotting this ratio against potential (mV). The smooth sigmoidal curve is drawn according to the equation

$$I/I_{max} = \frac{1}{1+\exp((V-0{\cdot}084)/0{\cdot}01)},$$

which was obtained by a least-squares fit to the points as described in Methods. V is in volts. Different symbols represent different experiments.

mean). True reversal of i could not usually be obtained by depolarization to the appropriate range due to the activation of a contaminating inward calcium current and a large outward potassium current. However, it was clear that the current observed upon depolarization to −50 mV from potentials at which this current was strongly or fully activated (−110 mV) did not reverse. The estimate of the reversal potential in these experiments (−24·5 mV) is slightly positive to that obtained by Mayer & Westbrook (1983) (−34 mV) perhaps reflecting a difference in potassium gradients. These authors suggested that such a positive reversal potential reflected the fact that g represents the activation of a non-specific conductance to sodium and potassium ions (see below).

Voltage dependence of i

The potential dependence of i activated upon hyperpolarization was estimated from the amplitude of the tail currents recorded upon repolarization to the holding potential, usually -40 to -50 mV. The initial amplitude of these tail currents was measured by extrapolating the exponential part of their time course to zero time and was used as an indication of the activation of g during the preceding potential step. In Fig. 3*A* these inward tail currents grew in amplitude as increasingly hyperpolarizing command steps were used and were maximal at command potentials negative to -100 mV. Assuming that activation had reached a steady state at the end of the command pulse (9–15 s), a steady-state activation curve was generated by expressing the tail current size (I, amperes) after any command potential (V, volts) as a fraction of the maximum tail current (I_{max}) and plotting the ratio against the membrane potential (Hodgkin & Huxley, 1952). In Fig. 3*B* the relative amplitude (I/I_{max}) of the tail currents is plotted for five experiments. The continuous line fitted through the data, by a recursive least-squares method, is a Boltzmann distribution given by:

$$I/I_{max} = \frac{1}{1+\exp((V-V_0)/k)}. \quad (1)$$

This steady-state activation curve (Hodgkin & Huxley, 1952) has values of V_0, the membrane potential at which g is half-activated, of -84 mV and k, a slope factor, of 0·010. Therefore:

$$I/I_{max} = \frac{1}{1+\exp((V-0{\cdot}084)/0{\cdot}010)}. \quad (2)$$

Effect of varying $[K]_o$ *and* $[Na]_o$ *on i*

The inward rectification recorded in starfish egg membranes (Hagiwara, Miyazuki, Moody & Patlak, 1978) and skeletal muscle fibres (Standen & Stanfield, 1978) is due to a potassium-specific conductance which is dependent on the potential and the concentration of potassium in the bathing solution, $[K]_o$. Raising $[K]_o$ not only potentiated the inward currents triggered upon hyperpolarization due to an increase in driving force (potassium reversal potential, E_K, becoming more positive) but also shifted the activation range of the inward rectifier. The inward rectifier current of these preparations was recorded at potentials negative of E_K no matter where E_K was set (Hagiwara *et al.* 1978; Kurachi, 1985).

The inward current recorded in our jejunal cells triggered upon hyperpolarization was inward at potentials both positive and negative of E_K (-78 mV) suggesting that other ions, presumably sodium or chloride, contribute to i (Fig. 2*B*). Experiments were done in which the external sodium concentration, $[Na]_o$, was reduced from 126 to 12 mM by replacement of NaCl with Tris chloride titrated to pH 7·2. In a $[Na]_o$ of 126 mM, the instantaneous current–voltage relationship was plotted upon stepping from a holding potential of -50 mV and again upon stepping from a holding potential of -110 mV. In the first case i is not significantly activated and in the second it is virtually fully activated. The extrapolation of the current–voltage relationships to their point of intersection gave a reversal potential of $-28{\cdot}0 \pm 0{\cdot}8$ mV ($n = 3$) in a $[Na]_o$ of 126 mM and $-50{\cdot}7 \pm 1{\cdot}7$ mV ($n = 3$) in the same

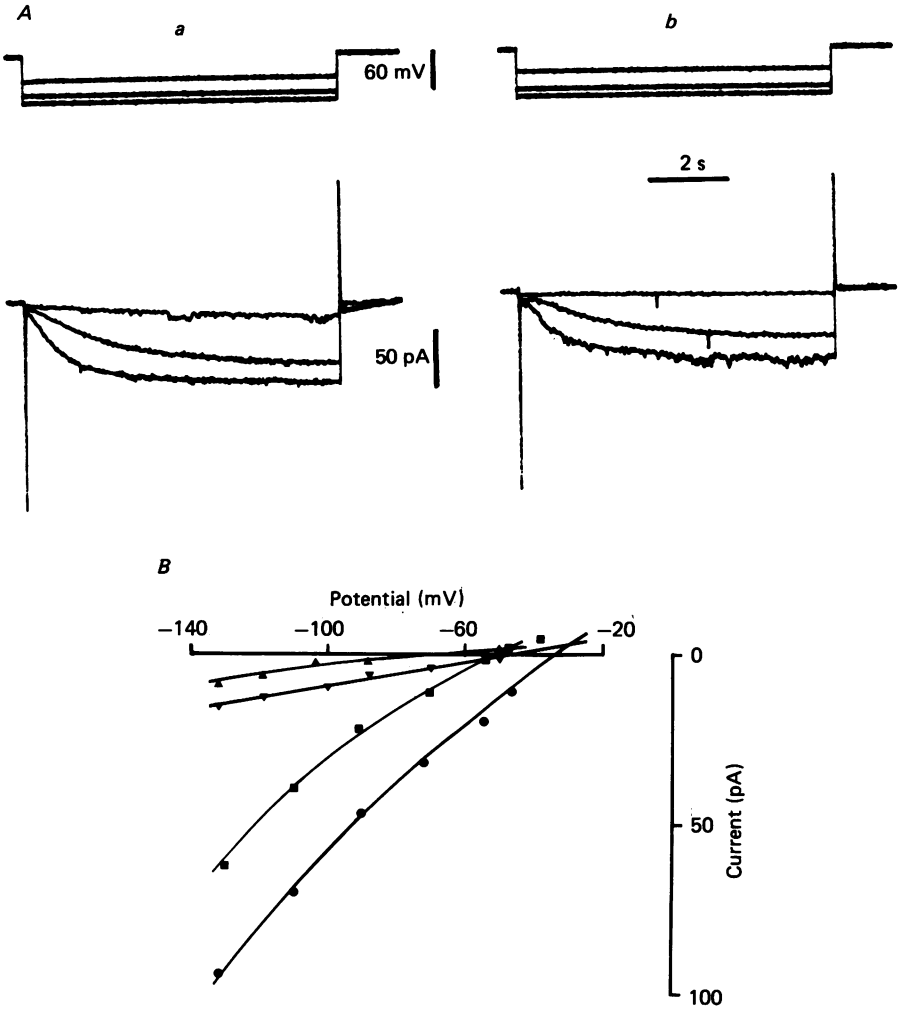

Fig. 4. The effect of reducing $[Na]_o$ from 126 to 12 mM on i and its reversal potential. *A*, the holding potential was −50 mV and hyperpolarizing steps were applied in normal solution ($[Na]_o$, 126 mM) (*a*) and in sodium-deficient (Tris-substituted: $[Na]_o$, 12 mM) solution (*b*). In *B* the instantaneous current–voltage relationship (▲, ▼) upon stepping from a holding potential of −50 mV (shown in *A*) and a holding potential of −110 mV (■, ●) are plotted in normal (▼, ●) and 12 mM-external sodium concentration (▲, ■). The intersection of these lines gives an estimate of the reversal potential of i in each solution. Note that in a $[Na]_o$ of 12 mM no tail currents are seen upon returning to −50 mV as predicted from the instantaneous current–voltage relationship.

cells in a $[Na]_o$ of 12 mM. The 22 mV shift in the reversal potential for an approximately tenfold shift in $[Na]_o$ suggests a substantial sodium contribution to i (Fig. 4*A* and *B*). Note also (Fig. 4*Ab*) that upon returning to the holding potential of −50 mV, tail currents are not seen in a $[Na]_o$ of 12 mM as predicted from the instantaneous current–voltage relationship.

Similar experiments were done in which the $[K]_o$ was varied and the reversal

potential of i obtained from the intersection of the extrapolated instantaneous current–voltage relationship. In a $[K]_o$ of 6 mM the reversal potential was −28 and −32 mV in two cells and in a $[K]_o$ of 36 mM it changed to −20 and −22 mV. Larger tail currents were seen upon returning to the holding potential −50 mV in a $[K]_o$ of 30 mM (Fig. 5*A*). The size of the tail current upon stepping from a holding potential

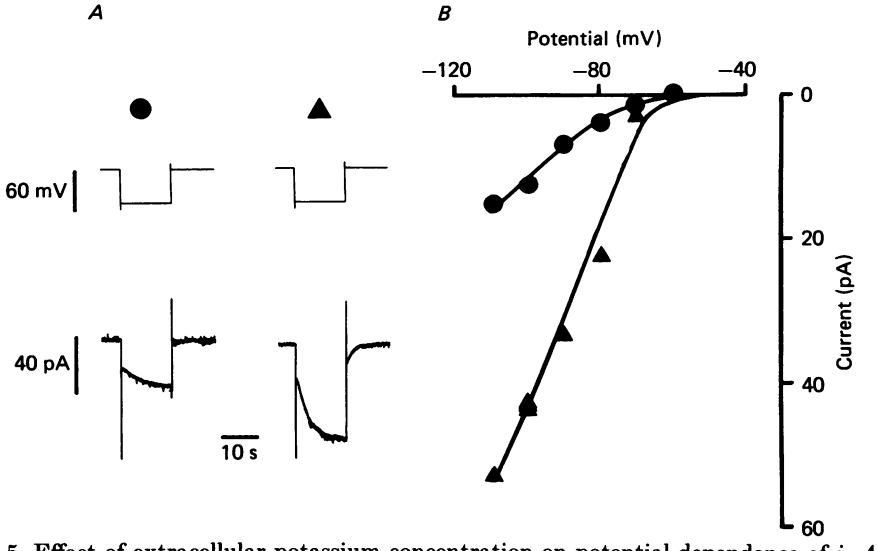

Fig. 5. Effect of extracellular potassium concentration on potential dependence of i. *A*, single command pulses from −50 to −100 mV in 6 (●) and 30 mM-potassium (▲). The instantaneous current (i_{inst}) and the current at the end of the pulse (i_{ss}) both increase with an increase in $[K]_o$. *B*, plot of i ($i_{ss}-i_{inst}$) (pA) against potential (mV) in 6 (●) and 30 (▲) mM-potassium. Note similar activation range of i at both potassium concentrations.

of −110 mV to various depolarized potentials also yielded an estimate of the reversal potential of i. In the experiment of Fig. 5 this method of estimating the reversal potential gave values of −37 mV ($[K]_o$, 6 mM) and −26 mV ($[K]_o$, 30 mM). In these experiments $[K]_o$ was increased by equimolar substitution for $[Na]_o$ so some change in the driving force on the sodium ion also occurred.

In raised $[K]_o$ the rectifier current i increased considerably in size but its activation range and kinetics were not noticeably altered (Fig. 5*B*). When $[K]_o$ was increased, the instantaneous current (i_{inst}) was increased upon stepping in a hyperpolarizing direction from −50 mV. The current at the end of the pulse (i_{ss}) was also increased substantially but $i = (i_{ss}-i_{inst})$ was still activated in the range of −60 to −120 mV (Fig. 5*B*).

Similar potentiation with no shift in the activation range of a non-specific inward rectifier current upon raising $[K]_o$ has been reported in cultured mouse sensory ganglia (Mayer & Westbrook, 1983) and in the sino-atrial node cells of the rabbit (DiFrancesco & Ojeda, 1980). Also the reversal potential of the inward rectifier current, i_f, in Purkinje fibres bathed in barium ions, became more positive as the $[K]_o$ was raised ($[Na]_o$ being fixed) or as the $[Na]_o$ was raised ($[K]_o$ being fixed)

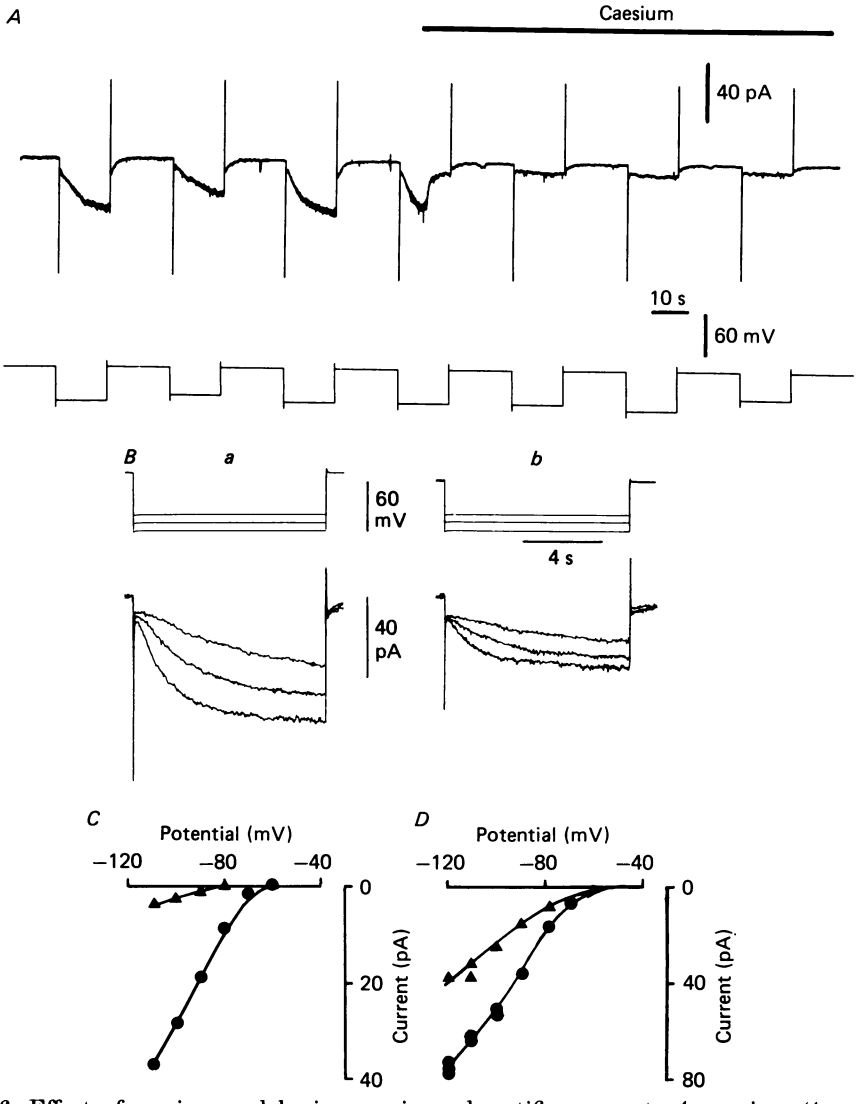

Fig. 6. Effect of caesium and barium on inward rectifier current. *A*, caesium (1 mM) rapidly blocks *i* during hyperpolarizing command pulses of various amplitudes from a holding potential of −50 mV. *B*, rectifier current activated by three hyperpolarizing pulses from a holding potential of −40 mV in normal solution (*a*) and after 30 min in the presence of 10 mM-barium from a holding potential of −50 mV (*b*). *C*, plot of *i* against potential in the absence (●) and the presence (▲) of 1 mM-caesium. *D*, plot of *i* against potential (mV) in the absence (●) and the presence (▲) of 10 mM-barium.

(DiFrancesco, 1981). Attempts to replace the extracellular sodium or chloride ions bathing mouse sensory neurones showed that removal of either ion reduced the inward current (Mayer & Westbrook, 1983). An effect of chloride ions on the pace-maker current has also been demonstrated in cardiac preparations (see Seyama, 1979 and van Bogaert & Carmeliet, 1985).

Effect of potassium channel blockers, caesium and barium

Barium ions have been previously demonstrated to be good blockers of potassium-selective inward rectifying channels in muscle (Standen & Stanfield, 1978) and starfish egg membrane (Hagiwara *et al.* 1978) at concentrations less than 1 mM but not to affect dramatically the non-selective channels responsible for the inward rectification in sensory neurones (Mayer & Westbrook, 1983) nor in single Purkinje cells (Callewaert, Carmeliet & Vereecke, 1984). Caesium on the other hand blocks both types of channel at 1 mM (Mayer & Westbrook, 1983; Constanti & Galvan, 1983). The relative insensitivity of the non-selective channels to barium therefore allows another means of characterizing the channels in single jejunal cells.

Fig. 6 shows the effects of caesium and barium on separate preparations. Caesium (1 mM) added to the bathing solution rapidly blocked the inward current triggered upon hyperpolarization (Fig. 6*A*). The current, *i*, activated upon hyperpolarization decreased approximately 80% in the presence of 1 mM-caesium (Fig. 6*C*). These effects of caesium were rapidly reversed by exchanging the bathing solution for a solution with no added caesium (not shown).

The current, *i*, was only slightly affected by barium. In Fig. 6*Bb* the cell had been bathed in 10 mM-barium for at least 30 min. Inward current and the following outward currents, recorded upon step depolarization from a holding potential of −50 mV in normal solutions, were enhanced and abolished respectively under these conditions (Bolton *et al.* 1985). The current, *i*, could still be recorded in 10 mM-barium (Fig. 6*Bb*) but was reduced to about 60% of control (Fig. 5*D*). These inward rectifier currents were not affected by tetraethylammonium (TEA, 20 mM) (see also Mayer & Westbrook, 1983) nor by calcium entry blockers such as cadmium (1 mM).

Kinetics of i

Upon stepping from a holding potential at which *i* was not significantly activated to a more negative potential, the current *i* developed after a short delay with a time course which could be fitted by a single exponential. DiFrancesco (1985) has shown that single exponentials can adequately describe the time course of the current responsible for inward rectification in Purkinje fibres at potentials near the potential of half-maximal activation. At more extreme potentials, positive or negative, the time courses of activation and deactivation required a more complicated description.

The initial delay before the exponential development of a current is reminiscent of other currents (e.g. potassium current in squid axon, Hodgkin & Huxley, 1952). It is likely to reflect at a single-channel level, the bursting characteristics of the channels. Decline of current upon deactivation was also more complex than a single exponential (Fig. 3*A*). The initial delay in the development of the current, *i*, introduced a complication in the fitting procedure which did not seem worthwhile to pursue in view of what is now known about single-channel behaviour, since the mathematical description has no simple physical interpretation. However, it was important to have some quantitative measure of the speed at which *i* developed at different potentials, and this could be provided for the greater part of its time course by fitting a single exponential function although it was clear that at early times this did not adequately describe the delay seen before the development of the current.

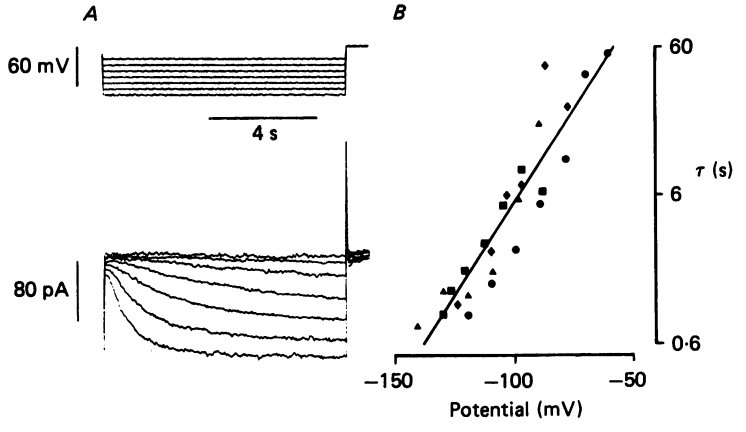

Fig. 7. Effect of potential on the activation kinetics of the rectifier current. *A*, averaged (two to four) records of i upon stepping to various hyperpolarized potentials, from a holding potential of −50 mV. Time courses of i were fitted by a least-square method to a single exponential to obtain a time constant of activation (τ) (see text). *B*, plot of τ against potential (mV) for four separate experiments. Straight line was fitted by least-squares method, the slope was such that 17·5 mV was required for an e-fold change in τ.

If the membrane is held at a potential (e.g. −50 mV) at which i is negligibly small, upon stepping to some potential where i flows, its time course will be described by:

$$i = i_{\infty}(1-\exp[-t/\tau]), \tag{4}$$

where i_{∞} is the steady-state size of i and τ is a time constant (s). The estimates of τ in Fig. 7 were obtained by fitting eqn. (4) to the time course of i at any potential. In Fig. 7*B* the log of τ for four separate experiments has been plotted against potential. The straight line plotted represents the least-square fit and has a slope that changes e-fold with 17·5 mV. It will be observed that τ varied between 1·0 and 60 s over the range −130 to −60 mV.

DISCUSSION

Time-dependent decline in the size of the electrotonic potentials recorded in response to hyperpolarizing currents, often followed by a transient depolarizing overshoot, have been recorded in a number of neuronal, cardiac and skeletal muscle preparations and at least three mechanisms have been identified which might cause such inward (anomalous) rectification. The activation of a potassium-specific conductance at potentials negative of E_K, which is sensitive to barium (<1 mM) and caesium (1 mM), has been described in starfish eggs (Hagiwara *et al.* 1978) frog skeletal muscle (Standen & Stanfield, 1978) and olfactory cortex neurones (Constanti & Galvan, 1983). The activation of this inward rectifier is dependent on $V-E_K$ and not just potential. In sympathetic ganglia the deactivation of a voltage-sensitive potassium conductance sensitive to muscarinic agonists (M-current) also produces an inward rectification of the hyperpolarizing electrotonic potential (Brown & Adams, 1980; Adams, Brown & Constanti, 1982). This rectification, however, becomes

smaller with larger hyperpolarizing currents (Adams *et al.* 1982). Such a mechanism is presumably responsible for the 'sag' of the electrotonic potential and rebound depolarization seen in single cells of the toad stomach (Fig. 2 in Sims *et al.* 1985). In cultured mouse sensory ganglia (Mayer & Westbrook, 1983), hippocampal pyramidal neurones (Halliwell & Adams, 1982) and cardiac sino-atrial cells (DiFrancesco & Ojeda, 1980) an inward rectifier selective for potassium and sodium ions, blocked by caesium but not by barium and activated at potentials negative of −50 to −60 mV, has also been described.

The inward currents triggered upon hyperpolarization in freshly isolated cells of the rabbit jejunum presumably arise from the activation of a non-selective conductance, g, perhaps similar to those in sensory neurones or cardiac cells. It was relatively insensitive to barium, up to 10 mM being required to reduce the current by 40%, but it was blocked by caesium (1 mM). Caesium has been shown to block the potassium-selective inward rectifier current in a voltage-dependent manner, the block increasing with hyperpolarization (Hagiwara *et al.* 1978). The use of 1 mM-caesium in the present experiments was perhaps too high a concentration to readily demonstrate a voltage-dependent block. A lower concentration of caesium (200 μM) applied to sensory ganglia does show a voltage-dependent block of the inward rectifier (Mayer & Westbrook, 1983). It seems possible that caesium blocks in a manner similar to the potential-dependent caesium block of calcium-activated potassium channels described previously in this jejunal preparation (Benham *et al.* 1986).

Raising the extracellular potassium concentration from 6 to 36 mM increased i without much shift in the steady-state activation curve (Fig. 5) (cf. Mayer & Westbrook, 1983). The measured reversal potential, however, moved positive by approximately 10 mV. The potential of half-maximal activation in the present experiments was identical to that obtained by Mayer & Westbrook (1983) (−84 mV). Previous attempts to remove the extracellular chloride resulted in a blockage of the channels carrying I_h, the current responsible for the inward rectification shown by sensory ganglia (Mayer & Westbrook, 1983). This block was suggested to be due to a direct block of channels carrying I_h by the impermeable chloride substitutes rather than an indication of a contribution of chloride to the current they carried (Mayer & Westbrook, 1983). We do not discount a possible contribution of chloride to the current, i, responsible for rectification in jejunal cells. It is of interest that the reversal potential of i (−24·5 mV) in the present experiments was positive to that obtained in sensory ganglia (−34 mV, Mayer & Westbrook, 1983). In our experiments the chloride reversal potential (E_{Cl}) is known to be 0 mV whereas in sensory ganglia it may well be different. A small chloride contribution to g could well explain the difference (particularly as E_K differed only by 6 mV in these two preparations).

The activation of i at potentials negative of −60 mV (Fig. 3) has some direct consequences for the establishment of the resting membrane potential and the duration of the quiescent periods between spontaneous action potential discharge. The activation of g would have a time-dependent depolarizing effect on the membrane potential so that it seems likely that the resting membrane potential of the longitudinal jejunal smooth muscle cell would be just positive of −60 mV. Intracellular recordings from the longitudinal jejunal muscles of the rabbit (El-Sharkawy & Daniel, 1976; Kitamura, 1978) and the guinea-pig (Bolton, Clark,

Kitamura & Lang, 1981) show regular slow potential changes up to 20 mV in size. Despite the fact that *in vitro* preparations are generally stretched to immobilize them, they usually show membrane potentials which reach −50 to −60 mV. *In vivo* a more negative potential may exist so that the large regular slow waves of potential will enter the range of potential where the current, i, is activated.

After action potential discharge in single jejunal cells there was usually a substantial after-hyperpolarization due to the activation of both a calcium-dependent and a calcium-independent, voltage-dependent potassium conductance. Cadmium (200 μM) blocks the inward calcium current under voltage clamp but only partially blocks the following outward potassium currents (R. J. Lang, C. D. Benham & T. B. Bolton, unpublished observations). The duration of the after-hyperpolarization, if it depends on the voltage- and calcium-dependency of these two potassium conductances, might well be shortened by the activation of g as it would promote depolarization and action potential discharge. Assuming a Q_{10} of i of 3–4 (DiFrancesco & Ojeda, 1980) at the potential of half-maximal activation (−84 mV) i would activate with a time constant of about 3 s (Fig., 7*B*) at 35 °C. At −60 mV, g would deactivate with a time constant of about 3 s also (at 35 °C), perhaps consistent with the mean frequency (23 min^{-1}) of action potential bursts (and after-hyperpolarizations) recorded in rabbit jejunum by Kitamura (1978).

Currents corresponding to i were not recorded in every cell studied in the present experiments (approximately 50 % of the cells). Intact smooth muscles that spontaneously generate slow waves and action potentials generally have regions of pace-maker activity (Publicover & Sanders, 1984). These regions that have unstable or readily depolarizable membranes may well have cells which exhibit a well-developed inward rectification.

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