J. exp. Biol. **123**, 175–189 (1986)
Printed in Great Britain © *The Company of Biologists Limited 1986*

A POTASSIUM-SELECTIVE CHANNEL IN ISOLATED *LYMNAEA STAGNALIS* HEART MUSCLE CELLS

BY B. L. BREZDEN, D. R. GARDNER

Department of Biology, Carleton University, Ottawa, Ontario K1S 5B6

AND C. E. MORRIS

Department of Biology, University of Ottawa, Ottawa, Ontario K1N 6N5, Canada

Accepted 11 November 1985

SUMMARY

Lymnaea stagnalis heart ventricle muscle cells, isolated by enzymatic dispersion, are suitable for patch clamp recording. A channel has been identified which is primarily selective for potassium, although it also appears to conduct sodium. This channel is not blocked by 4-aminopyridine or tetraethylammonium but is sensitive to quinidine. The relationship between the membrane potential, the external potassium concentration and the channel currents, when compared with results obtained from whole cell recording, suggests that this channel could mediate a large part of the cell's resting conductance.

The probability of this channel being open is increased by stretching the patched cell membrane. This has led to speculations about further possible functional roles for this channel.

INTRODUCTION

The resting and active conductances of excitable cell membranes are mediated by protein channels which are specific to varying degrees (Latorre & Miller, 1983) for particular ions. The resting conductance constitutes the framework against which transiently activated conductances must operate to alter the membrane potential. For molluscan muscle cells, the nature of the resting conductance mechanisms is largely unknown, although some studies have indicated that the resting membrane is permeable to potassium ions (e.g. Burnstock, Greenberg, Kirby & Willis, 1967; Twarog, 1967; Hill, Greenberg, Irisawa & Nomura, 1970). A more detailed examination of the ionic basis of the resting potential in the heart ventricle muscle cells of the freshwater snail *Lymnaea stagnalis* has suggested that the resting conductance has potassium, chloride and sodium components and can be adequately described by a form of the Goldman–Hodgkin–Katz equation. The ratio of potassium to sodium permeability (P_K/P_{Na}) was found to average about 14, and the slope over a wide range of potassium concentrations was about 50 mV/10-fold change (Brezden & Gardner, 1984).

Key words: *Lymnaea stagnalis*, isolated heart muscle cells, single-channel recording, potassium channel, stretch-sensitive channel, quinidine.

In this study, we have examined the conductances of these *Lymnaea* cells using the gigaohm seal patch clamp technique (Hamill *et al.* 1981). To facilitate the investigations, enzymatic dispersion was used to isolate the heart ventricle cells. The isolated cells are suitable for patch clamping (and, therefore, presumably free of basement membrane) and can be maintained for several days *in vitro*.

Single-channel recording from the ventricle cells has allowed us to begin addressing the following questions. (1) Are there ion channels which are spontaneously active at the cell's resting voltage, and, if so, to which ions are these channels permeable? (2) What factors modulate the kinetics or conductance of these channels? (3) How are these channels distributed in the cell membrane? Answers to these questions are particularly important in understanding the behaviour of myogenic tissue such as the *Lymnaea* heart ventricle.

MATERIALS AND METHODS

The animal and preparation

Hearts were removed from adult *Lymnaea stagnalis* (see Brezden & Gardner, 1984, for details). The atria and aortae were cut away and the ventricles were placed in normal *Lymnaea* saline (Table 1) containing 5 mmol l^{-1} glucose, 200 i.u. ml^{-1} of penicillin and 2 i.u. ml^{-1} of bacitracin (antibiotic/glucose saline, AGS). The ventricles were cut into approximately 1 mm pieces with Vannas spring scissors and placed in 15 ml conical centrifuge tubes containing 5 ml of 0·25% trypsin (Sigma, type XII-S) in AGS (2–4 ventricles per tube). The tissue fragments were treated for 30 min at room temperature and subsequently centrifuged at 115 ***g*** for 10 min. The trypsin was replaced with a 0·1% solution of collagenase (Sigma, type II-S) in AGS and digested for a further 2 h. Throughout these procedures, the tubes were gently agitated in a vortex mixer to facilitate dispersion of the cells. Following collagenase treatment, the tubes were centrifuged and the dispersed cells were resuspended in 5 ml of AGS. The cells were plated on sterile glass coverslips in 35 mm Petri dishes. The coverslips had been previously treated with 3% (v/v) HCl for 30 min and 0·1% (w/v) Na_2CO_3 for 30 min. They were sterilized by exposure to dry heat (200°C) for

Table 1. *Composition of the saline solutions*

	KCl	NaCl	$CaCl_2$	$MgCl_2$	BDAC	Hepes*	Glucose
Normal saline	1·6	50·0	3·5	2·0	0	5·0	0
30 mmol l^{-1} K^+ saline	30·0	21·6	3·5	2·0	0	5·0	0
100 mmol l^{-1} K^+ saline	100·0	0	3·5	2·0	0	5·0	0
Zero Na^+ saline	1·6	0	3·5	2·0	50·0	5·0	0
AGS saline†	1·6	50·0	3·5	2·0	0	5·0	5·0

Concentrations given in mmol l^{-1}.
* Hepes adjusted to pH 7·6 with 10 mol l^{-1} NaOH.
† AGS, antibiotic/glucose saline contained 200 i.u. ml^{-1} penicillin and 2 i.u. ml^{-1} bacitracin.
BDAC, bis(2-hydroxyethyl)dimethylammonium chloride.

12 h (Jacobson, 1977). The Petri dishes were plated at a density of about two hearts per dish. The isolated cells were kept at ambient room conditions.

Resting potential measurements

The resting potential of 2-day-old isolated cells was measured using 3 mol l^{-1} KCl-filled glass microelectrodes and standard electrophysiological techniques (WPI model KS 700 microelectrode amplifier). Prior to recording, the AGS saline was replaced with normal saline.

Single-channel recordings

Patch microelectrodes were fabricated from Corning 7052 (Kovar sealing) glass (1·65 mm o.d., 1·15 mm i.d.). After coating with Sylgard 184 (Dow Corning) the tips were fire-polished. It was necessary to apply a few cmHg of negative pressure to obtain gigaohm seals (gigaseals). We had much less success in obtaining gigaseals using Pyrex or soda glass pipettes. Recordings were made from cell-attached patches using standard techniques (Hamill *et al.* 1981) with a List EPC-5 patch clamp amplifier at a gain of 50 mV pA^{-1}. This amplifier was also used to establish and maintain transpatch potentials. Data were filtered at 4 kHz with a four-pole Bessel filter and recorded on a Racal FM tape recorder for subsequent digitization and analysis on a Digital PDP-11 computer using threshold detection software. Event selection and measurement were done using semi-automated, user-inspected routines.

The effects of variations in the external (intra-pipette) potassium, chloride and sodium concentrations on channel currents and conductances were examined. The saline compositions are given in Table 1. The 100 mmol l^{-1} potassium saline necessarily produces a higher ionic strength than the other salines [the ionic concentrations of 100 mmol l^{-1} potassium saline (zero sodium) was 221·5 mmol l^{-1} whilst that of the other salines was 124·7 mmol l^{-1}]. 30 mmol l^{-1} potassium saline contained 21·6 mmol l^{-1} sodium (*vs* 50 mmol l^{-1} in normal saline). In all experiments, the bath solution was normal *Lymnaea* saline. Note that this saline has a low ionic strength (i.e. about one-tenth that of sea water), and, therefore has a relatively low conductance.

The effects of 4-aminopyridine (4-AP, 1–10 mmol l^{-1}, Sigma), tetraethylammonium (TEA, 1–10 mmol l^{-1}, Sigma) and quinidine (0·25–1 mmol l^{-1}, Sigma) on channel currents were also investigated. Prior to patch clamping, the AGS saline was replaced with normal saline. The experiments were performed at room temperature (20–23 °C).

Effect of membrane stretch on channel currents

Following the formation of a gigaseal, the cell membrane was stretched by applying suction through the patch electrode *via* a side-port on the pipette holder; suction was created with a syringe attached in series to a mercury manometer for monitoring pressure.

RESULTS

The isolated cells

The isolated cells attached to the glass substrate within minutes of plating and remained viable for up to 5 days at room temperature with no interim changes of the medium. Within the first day *in vitro*, they formed projections which attached to the glass substrate. The cells, as they appeared on the first and third day following isolation, are shown in Fig. 1. Cells of this morphology were selected for patch clamping.

The resting potential

The resting potentials of *L. stagnalis* cells 2 days after isolation ranged from -50 mV to -67 mV [$N = 30$, mean $= -59 \pm 5$ (S.D.) mV]. This value is almost identical to the resting potential of these cells *in situ* (-61 ± 5 mV, Brezden & Gardner, 1984).

Single-channel properties

In the following results, the 'transpatch' potential (membrane potential = V_m) is given as the mean resting potential (we used a rounded value of -60 mV) minus the pipette potential. Generally, patching was more successful on 2-day-old or older cultures than on freshly isolated cells.

Channel activity in normal saline

With normal *Lymnaea* saline (1·6 mmol l^{-1} potassium) in the pipette, channel currents were not evident at the resting potential, but were detected upon depolarization of the membrane potential (Fig. 2). The predominant channel species, which was observed in every patch, was characterized by a conductance of 33 pS (ranging from 29 to 36 pS, $N = 5$ patches). The channel openings often appeared in bursts with the current magnitude increasing steadily with increased depolarization. There was no apparent inactivation during the sustained voltages used to clamp the membrane potential.

Channel current reversal

Only about 20% of the patches examined exhibited inward currents with normal saline (1·6 mmol l^{-1} potassium) in the patch pipette, even with the membrane hyperpolarized to -120 mV. Generation of I/V relationships was restricted to data from these patches. Both inward and outward channel currents were, however, readily seen with elevated extracellular potassium (Fig. 3). Fig. 4 shows data for the patches where current reversals were seen with normal saline in the pipette. The reversal potential of these channels (as determined from the interpolated zero-crossing point of the I/V relation) ranged from -50 to -74 mV [mean $-60{\cdot}4 \pm 10{\cdot}1$ (S.D.) mV, assuming a mean resting potential of -60 mV].

Effect of variations in external potassium

The potassium and probably chloride equilibrium potentials in these cells are close to the resting potential (Brezden & Gardner, 1984). To distinguish which of these

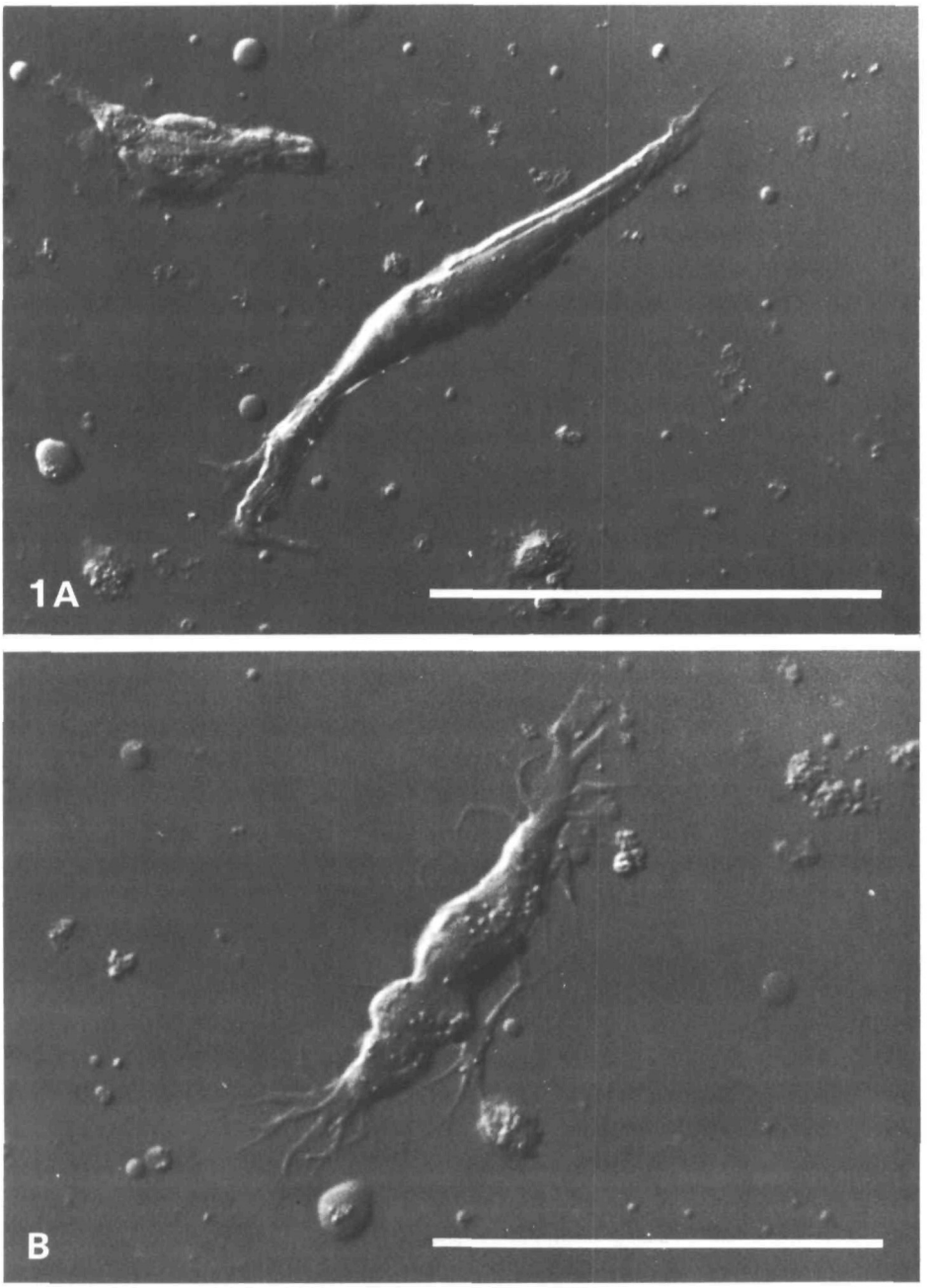

Fig. 1. The isolated *Lymnaea stagnalis* heart ventricle muscle cells (A) 1 day and (B) 3 days after isolation (Nomarski optics). Note the processes by which the 3-day-old cells are attached to the substrate. Scale bars, 100 μm.

ionic species is the predominant charge carrier through this channel, the potassium concentration in the pipette was varied while maintaining a constant chloride concentration.

An increase in the extracellular potassium concentration from 1·6 to 30 mmol l^{-1} (constant chloride and osmolarity) resulted in a 35 mV shift of the equilibrium potential in a depolarizing direction from −60 to −25 mV (25·2 ± 7·6 mV). With 100 mmol l^{-1} potassium in the pipette (elevated chloride and osmolarity, zero sodium), the reversal potential was found to be at about +4 mV (4 ± 3 mV) (Figs 4, 5, 6). These changes represent a 27 mV slope per decade change in the external potassium concentration between 1·6 and 30 mmol l^{-1}, and a slope of 57 mV

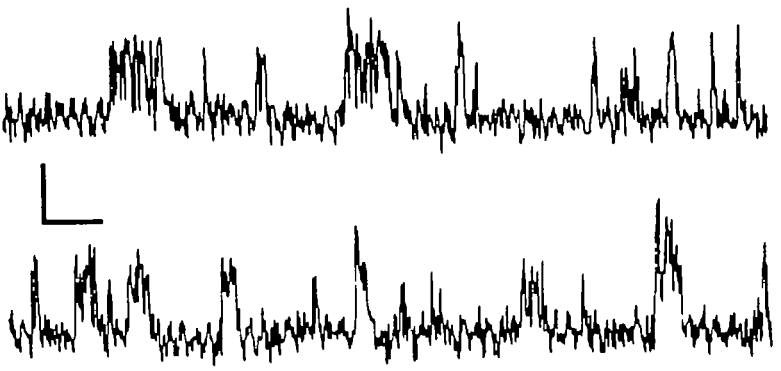

Fig. 2. Single-channel currents recorded from *Lymnaea* heart cells. Upward deflection = outward current; pipette solution, normal saline; V_m = +20 mV. Scale bars, 2 pA, 150 ms. Note the frequent grouping of channel openings into 'bursts'. Both traces are from the same patch.

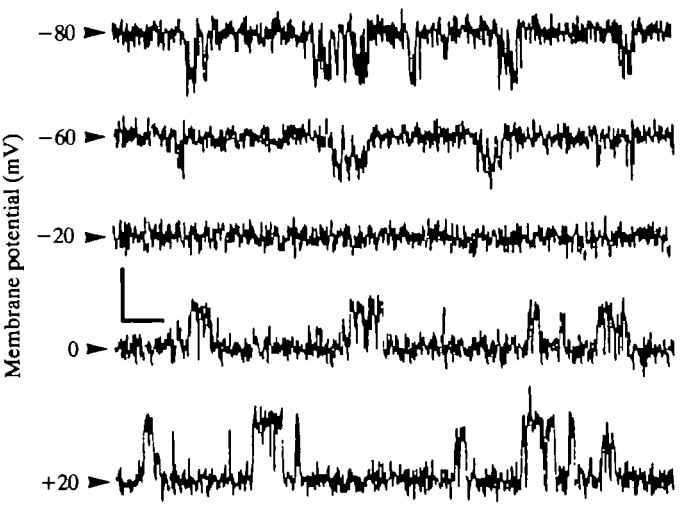

Fig. 3. Channel currents at different pipette potentials. Pipette solution, 30 mmol l^{-1} potassium saline; channel conductance, 30 pS. Scale bars, 1 pA, 25 ms.

per decade change between 30 and 100 mmol l^{-1} potassium. The channel conductances (as determined from the slope of the I/V relationships) in 1·6 and 30 mmol l^{-1} potassium were 33 pS (ranging from 29 to 36 pS, $N = 5$ patches) and 30 pS (ranging from 20 to 37 pS, $N = 4$ patches), respectively, whereas the conductance in 100 mmol l^{-1} potassium was 84 pS (ranging from 64 to 90 pS, $N = 4$ patches). In changing from 1·6 to 100 mmol l^{-1} potassium, the chloride concentration was increased from 62·6 to 111 mmol l^{-1}. Consequently, if the channel was chloride-

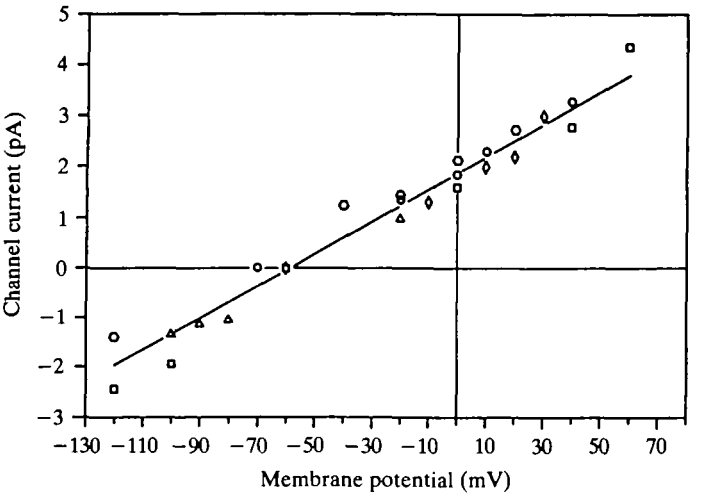

Fig. 4. Current–voltage relationship. Pipette solution, normal saline (1·6 mmol l^{-1} potassium); reversal at $V_m = -60$ mV (line fitted by linear regression); conductance, 33 pS. Different symbols denote different patches.

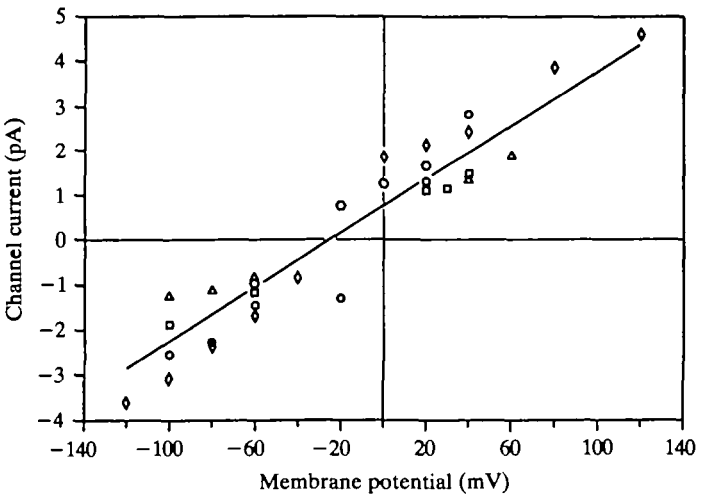

Fig. 5. Current–voltage relationship. Pipette solution, 30 mmol l^{-1} potassium saline; reversal at $V_m = -25$ mV (line fitted by linear regression); conductance, 30 pS. Different symbols denote different patches.

selective, a hyperpolarizing shift of the reversal potential would be expected. However, the shift in the reversal potential was in a depolarizing direction, as expected for a potassium-selective channel. Furthermore, a change in the potassium concentration from 1·6 to 30 mmol l^{-1} (keeping the chloride concentration constant) also shifted the reversal potential in a depolarizing direction; no shift would have been expected for a chloride channel. These results preclude the possibility that the channel is chloride-selective.

Elevated external potassium concentration also resulted in the appearance of inward currents at the resting potential (Fig. 7), showing directly that gating can occur in the absence of membrane depolarization.

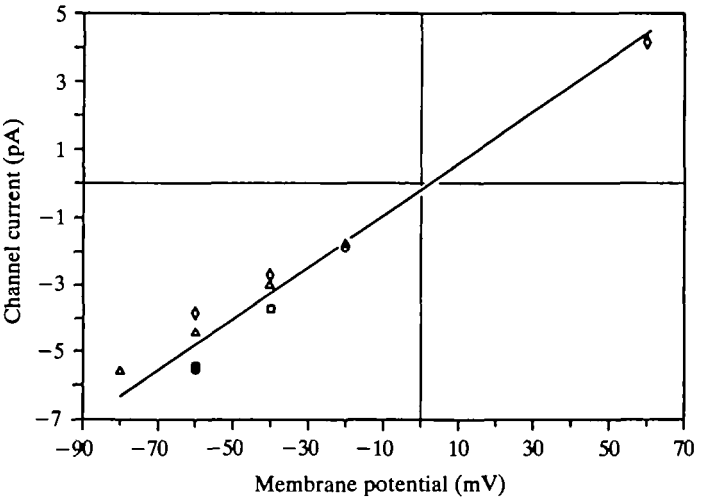

Fig. 6. Current–voltage relationship. Pipette solution, 100 mmol l^{-1} potassium saline; reversal at $V_m = +4$ mV (line fitted by linear regression); conductance, 84 pS. Different symbols denote different patches.

Fig. 7. Channel currents at the resting potential with (A) 1·6, (B) 30 and (C) 100 mmol l^{-1} potassium in the pipette. Scale bar, 10 ms, 1 pA.

Effect of zero sodium on channel conductance

The substitution of bis(2-hydroxyethyl) dimethylammonium chloride (BDAC) for sodium (potassium = 1·6 mmol l^{-1}) resulted in an increase in channel conductance from 33 to 37 pS and a shift of the equilibrium potential from −60 to −68 mV (Fig. 8). An analysis of the residual mean squares about the fitted regression lines shows that these differences in the conductance and reversal potential are significant at the 0·1% level (F-distribution). The significance of these results is further supported by the observation that under zero sodium conditions outward channel currents were detected at the resting potential, whereas they were never detected with normal saline (50 mmol l^{-1} sodium) in the pipette.

Effect of pressure on channel behaviour

The application of 4–12 cmHg suction to the patch through the patch electrode increased the probability of the channel being open, resulting in an increased frequency of simultaneous openings (Fig. 9). Suction had no effect on the amplitude of the channel currents. The channel activity persisted during sustained suction and returned to baseline levels upon the release of suction. Increased channel activity in response to stretch was seen at all membrane potentials and at all potassium concentrations used, inclusive of physiological saline.

Distribution of channels

An important characteristic of these stretch-activated potassium (SAK) channels is their apparently homogeneous distribution in the sarcolemma. Every patch on

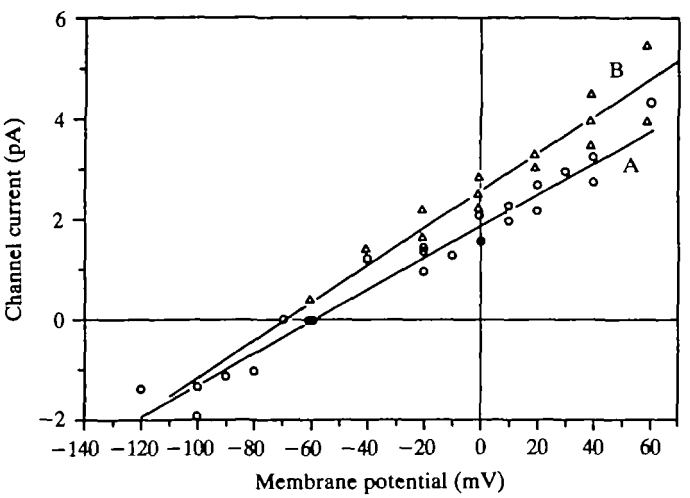

Fig. 8. A comparison of the current–voltage relationship for normal saline (1·6 mmol l^{-1} potassium) and zero sodium saline (1·6 mmol l^{-1} potassium). (A) Pipette solution, normal saline; reversal potential at $V_m = -60$ mV; conductance, 33 pS (replotted from Fig. 5). (B) Pipette solution, zero sodium (data from four patches); reversal potential at −68 mV; conductance, 37 pS.

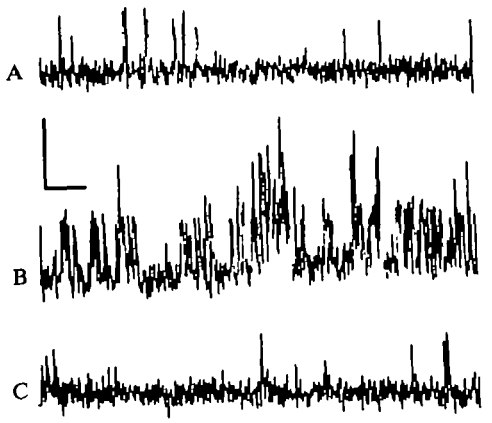

Fig. 9. Effect of membrane stretch on channel activity. 30 mmol l^{-1} potassium in pipette; $V_m = +20$ mV. (A) Activity before suction; (B) activity during 12 cmHg suction; (C) activity after release of suction. This patch contained at least three channels. Scale bars, 1 pA, 500 ms.

which we obtained a gigaseal contained more than one SAK channel. A minimum number of channels in a patch is given by the maximum number of simultaneous openings observed, which, in the patches obtained most recently, for which we have a measure of the pipette tip dimensions, was usually four or more (stretch was applied to activate as many channels as possible). Given a patch area of 10 μm^2 (a generous estimate based on measurements of our pipette tip sizes; cf. Guharay & Sachs, 1984) and an average of four channels per patch, the minimum SAK channel density is one per 2·5 μm^2 of membrane. At this density a cylindrical cell of 10 $\mu m \times 100$ μm (a typical size for *Lymnaea* ventricle cells) would have about 1200 SAK channels.

Effect of TEA, 4-AP and quinidine

The potassium channel blockers TEA (Stanfield, 1983) and 4-AP (cf. Adams, Smith & Thompson, 1980) had no effect on the channel currents at concentrations up to and including 10 mmol l^{-1}. Conversely, 1 mmol l^{-1} quinidine completely abolished the channel conductance in about 75% of the cells examined. It is unlikely that the failure to observe currents in these cases was due to the absence of SAK channels as they were always detected in control patches (no quinidine) obtained on cells in the same culture dish. In the instances where 1 mmol l^{-1} quinidine did not completely abolish the channel currents, they were reduced by up to 90% of normal within a few minutes of exposure. At a quinidine concentration of 0·25–0·5 mmol l^{-1}, channel currents were reduced by 30–70% within about 5 min of exposure.

Quinidine (0·25 mmol l^{-1}) gradually reduced the probability of the channel being open. After several minutes of exposure to this drug, it was possible to obtain sufficient channel openings for current measurements only by applying suction to the patch. When the patched membrane was stretched after about 4 min of exposure to 0·25 mmol l^{-1} quinidine, the channel current magnitude declined within several

seconds to about 20% of the value measured just after the onset of suction (Fig. 10). Under these conditions the decline in the current magnitude was much more rapid than the decline observed in the unstretched membrane.

DISCUSSION

The isolated cells

A problem inherent in physiological studies of disaggregated tissue is the difficulty of assessing the degree of cell damage incurred during enzyme treatment. This leads to uncertainty about the extent to which results obtained with isolated cells represent their behaviour *in vivo*. A good indicator of the integrity of the cell membrane is the transmembrane potential difference. In the isolated *Lymnaea stagnalis* heart cells, the resting potential was virtually identical to that reported for these cells *in situ* (about −60 mV in both cases) (Brezden & Gardner, 1984). Consequently, it is likely that the treatment used in this study did not result in serious disruption of cell function. Furthermore, the cells remained viable and were able to contract upon mechanical stimulation for at least 5 days following isolation. We have not examined older cultures, but it is likely that the cells would survive for considerably longer periods with periodic changes of the culture medium.

Channel characteristics

Inspection of the single-channel current records (Fig. 2) makes it immediately evident that the SAK channel has at least one open state and two closed states, since events occur as a burst of openings separated by longer closed intervals.

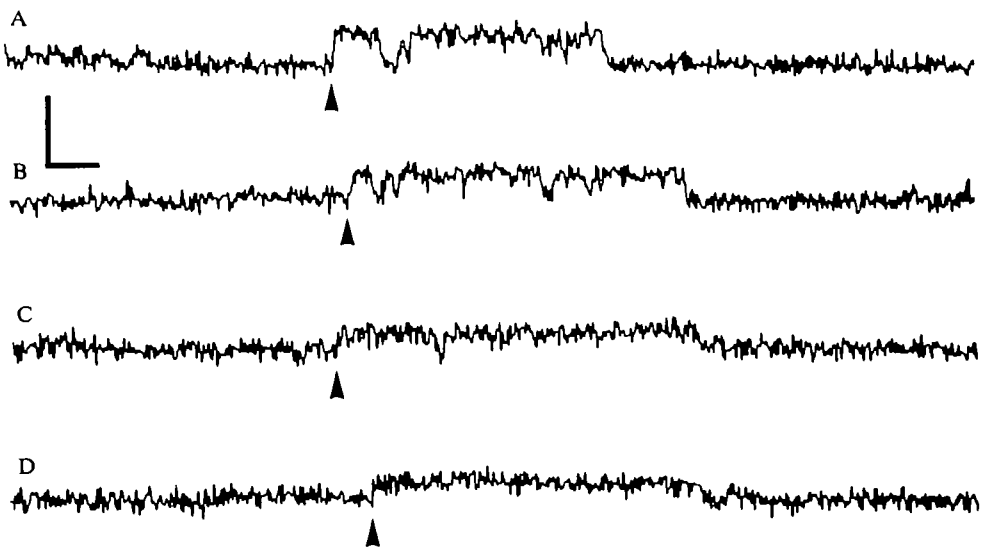

Fig. 10. Effect of 0·25 mmol l^{-1} quinidine on channel currents. 30 mmol l^{-1} potassium in pipette; $V_m = +20$ mV; 6 cmHg suction applied 4 min after making seal. Note rapid, progressive reduction of channel currents (arrowheads). Elapsed time between (A) and (D) is about 6 s. Scale bars, 1 pA, 10 ms.

The 35 mV shift in the reversal potential caused by an increase of extracellular potassium from 1·6 to 30 mmol l^{-1} corresponds to a slope of 27 mV per 10-fold change in the potassium concentration. Although this is considerably lower than the 58 mV slope expected for a perfect potassium-selective channel, whole cell recording also shows a deviation from an ideal potassium electrode at low extracellular potassium concentrations (Brezden & Gardner, 1984).

When the pipette potassium concentration was raised to 100 mmol l^{-1} (zero sodium, 111 mmol l^{-1} chloride), the reversal potential shifted to about +4 mV. This is equivalent to a 57 mV slope per 10-fold change in the extracellular potassium between 30 and 100 mmol l^{-1}. Again, these results are consistent with those obtained with changes in external potassium during whole-cell recording (Brezden & Gardner, 1984), and suggest that this channel contributes to the resting conductance in these cells.

The hyperpolarizing shift in the reversal potential observed upon removal of external sodium suggests that the SAK channel is permeable to sodium as well as to potassium. Under physiological conditions (1·6 mmol l^{-1} potassium, 50 mmol l^{-1} sodium), application of the modified Goldman–Hodgkin–Katz equation (Moreton, 1968), using the measured reversal potential of −60 mV and an estimated intracellular potassium concentration of 51·5 mmol l^{-1} (Brezden & Gardner, 1984), gives a $P_K:P_{Na}$ ratio of approximately 16. This is close to the ratio of 14 estimated for intact *Lymnaea* heart cells.

The effects of extracellular ion changes on the channel conductance over the range of potassium concentrations examined is not straightforward. Unavoidably, the 100 mmol l^{-1} potassium saline had a higher ionic strength than the other salines. The effects of a generalized increase in ionic strength have not been investigated independently. Nevertheless, the channel conductance increase between 1·6 and 100 mmol l^{-1} potassium is to be expected. Over this range, the conductance of a potassium-selective channel from sarcoplasmic reticulum, for example, increases sharply and reaches half saturation at 54 mmol l^{-1} (Coronado, Rosenberg & Miller, 1980). The similarity of channel conductance at 1·6 and 30 mmol l^{-1} potassium for the *Lymnaea* cells is difficult to explain. The 30 mmol l^{-1} potassium saline contained less sodium (21·6 mmol l^{-1} in 30 mmol l^{-1} potassium saline *vs* 50 mmol l^{-1} in normal saline). Since sodium appears to interfere with potassium permeation through this channel (Fig. 8), sodium reduction would have been expected to increase the channel conductance. Further investigations using excised patches are required to determine the source of these anomalies. It is possible that the I/V relationships contain as yet undetected sublinearities (Yellen, 1984*a*).

Effect of potassium channel blockers

The potassium channel blockers 4-AP and TEA, applied on the external side of the membrane, had no effect on the channel conductance at concentrations as high as 10 mmol l^{-1}. Quinidine, on the other hand, did block the channel currents at a concentration of 0·25–1 mmol l^{-1}. Hermann & Gorman (1984) reported that 0·1 mmol l^{-1} quinidine blocks primarily the delayed potassium current in *Aplysia*

californica neurones. The calcium-activated potassium current was blocked only with 20- to 50-fold higher concentrations. By contrast, Fishman & Spector (1981) found that on neuroblastoma cells quinidine completely blocked calcium-activated potassium currents at 0·04 mmol l^{-1}.

Rather than producing an all-or-none block (cf. tetrodotoxin on sodium channels), quinidine (0·25 mmol l^{-1}) reduces the SAK channel amplitude. This suggests that it may produce a fast local anaesthetic-like block (Yellen, 1984*b*). However, quinidine is probably membrane-permeable (Hermann & Gorman, 1984), so additional effects are also possible. The fact that the full effect of quinidine usually took several minutes to develop may reflect a slow increase in the submembrane quinidine concentration, or perhaps indirect effects induced by this drug. The interpretation of the action of quinidine is further complicated by the acceleration of channel current inhibition during stretch activation of the SAK channels. This suggests that there may be a use-dependent component in the action of quinidine.

Quinidine-sensitive, stretch-sensitive channels may be present in other tissues as well. For example, Germann & Dawson (1984) have recently reported that the colon cells of turtle exhibit quinidine-sensitive, stretch-activated potassium currents.

Relationship of the SAK channel to the resting conductance

Since the reversal potential of the SAK channel coincides with the cellular resting potential, and as both the channel and the resting membrane have similar $P_K:P_{Na}$ ratios, it is likely that the SAK channel is largely responsible for the resting conductance in *Lymnaea* heart ventricle cells. For this conclusion to be valid, however, it is necessary to show that the channel spends some time in the open state when V_m is at the resting potential. This poses a dilemma since a channel has no net flux at its reversal potential, and hence carries no current even though it may be open and conducting. One approach to this problem is to examine whether steady-state channel activation can be detected at voltages both more depolarized and more hyperpolarized than the resting potential. If so, it would be reasonable to assume that activation also occurs at the resting potential. Patches exposed to normal saline all exhibited outward currents when depolarized 20 mV or more from rest, but only in about 20% of the patches examined were inward currents resolved when the membrane was hyperpolarized. The probability of being open is apparently low, therefore, with hyperpolarization in the presence of normal saline. To obtain more information about the gating properties of the SAK channel at $V_m = -60$ mV (resting potential), we used elevated potassium concentrations in the pipette, which would be expected to produce inward currents at rest given an open channel. Under these conditions it was readily apparent that the SAK channel is capable of conduction at the resting potential. Furthermore, it was possible to see outward currents at rest with normal (1·6 mmol l^{-1}) potassium when sodium was absent from the pipette. On a few occasions (usually in the presence of an elevated potassium concentration in the pipette) a smaller conductance channel was also observed, but it was not ubiquitous like the SAK channel and would be unlikely to contribute substantially to the resting conductance. This set of observations leads us to conclude

that the SAK channel probably plays a major role in establishing the resting conductance.

Sensitivity to stretch

The SAK channel does not reveal itself at rest, so its stretch-sensitive character is easily overlooked. Certainly, we discovered its nature by chance through the use of high-potassium pipette solutions which allowed for inward currents at rest: we observed that the channel became very active while suction was being applied to make a gigaseal, then became more quiescent when suction was released.

Guharay & Sachs (1984) describe a stretch-sensitive channel in cultured chick skeletal muscle. This channel, however, is less selective ($P_K:P_{Na} = 4:1$) than the channel we describe here. Like Guharay & Sachs (1984), we can only speculate about possible physiological roles for stretch-activated channels. Since the *Lymnaea* SAK channel is more potassium-selective than the one in chick skeletal muscle, it would be expected to produce rather different effects upon activation. It could, for example, reduce membrane excitability by increasing the sarcolemmal potassium permeability. In a myogenic tissue such as the *Lymnaea* heart, a reduction in membrane excitability might allow the ventricle to fill to a greater degree than would be possible in the absence of SAK channels. Alternatively (or in addition), these channels may play a role in osmoregulation in this freshwater species by releasing osmotically active ions when the cells swell during hypo-osmotic stress. We calculate that for a cell having dimensions of $10\,\mu m \times 100\,\mu m$, containing $50\,mmol\,l^{-1}$ potassium and having 1200 SAK channels, conditions which create a 0·5 pA current through each of the channels for 1 s would lead to the loss of about 1 % of the intracellular potassium in that time (using a probability of being open = 0·5). This 'order-of-magnitude' calculation suggests that it is worthwhile to investigate the possible role of the SAK channel in osmoregulation. That osmoregulating channels may exist in other tissues is suggested by Hamill (1983), who has provided preliminary data on the single-channel activity of an erythrocyte potassium channel which is activated by hypo-osmotic stress. Also, quinine, a potassium blocker and stereoisomer of quinidine, has been shown to interfere with cell swelling-induced potassium efflux in lymphocytes (Sarkadi, Mack & Rothstein, 1984).

We thank the Natural Sciences and Engineering Research Council of Canada for operating grants to DRG (A-4290) and CEM (A-8402), and the Muscular Dystrophy Association of Canada for a grant to CEM. We thank Mr Wade Sigurdson for photographing the isolated *Lymnaea* cells.

REFERENCES

ADAMS, D. J., SMITH, S. J. & THOMPSON, S. H. (1980). Ionic currents in molluscan soma. *A. Rev. Neurosci.* **3**, 141–167.

BREZDEN, B. L. & GARDNER, D. R. (1984). The ionic basis of the resting potential in a cross-striated muscle of the aquatic snail *Lymnaea stagnalis*. *J. exp. Biol.* **108**, 305–314.

BURNSTOCK, G., GREENBERG, M. J., KIRBY, S. & WILLIS, A. G. (1967). An electrophysiological and pharmacological study of visceral smooth muscle and its innervation in a mollusc, *Poneroplax albida*. *Comp. Biochem. Physiol.* **23**, 407–429.

CORONADO, R., ROSENBERG, R. L. & MILLER, C. (1980). Ionic selectivity, saturation, and block in a K^+-selective channel from sarcoplasmic reticulum. *J. gen. Physiol.* **76**, 425–446.

FISHMAN, M. C. & SPECTOR, I. (1981). Potassium current suppression by quinidine reveals additional calcium currents in neuroblastoma cells. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5245–5249.

GERMANN, W. J. & DAWSON, D. C. (1984). Quinidine-sensitive basolateral potassium conductance in turtle colon: activation by cell swelling? *Fedn Proc. Fedn Am. Socs exp. Biol.* **43**, 892.

GUHARAY, F. & SACHS, F. (1984). Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. *J. Physiol., Lond.* **352**, 685–701.

HAMILL, O. P. (1983). Potassium and chloride channels in red blood cells. In *Single Channel Recording* (ed. B. Sakmann & E. Neher), pp. 451–471. New York: Plenum Press.

HAMILL, O. P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F. J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch. ges. Physiol.* **391**, 85–100.

HERMANN, A. & GORMAN, A. L. F. (1984). Action of quinidine on ionic currents of molluscan pacemaker neurons. *J. gen. Physiol.* **83**, 919–940.

HILL, R. B., GREENBERG, M. J., IRISAWA, H. & NOMURA, H. (1970). Electromechanical coupling in a molluscan muscle, the radula protractor of *Busycon canaliculatum*. *J. exp. Zool.* **174**, 331–348.

JACOBSON, S. L. (1977). Culture of spontaneously contracting myocardial cells from adult rats. *Cell Struct. Funct.* **2**, 1–9.

LATORRE, R. & MILLER, C. (1983). Conduction and selectivity in potassium channels. *J. Membrane Biol.* **71**, 11–30.

MORETON, R. B. (1968). An application of the constant field theory to the behaviour of giant neurones of the snail, *Helix aspersa*. *J. exp. Biol.* **48**, 611–623.

SARKADI, B., MACK, E. & ROTHSTEIN, A. (1984). Ionic events during the volume response of human peripheral blood lymphocytes to hypotonic media. I. Distinction between volume-activated Cl^- and K^+ conductance pathways. *J. gen. Physiol.* **83**, 497–512.

STANFIELD, P. R. (1983). Tetraethylammonium ions and potassium permeability of excitable cells. *J. Membrane Biol.* **97**, 1–67.

TWAROG, B. M. (1967). Excitation of *Mytilus* smooth muscle. *J. Physiol., Lond.* **257**, 531–547.

YELLEN, G. (1984*a*). Relief of Na^+ block of Ca^{2+}-activated K^+ channels by external cations. *J. gen. Physiol.* **84**, 187–199.

YELLEN, G. (1984*b*). Ionic permeation and blockade in Ca^{2+}-activated K^+ channels of bovine chromaffin cells. *J. gen. Physiol.* **84**, 157–186.