Two Types of Voltage-Gated K\textsuperscript{+} Currents in Dissociated Heart Ventricular Muscle Cells of the Snail Lymnaea stagnalis

M. S. YEOMAN\textsuperscript{1,2} AND P. R. BENJAMIN\textsuperscript{1}
\textsuperscript{1}Sussex Centre for Neuroscience, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG; and \textsuperscript{2}School of Pharmacy and Biomolecular Sciences, University of Brighton, Moulsecoomb, Brighton BN2 4GJ, United Kingdom

Yeoman, M. S. and P. R. Benjamin. Two types of voltage-gated K\textsuperscript{+} currents in dissociated heart ventricular muscle cells of the snail Lymnaea stagnalis. J. Neurophysiol. 82: 2415–2427, 1999. We have used a combination of current-clamp and voltage-clamp techniques to characterize the electrophysiological properties of enzymatically dissociated Lymnaea heart ventricle cells. Dissociated ventricular muscle cells had average resting membrane potentials of \(-55 \pm 5\) mV. When hyperpolarized to potentials between \(-70\) and \(-63\) mV, ventricle cells were capable of firing repetitive action potentials (8.5 \pm 1.2 spikes/min) that failed to overshoot 0 mV. The action potentials were either simple spikes or more complex spike/plateau events. The latter were always accompanied by strong contractions of the muscle cell. The waveform of the action potentials were shown to be dependent on the presence of extracellular Ca\textsuperscript{2+} and K\textsuperscript{+} ions. With the use of the single-electrode voltage-clamp technique, two types of voltage-gated K\textsuperscript{+} currents were identified that could be separated by differences in their voltage sensitivity and time-dependent kinetics. The first current activated between \(-50\) and \(-40\) mV. It was relatively fast to activate (time-to-peak: 13.7 \pm 0.7 ms at +40 mV) and inactivated by 53.3 \pm 4.9\% during a maintained 200-ms depolarization. It was fully available for activation below \(-80\) mV and was completely inactivated by holding potentials more positive than \(-40\) mV. It was completely blocked by 5 mM 4-aminopyridine (4-AP) and by concentrations of tetraethylammonium chloride (TEA) > 10 mM. These properties characterize this current as a member of the A-type family of voltage-dependent K\textsuperscript{+} currents. The second voltage-gated K\textsuperscript{+} current activated at more depolarized potentials (\(-30\) to \(-20\) mV). It activated slower than the A-type current (time-to-peak: 74.1 \pm 3.9 ms at +40 mV) and showed little inactivation (6.2 \pm 2.1\%) during a maintained 200-ms depolarization. The current was fully available for activation below \(-80\) mV with a proportion of the current still available for activation at potentials as positive as 0 mV. The current was completely blocked by 1–3 mM TEA. These properties characterize this current as a member of the delayed rectifier family of voltage-dependent K\textsuperscript{+} currents. The slow activation rates and relatively depolarized activation thresholds of the two K\textsuperscript{+} currents are suggestive that their main role is to contribute to the repolarization phase of the action potential.

INTRODUCTION

Invertebrate preparations with their identified neurons and accessible muscle systems have proved suitable preparations for understanding transmitter complexity and relating cellular changes to behavioral plasticity (Kupfermann 1991). A large number of studies have examined how transmitters can alter muscle types, but how these substances affect the detailed ionic conductances of muscle cells has been relatively little studied, particularly in rhythmically active systems like the heart. The recent development of methods for dissociating muscles in to their constitutive fibers in invertebrate preparations (Brezden and Gardner 1986; Brezina et al. 1994a; Dorsett and Evans 1991; Laurienti and Blankenship 1996a) has facilitated the study of muscle electrophysiology, but there are still very few detailed reports of the ion current complement of invertebrate muscle cells. Those that have been published have concentrated on the unstriated muscles of invertebrates, which are most similar to vertebrate smooth muscle. These include studies on the accessory radula closer (ARC) muscle of Aplysia (Brezina et al. 1994a–c; Brezina and Weiss 1995; Ram et al. 1990, 1991) and its antagonist the accessory radula opener (ARO) (Scott et al. 1997), the buccal mass retractor muscles of Philine (Dorsett and Evans 1991) and most recently the parapodial swim muscles of Aplysia (Laurienti and Blankenship 1996a,b). These muscles contained a mixture of Ca\textsuperscript{2+} and K\textsuperscript{+} currents that was similar to those recorded in invertebrate neurons and are related to those present in vertebrate muscle. These muscles were conspicuous by the absence of a voltage-gated Na\textsuperscript{+} current, a property they have in common with the majority of vertebrate smooth muscles that have been characterized to date. On the other hand, invertebrate heart muscle has been classified by Hoyle (1964) as cross-striated because it appears identical under the light microscope to the skeletal muscle of vertebrates and arthropods (Brezden and Gardner 1992). It is therefore different from those invertebrate muscles that have been studied previously. Early work using extracellular recordings from the whole heart of a variety of bivalve mollusks showed that this organ like its vertebrate counterpart fired regular action potentials (for review see Deaton and Greenberg 1980). The shape of these action potentials was extremely variable, but two components were usually identifiable. These were a fast spike followed by a slower plateau. Ionic substitution experiments have shown that in the majority of species tested the spike phase was Ca\textsuperscript{2+}-dependent, whereas the plateau phase was dependent on the presence of extracellular Na\textsuperscript{+} ions. Thus it appears that the majority of bivalve hearts contain both Na\textsuperscript{+}-permeable and Ca\textsuperscript{2+}-permeable ion channels.

Action potentials have also been recorded from single muscle fibers of the auricle of another type of mollusk, the snail Lymnaea (Buckett et al. 1990c). Using intracellular recording techniques on the whole heart, these authors demonstrated the presence of simple spikelike events, although the ionic depen-
dence of these events was not examined. Other work by Brezden and colleagues, using the cell-attached patch-clamp technique, has demonstrated the presence of several ion channel types in dissociated *Lymnaea* ventricle cells (for review see Brezden and Gardner 1992). These included at least one type of K+ current that probably contributed significantly to the resting membrane potential of the cell. This channel also showed some sensitivity to stretch, indicating that it may have other functions (Brezden and Gardner 1986).

The innervation of the heart of *Lymnaea* is extremely complex, and previous work by Buckett (1990a–c) has shown that this organ is regulated by a variety of classical and peptide neurotransmitters. Our detailed knowledge of the motor neurons innervating the heart and their transmitter content makes this system extremely tractable as a model for examining the mechanisms of action of transmitters that modulate muscle contractility. In particular, we are interested in understanding the role of multiple co-localized peptides that are encoded by a single neuropeptide gene. The gene and its products have been shown to be present in a single motor neuron that innervates the heart (Benjamin and Burke 1994).

To determine the detailed effects of this complex set of neuropeptides, it was important to first characterize the ion channels present in the *Lymnaea* heart muscle fibers. To date, nothing is known about the voltage-gated currents present in these cells and their relationship to the generation of the spontaneous action potentials. The data presented in this and the following paper (Yeoman et al. 1999) on *Lymnaea* provides the first detailed description of the voltage-gated ionic currents present in the heart of an invertebrate and provides the basis for a future detailed examination of the mechanism of action of multiple peptides in cell signaling.

**METHODS**

**Preparation**

Membrane currents were characterized in dissociated heart ventricle cells. These were chosen in preference to auricle cells because they are larger and easier to record using the single-electrode voltage-clamp (SEVC) technique employed here.

**Dissociation of ventricle muscle fibers**

Ventricle muscle fibers were dissociated from hearts of 2- to 3-g *Lymnaea stagnalis* (supplied by Blades Biological, Kent) kept on a 12 h/12 h light/dark cycle at 19°C and fed ad libitum on lettuce. The technique for producing dissociated cells was modified from that described by Brezden and Gardner (1986). Briefly, the heart was dissected from the animal, and the auricle and the remains of the aorta removed. The ventricle was carefully cut in to small pieces (≈1 mm square). The cells were then dissociated by digestion for 20 min in 0.25% trypsin wt/vol (Sigma Type XII S) and subsequently for 75 min in 0.1% wt/vol collagenase (Sigma Type II S), both made up in 0.5 mM Ca2+ Leibowitz medium containing gentamycin (Sigma, 500 μg/ml) and 30 mM glucose. After the digestion the cells were centrifuged at 300 rpm for 5 min, the supernatant removed, and the pellet resuspended in 3.5 mM Ca2+ Leibowitz medium containing gentamycin (Sigma, 500 μg/ml), 30 mM glucose, and 2% vol/vol fetal calf serum. This process was repeated three times. The cells were then stored overnight at 19°C or until required. Cells remained viable in suspension for up to 5 days.

Agar embedding of muscle fibers and drug application

The fibers were immobilized for recording as described originally by Brezina et al. (1994a). Briefly, an aliquot of the cell suspension was mixed with an equal quantity of low melting point agarose gel (GIBCO-BRL: 0.8% wt/vol) and the mixture pipetted into the experimental chamber (35-mm Falcon 3001 Petri dishes). The chamber was then inverted to allow the fibers to move to the surface of the agar, thus facilitating recording. Agar embedding did not appear to alter the properties of ventricle cells. Morphologically, dissociated ventricle cells embedded in agar did not differ from those cells plated directly on glass cover slips (Brezden et al. 1986). Fibers were 40–120 μm long and 10–20 μm wide. The surface of healthy relaxed fibers, unlike those described recently by Brezina et al. (1994a) and Laurienti and Blankenship (1996a), were covered with numerous invaginations as previously described (Brezden and Gardner 1986) and contracted repeatedly in response to injection of short (200 ms) depolarizing current pulses. Injection of large amounts of current (≥0.5 nA) caused the cells to become irreversibly contracted with their membrane taking on a “bleblike” appearance. However, this irreversible contraction did not appear to disrupt the integrity of the cells because both their input resistance and the amplitude of evoked voltage-gated currents were unaltered (data not shown). Further confirmation that the agar embedding was not having any deleterious effects on the cells came from recordings of the resting membrane potential. Resting membrane potentials of the cells varied between −40 and −60 mV with a mean of −55 ± 5 mV (mean ± SE, n = 25). This mean value was similar to that recorded in the intact ventricle (Yeoman, unpublished observations) (−53 mV) and Brezden and Gardner (1986) (−59 mV), who recorded from dissociated ventricle cells in *Lymnaea*. Cells that appeared to be physically damaged usually had membrane potentials much lower than −55 mV and were not used for further experimental analysis. The input resistance of healthy cells calculated over a hyperpolarizing range of potentials −80 to −120 mV varied between 450 and 1,890 MΩ (939 ± 73.9 MΩ, n = 46). The input resistance was dramatically reduced over the depolarized range of potentials (−20 to +50 mV) where currents as large as 20 nA were routinely recorded.

**Drug application**

During recordings the chamber was continually perfused with normal *Lymnaea* saline (composition shown below). The level of fluid in the bath was kept constant by removing the excess using a suction pipette attached to a vacuum pump. Drugs and modified salines were applied by means of a local superfusion pipette, tip diameter 75 μm, placed ∼100 μm away from the cell. Quantitatively similar effects were obtained when the effects of drugs applied via the superfusion pipette were compared with those applied grossly to the bath, indicating that the perfusate from the superfusion pipette was reaching the whole cell. Using dyes in the superfusate, solutions were seen to be fully exchanged within 15 s. In these experiments drugs were applied for at least 30 s before commencing voltage-clamp recording.

**Electrophysiological techniques**

**CURRENT-CLAMP RECORDINGS.** Isolated cells were impaled with glass microelectrodes pulled from 1-mm borosilicate glass (100FT Clark Electromedical) and filled with 3 M KCl to give final resistances of 20–30 MΩ. Cells were impaled, and recordings of the voltage changes in the cell were made using the discontinuous current-clamp configuration on the Axoclamp 2B (Axon Instrument). Data were recorded simultaneously on a Gould 2 channel pen recorder and on to digital audio tape (DAT) using a Biologic 8 channel recorder. Spontaneous action potentials were only generated from membrane potentials between −70 and −63 mV. In the majority of cells this meant
injecting a small amount of constant hyperpolarizing current, via the recording electrode.

VOLTAGE-CLAMP EXPERIMENTS. The surfaces of dissociated Lymnaea ventricle cells have numerous invaginations, making whole cell recordings using a patch pipette extremely difficult. It was therefore decided to use sharp microelectrodes to perform a voltage-clamp analysis of membrane ion currents (cf. Brezina et al. 1994a). Due to the small size of the muscle fibers (~40–120 μm long by 10–20 μm wide), it was extremely difficult to impale these cells with two microelectrodes and maintain the integrity of the cell for more than a few seconds. We therefore chose to use the discontinuous SEVC technique to analyze the ion currents in these fibers. Electrodes were pulled from 1-mm, thin-walled filamented borosilicate glass (100FT, Clark Electromedical) and filled with 3 M KCl. Electrodes had resistances of between 20 and 30 MΩ. Unlike the ARC muscle of Aplysia where isolated muscle cells showed a slowly developing hyperpolarization-activated Cl− current following prolonged recording with KCl electrodes (Brezina et al. 1994a), we did not observe any change in current amplitude during our recordings, and it was therefore decided to use 3 M KCl as our pipette solution. The bath was grounded via an agar bridge containing 3 wt/vol agar made up in 3 M KCl, connected to a Ag-AgCl half-cell that was filled with 3 M KCl. Voltage-clamp recordings were made using an Axoclamp 2B amplifier with data sampled at 20 kHz and recorded directly on to the computer using a Digidata 1200 data acquisition system and pClamp software (Axon Instruments, Foster City, CA). The switching frequency used in these experiments varied between 13 and 18 kHz depending on the cell, producing voltage steps that settled within 2–3 ms of the depolarizing pulse. However, to eliminate any disparities between the command voltage and the actual voltage of the cell, all records of currents shown in this and the following paper use the command voltages. The final statistical analysis of the currents based on current-voltage (I-V) curves were constructed using actual voltages. Voltage-activated currents were initially identified using ramp protocols and then further characterized using voltage step protocols. Voltage protocols were given at regular intervals (1 s). This time allowed identical I-V relationships with sequential voltage protocols. Currents of different types were separated both by means of voltage protocols where appropriate and/or by the use of pharmacological blocking agents.

Reversal potentials for the potassium currents were calculated using tail currents (see Fig. 10 for specific protocol). All data were initially recorded unfiltered and without leak subtraction. Data were filtered off-line at 200 Hz. Leak subtraction of records was also performed later in one of two ways. In some cells the leak current was calculated after all other currents had been blocked by the application of pharmacological agents. This was achieved by bathing the cells in a saline containing (in mM) 3.5 NaCl, 1.7 KCl, 3.5 CaCl2, 4 MgCl2, and 10 N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES). The pH was adjusted to 7.8 using NaOH. In current-clamp experiments where we examined the effects of removing either Na+ or Ca2+ ions on action potential generation, Na+ ions were replaced by an equimolar concentration of choline (zero Na+ saline), or Ca2+ ions were replaced by an equimolar concentration of Cd2+ (zero Ca2+ saline). For the voltage-clamp experiments, pharmacological agents (TEA or 4-AP) were added to the saline at the expense of an equimolar concentration of Na+ ions. To calculate the selectivity of channels for K+ ions, extracellular K+ concentrations were raised from 1.7 mM to either 4.76 or 17 mM at the expense of Na+ ions, to give 4.76K or 17K saline. In some experiments extracellular K+ concentrations were raised to 100 mM (100K saline). The osmolarity change associated with this increase was partly offset by removing all of the Na+ (50 mM) and Ca2+ (3.5 mM) ions from the normal saline. However, it should be noted that the final osmolarity of this saline is approximately one-third higher than that of the normal saline. All chemicals and drugs used in this paper were obtained from Sigma Chemical Co., Poole, U.K.

Adequacy of space clamp

Lymnaea ventricle muscle cells are long and thin, and it was therefore important to check whether they could be adequately space clamped. Fibers were injected with hyperpolarizing square-wave current pulses and the shape of the voltage response during the onset of the current pulse was examined (Brezina et al. 1994a). In general the voltage trace could be fitted by a single exponential at both depolarized (~30 mV) and hyperpolarized (~80 mV) potentials, indicating that the fibers were being adequately clamped. It would have been preferable to have confirmed this finding by directly recording the voltage response of the cell at two different locations along the length of the fiber under voltage-clamp conditions. However, due to the small size of the fibers, it proved impossible to impale them with more than one electrode.

To substantiate these results, we used the following equation to estimate the length constant (λ) in an average Lymnaea ventricle muscle cell

\[ \lambda = \sqrt{r \times R_m/r_i} \]

where \( r \) is the radius, \( R_m \) is the membrane specific resistance, and \( r_i \) is the internal resistance. As the value of \( r_i \) increases as the ionic strength of the cytoplasm decreases, we estimated \( r_i \) to be between 250 and 500 Ω/cm (i.e., greater than that of the frog). The length constant for an average cell, 100 μm long and 15 μm wide and with an average input resistance (~40 to ~20 mV) of 65 MΩ was calculated to be between 1.03 mm and 730 μm. This suggests that the response of the cell to an electrode placed in the center of the fiber will decrement by between 1.8 and 2.5% depending on the internal resistance. Thus, with a voltage step from −100 to 0 mV at the tip of the electrode, the voltage step at the ends of a typical muscle cell will be between 98.2 and 97.5 mV. The voltage range over which the membrane resistance of the cell was measured is the upper operating range of voltages seen in these cells. At these potentials the muscle fibers shorten significantly (up to 50%) thereby increasing the quality of the space clamp. At more depolarized potentials (~30 to ~50 mV) the specific membrane resistance decreases to values close to 6 MΩ. Under these conditions the length constant is reduced to ~300 μm. This is still six times the length of the original cell, and thus the data we present at these membrane potentials may be less quantitative but are still valuable.

Statistical analysis of data

All values quoted in the text represent means ± SE (standard error of the mean). Unless stated otherwise in the text, groups of data were compared using an unpaired t-test assuming unequal variances. Probability values <0.05 were taken as significant.
spikelike action potentials could be generated on the rebound from the injection of constant current leads to the generation of spontaneous action potentials (A1). Two types of action potential, spike (A2) and spike/plateau (A3), were recorded. N.B.: these spikes fail to overshoot 0 mV. B: application of 5 mM tetraethylammonium chloride (TEA) caused an increase in the duration of the action potential. This increase was initially apparent 10 s after the application of the TEA (B2) and continued to increase before reaching a steady state at 30 s (B3). The effects of TEA were almost completely reversible on washing (B4). C: the spikelike action potentials were blocked by the application of 3.5 mM Cd$^{2+}$ (C2), indicating that they were probably due to the influx of Ca$^{2+}$ ions. This effect was also reversed on washing with normal saline (C3). D: regular spikelike action potentials could be generated on the rebound from the injection of a 2-s hyperpolarizing current pulse (100 pA; see arrow D1). These spikes were unaffected by the application of a zero Na$^{+}$ saline (D2) but could be blocked by the application of 3.5 mM Cd$^{2+}$ (D3). The effects of the Cd$^{2+}$ could be reversed by washing the cell with normal saline (D4). Action potentials in the cells shown in A–C were elicited by hyperpolarizing the cells to −65 mV by injection of steady current. Recordings in A–D were all from different ventricle cells.

**RESULTS**

*Dissociated Lymnaea heart ventricle cells generate two types of action potential*

Isolated heart ventricle cells were capable of generating repetitive spontaneous action potential–like events at membrane potentials between −70 and −63 mV (Fig. 1A1; n = 20). Two distinct types of spikelike events could be recorded at these membrane potentials. The first had a simple waveform with a “fast” rising and slower repolarizing phases and was 2–4 s in duration (Fig. 1A2). These events were never associated with visually observed muscle contractions. The second type (Fig. 1A3) had a more complicated waveform. It consisted of an initial brief spike followed by a sustained plateau phase of varying duration (2–4 s). The presence of the plateau phase was always associated with a strong contraction of the muscle fiber, which lasted for the duration of the plateau. Every muscle fiber that we recorded under current-clamp conditions (n = 20 fibers) showed both types of action potential–like event, indicating that these two different events were not cell specific. In 13 of the 20 cells recorded, there appeared to be no regularity in the pattern of firing. However, in the remaining seven cells, action potentials were generated with extreme regularity with rates ranging between 1 spike/5 s and 1 spike/15 s in different preparations (mean of 8.5 ± 1.2 spikes/min, n = 7 fibers). In all cases the rate in isolated cells was slower than the rate of spontaneous heart beat recorded in the intact animal which is ~1 beat/3 s but was comparable to rates seen in the isolated heart that was constantly perfused with saline in an organ bath (Buckett et al. 1990a–c). The ionic independence of these action potentials was examined under current-clamp conditions. Initially, the effects of applying the K$^{+}$ channel blocker TEA on the waveform of spontaneously generated action potentials was examined (Fig. 1B). Ten seconds after superfusion of the cell with 5 mM TEA, a noticeable broadening of the action potential was observed (Fig. 1B2), when compared with controls (Fig. 1B1). This effect reached a maximum by 30 s (Fig. 1B3) with spike widths increasing by 260 ± 32% (mean ± SE; n = 6; P < 0.01). The effects of the TEA were almost completely reversible after a 60-s wash with normal saline (Fig. 1B4). To test whether the influx of Ca$^{2+}$ was important in generating the spike, the heart was perfused with a saline in which the Ca$^{2+}$ ions had been replaced by 3.5 mM Cd$^{2+}$, a selective Ca$^{2+}$ channel blocker. An example of one such experiment (n = 5) is shown in Fig. 1C. Before the application of the Cd$^{2+}$ saline, cells were capable of generating fast action potentials (Fig. 1C1). Application of the Cd$^{2+}$ saline prevented the cells generating action potentials. Instead we observed a number of low-amplitude (10 mV) membrane depolarizations (Fig. 1C2). These lacked the fast rise and fall characteristic of the action potentials. On washing the preparation with normal saline, the fast action potentials returned. It appeared therefore that the fast spikelike events were dependent on extracellular Ca$^{2+}$ ions. Despite these results, it was still possible that Na$^{+}$ ions were important for spike generation. However, perfusion with a saline containing zero Na$^{+}$ ions had no observable effect on the amplitude of an evoked muscle cell spike (Fig. 1D2) when compared with controls (Fig. 1D1). In these experiments action potentials were reproducibly generated by applying a hyperpolarizing square-wave current pulse to the cell and generating an action potential on the rebound. As in the previous experiment, action potentials generated by this method were reversibly blocked by Cd$^{2+}$ ions (Fig. 1, D3 and D4). The ionic basis of the plateau potential was not investigated in the present experiments.

**Characterization of K$^{+}$ currents present in dissociated Lymnaea ventricle cells by voltage clamp**

Having determined that the efflux of K$^{+}$ ions was important in determining the duration of the spontaneously generated action potentials, we characterized the types of voltage-gated K$^{+}$ currents that might underlie this phase of the action potential.

In an initial series of experiments aimed at isolating the currents present in dissociated ventricle muscle fibers, a series of voltage ramp protocols were used. Although these types of protocol preclude a characterization of time-dependent currents, they can provide information about whether or not the cells show inward or outward rectification at different membrane potentials. Cells were held at −60 mV and stepped briefly to −90 mV (25 ms) to remove any channel inactivation that might have been present at the holding potential of the cell. Cells were then ramped to +40 mV over a 2.5-s period. The I-V plot shown in Fig. 2 shows a record of the ramp current from a single ventricle muscle cell. Over the hyperpolarized range (−90 to −60 mV), the I-V plot is flat, indicating that there was no voltage-dependent current flow across the mem-
brane. This therefore excludes the presence of an inward rectifier, which would appear as a steepening downward deflection of the I-V curve at these potentials. Hyperpolarizing the cell from its resting membrane potential using a series of voltage steps to potentials produced small linear leakage currents, again confirming the absence of an inward rectifier current. At more depolarized potentials (−50 mV) the cells started to show a pronounced outward current that increased as the membrane potential became more depolarized. The majority of cells (≈85%, n = 30) also had two regions of inward rectification. The first occurred around −60 mV (Fig. 2; I₁), whereas the second occurred at more depolarized potentials, −30 mV(Fig. 2; I₂). Although I₂ is difficult to visualize in ramps performed in normal saline, we will show in the following paper (Yeoman et al. 1999) that these regions of inward rectification are due to activation of two different classes of voltage-gated Ca²⁺ current and that I₂ recorded in normal saline is essentially masked by the large voltage-gated K⁺ currents and is thus barely evident.

**Muscle fiber types**

Although there was no obvious morphological differences between the muscle fibers, an analysis of the membrane ion currents in Lymnaea heart ventricle cells indicated the presence of two different fiber types (N.B. both these types of fiber could generate both the spike and spike/plateau type of action potentials). These differences did not manifest themselves clearly during voltage-ramp protocols but were clearly evident in cells that had undergone a series of voltage-step protocols designed to characterize time-dependent currents. An example of the current profile of each of the two types of fiber is shown in Fig. 3, A1 and A2. These cells were initially held at −90 mV and stepped to −120 mV for 25 ms and then in 10-mV steps from −70 to +40 mV (200 ms). Although there was no statistical difference in the peak amplitude of the outward currents recorded in the two cell types, several differences were observed in the current complement of the two fibers. Outward currents present in type I muscle cells were relatively slow to peak (23 ms with voltage steps to +40 mV) and showed marked inactivation during the duration of the 200-ms step that became increasingly more obvious as the membrane potential was stepped to more positive potentials (25.4 ± 2.9% at +40 mV). Inactivation was measured as the difference between the size of the peak current and the current flowing at the end of the

![FIG. 2. Slow voltage ramp protocols provide steady-state current-voltage (I-V) curves for Lymnaea ventricle cells. An I-V relationship of the total membrane current evoked using a fast voltage ramp protocol (52 mV/s) in normal saline. There is no change in the holding current between −90 and −70 mV. At −60 mV, there is an area of negative slope resistance (NSR; I₁). At potentials above −50 mV the current becomes outward increasing in amplitude as the voltage to which the cell is ramped becomes more depolarized. Between −30 and −20 mV, there is another region of NSR (I₂). I₁ represents the membrane current, whereas V_m represents the recorded membrane potential of the cell.](http://jn.physiology.org/)

![A1](http://jn.physiology.org/)

**A1**

**Cell Type I**

![A2](http://jn.physiology.org/)

**A2**

**Cell Type II**

![B](http://jn.physiology.org/)

**B**

![C](http://jn.physiology.org/)

**C**

**FIG. 3. Lymnaea ventricle muscle cells can be divided into 2 populations. A: currents evoked by holding cells at −90 mV, prestepping the cells briefly to −120 mV for 25 ms and then to a range of potentials between −70 and +40 mV (200 ms) could be divided into 2 populations according to their waveforms. Cell type I (A1) has outward currents that are relatively slow to peak and show significant inactivation during the 200-ms voltage step. Cell type II (A2) have outward currents that are much faster to peak and show relatively little inactivation during the voltage step. Both cell types also have marked inward currents indicated by the arrows. B: the I-V relationships of the 2 cells shown in A, illustrating the similar activation thresholds and amplitude of the total membrane currents. C: voltage steps (200 ms) to a variety of membrane potentials can evoke contractions of Lymnaea ventricle cells. Graph shows a plot of mean %contraction [(original length − contracted length)/original length × 100] vs. membrane potential for both cell types. N.B.: the stronger contractions in type II cells.**
Fig. 3B. Outward currents in both cell types activated between 50 and 20 mV. At potentials more positive than this, the degree of contraction became weaker presumably due to a combination of the increasing size of the outward current (see Fig. 3) and the decreasing size of the inward Ca$^{2+}$ current (see Yeoman et al. 1999). These experiments were all performed on the largest cells with lengths between 100 and 120 μm. The technique thus allowed us to resolve changes ≥1 μm.

The majority of cells we recorded could be classified as either type I or type II (82%). However, 18% of cells had intermediate properties, suggesting that this classification was not complete.

Isolation of $I_{K(A)}$

$I_{K(A)}$ was isolated from the total whole cell current in type I fibers by superfusing the cells with low concentrations of TEA (1–5 mM). An example of one such experiment is shown in Fig. 4A. Figure 4AI shows the series of currents evoked in a type I muscle cell initially held at −90 mV, stepped briefly to −120 mV (25 ms), and then depolarized in 10-mV steps from −70 to +40 mV (200 ms). Application of either 1 mM (Fig. 4A2) or 5 mM (Fig. 4A3) TEA, left a series of smaller transient currents that inactivated faster than the whole cell current gained by calculating how much the fiber had shortened as a percentage of the relaxed fiber length. Figure 3C shows a plot of the combined data from five experiments where %contraction was plotted against membrane potential of the cell. Two lines are plotted, based on the electrophysiological characterization of the fibers detailed above. Type I fibers (●) showed a maximum contraction of 11 ± 1% of their original length ($n = 5$), whereas type II fibers (■) showed average contractions of 48 ± 4% ($n = 5$). In both cases the muscle cells began to contract at around −50 mV and reached their peak contraction with voltage steps to −20 mV. At potentials more positive than this, the degree of contraction became weaker presumably due to a combination of the increasing size of the outward current (see Fig. 3) and the decreasing size of the inward Ca$^{2+}$ current (see Yeoman et al. 1999). These experiments were all performed on the largest cells with lengths between 100 and 120 μm. The technique thus allowed us to resolve changes ≥1 μm.

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A1 Type II Cell
Normal Saline

A2 5mM TEA

A3 10mM TEA

A4 Δ(5.0mM) TEA

B1 Normal Saline

B2 3.5mM Cd²⁺

B3 Δ (3.5, 0mM) Cd²⁺

FIG. 5. Isolation of $I_{K(A)}$ from a type II cell. A: currents evoked in a type II cell, in normal saline, from cells held at −90 mV, prestepped to −120 mV (25 ms) and then stepped positively in to a range of potentials between −70 and +50 mV (A1). Perfusion of the cell with 5 mM TEA turned the current waveform from that of a type I cell to one resembling a type I cell (A2), which contained a mixture of $I_{K(A)}$ and $I_{K(V)}$. Increasing the dose of TEA to 10 mM changed the current waveform to one resembling $I_{K(A)}$ (A3). Currents shown in A4 represent the difference currents between A1 and A2. B1: a different type II cell perfused with 3.5 mM Cd²⁺, yields a current (B2) that is qualitatively similar to that in A2. The current blocked by the Cd²⁺ (B3), i.e., B1 minus B2) is presumably Ca²⁺ dependent and has a similar waveform to that blocked by 5 mM TEA (A4).

recorded in Fig. 4A1. The lack of sensitivity to TEA and the significant inactivation seen during the 200-ms voltage steps are both characteristics of an A-type current. The residual current activated at potentials between −50 and −40 mV (−45 ± 1 mV, n = 14) and increased in amplitude as the cell was stepped to more positive potentials (Fig. 4B). This activation threshold was within the range of potentials seen for A-currents in a variety of tissues in different organisms (Rudy 1988). Increasing the TEA concentration to 10 mM blocked the residual transient current almost completely. (Fig. 4A5). Unlike a number of A-currents described in the literature, the time-to-peak current for $I_{K(A)}$ was relatively slow, increasing from 50.4 ± 5.2 ms at −10 mV to 12 ± 1.4 ms at +40 mV (n = 8; Fig. 4, A2 and A3). A decrease in the time-to-peak as the voltage step becomes more depolarized is characteristic of all “A”-type currents (Rudy 1988). The inactivation of $I_{K(A)}$ in type I cells was calculated by fitting an exponential curve to the declining phase of the current from its peak to the end of the voltage step. This inactivation phase fitted best to a single exponential with a mean time constant (τ) of 145 ± 31.5 ms (n = 12). The current blocked by the lower concentrations of TEA (1–5 mM) in type I cells showed all the characteristics of a delayed rectifier-type current [$I_{K(V)}$; Fig. 4A4]. It was slow to activate, showed very little inactivation during the 200-ms voltage pulse, and was sensitive to low concentrations of TEA. This current will be described in detail in the next section. Washing the preparation with normal saline removed the TEA block (cf. Fig. 4, A1 and A6).

Isolation of $I_{K(A)}$ from type II fibers was achieved in a similar way to type I fibers. Application of low concentrations of TEA (5 mM) altered the waveform of the outward currents from one of a type II cell (Fig. 5A) to that representative of a type I cell (see Fig. 5A2). Addition of 10 mM TEA allowed us to resolve $I_{K(A)}$ in this cell type (Fig. 5A3). The complex waveform of the current blocked by 5 mM TEA (Fig. 5A4) in the type II cell is suggestive that this is in fact two separate currents. Superfusion of type II cells with 3.5 mM Cd²⁺ (Fig. 5B2) a specific Ca²⁺ channel blocker was also shown to block current(s) with the same waveform as those blocked by 5 mM TEA (compare Figs. 5A4 and 5B3). We therefore conclude that as well as having both $I_{K(A)}$ and $I_{K(V)}$ type II cells also possess one or more Ca²⁺-dependent currents whose properties were not investigated further in the present experiments.

A comparison of $I_{K(A)}$ currents isolated from type I cells and type II cells demonstrated that there was no significant difference in the activation thresholds of the two currents (−41 and −45 mV, respectively; P > 0.05). Similarly, there were no differences in the time-to-peak with voltage steps to +40 mV (12 ± 1.4 ms and 15 ± 0.8 ms, respectively; P > 0.05; mean for both fiber types 13.7 ± 0.7 ms) or the inactivation rate recorded at the same potential (τ = 145 ± 31.5 and 94 ± 10.3 ms, respectively, for the 2 different cell types; P > 0.05; mean for both fiber types 102.6 ± 10.6 ms) of the two currents. This indicated that $I_{K(A)}$ isolated from both type I and type II fibers were probably the same current.

Holding potential dependence of $I_{K(A)}$

A-type currents, like all voltage-dependent currents, show increasing steadystate inactivation when the holding potential from which they are evoked becomes increasingly more positive (Hille 1992). To determine the holding-potential dependence of $I_{K(A)}$, the current was first isolated by perfusing type I cells with 5 mM TEA. Muscle fibers were then stepped from −70 to +50 mV from a series of different holding potentials (−90 to −30 mV) and I-V plots constructed from the peak current evoked at each membrane potential. Currents evoked from a variety of holding potentials are illustrated in Fig. 6. At a holding potential of −90 mV, $I_{K(A)}$ was fully available for activation (Fig. 6A). However, as the holding potential became more positive the amplitude of $I_{K(A)}$ was reduced (Fig. 6, A2–A5) until at potentials more positive than −40 mV $I_{K(A)}$ was almost completely eliminated (Fig. 6A7). Figure 6B shows I-V plots for the currents evoked at four different holding potentials (−90, −70, −50, and −30 mV). The overall holding potential dependence of $I_{K(A)}$ is illustrated in Fig. 6C, indicating maximal activation of the current from −90-mV holding potentials and complete loss of the current in cells stepped from a holding potential of −40 mV. The data were fitted with a Boltzmann curve giving a $V_{1/2}$ of −66 mV and a slope factor of 11.4 ± 2.1 mV. The residual current seen in Fig. 6, A6 and A7, probably represents an unblocked component of $I_{K(V)}$. 
Sensitivity of I\(_{K(A)}\) to 4-AP

Another characteristic of A-type currents is their sensitivity to the potassium channel blocker, 4-AP. To test the sensitivity of I\(_{K(A)}\) to 4-AP, the current was initially isolated by perfusing a type I muscle fiber with 5 mM TEA. Figure 7 shows an example of an experiment in which a muscle fiber was superfused with increasing concentrations of 4-AP. In normal saline, cells were initially held at \(-90\) mV and stepped briefly to \(-120\) mV (25 ms) and then in 10-mV depolarizing voltage steps from \(-70\) to \(+30\) mV to evoke a series of currents that showed inactivation during the 200-ms voltage step representative of a type I cell (Fig. 7A1). Application of 5 mM TEA left a transient outward current (Fig. 7A2) that was partially blocked by the application of 1 mM 4-AP (Fig. 7A3). Increasing the concentration of 4-AP to 5 mM caused a complete block of the outward current (Fig. 7A4).

The majority of transient currents isolated from Lymanaea heart ventricle cells were sensitive to low concentrations of 4-AP (100% block \(<5\) mM, \(n = 30\) fibers) and showed complete steady-state inactivation from holding potentials more positive than \(-40\) mV. However, in a small subset of cells (10%, \(n = 3\)), the currents appeared relatively insensitive to 4-AP (50% block at 10 mM) but were completely inactivated by holding potentials of \(-60\) mV. The combined data from these 33 cells is illustrated in Fig. 7B, where the mean %block of I\(_{K(A)}\) by 1 mM and 5 mM 4-AP is plotted. In the presence of 1 mM 4-AP, the peak amplitude of I\(_{K(A)}\) was reduced by 52.5 ± 9.6%, with the %block increasing to 84.5 ± 15.2% in the presence of 5 mM 4-AP (\(P < 0.05\)).

K\(^+\) selectivity of I\(_{K(A)}\)

A-type currents show a high selectivity for K\(^+\) ions. Usually, this can be determined by examining the reversal potentials of their tail currents in bath solutions containing different concentrations of K\(^+\) ions [see section on characterization of I\(_{K(V)}\)]. However, the tail currents of I\(_{K(A)}\) were extremely fast and impossible to resolve using the single-electrode voltage-clamp technique. We have therefore used two methods to indirectly illustrate the K\(^+\) selectivity of these channels. The current flowing through I\(_{K(A)}\) channels could either be due to an efflux of positive ions, presumably K\(^+\) or an influx of negative ions, namely Cl\(^-\). To test whether a significant proportion of the current flowing through I\(_{K(A)}\) channels was carried by Cl\(^-\) ions, we substituted 85% of the extracellular Cl\(^-\) ions with methanesulphonate and examined the effects on the amplitude of I\(_{K(A)}\). Figure 8B shows an example of such an experiment. I\(_{K(A)}\) has been isolated from a type I fiber by superfusing the cell with 5 mM TEA. The current was evoked by holding the cell at \(-90\) mV, prestepping the voltage to \(-120\) mV for 25 ms and then stepping the membrane potential to \(+40\) mV. Substitution of 85% of the Cl\(^-\) ions with methanesulphonate had no effect on the amplitude of I\(_{K(A)}\) (\(n = 4\)).
A4

FIG. 7. 4-Aminopyridine (4-AP) sensitivity of \( I_{KA} \). A: shows currents recorded in cells held at \(-90\) mV, prestepped to \(-120\) mV and then depolarized to a series of voltages between \(-70\) and \(+30\) mV for \(200\) ms. Currents recorded from a type I cell in normal saline are shown in A1. Perfusion with a 5-mM TEA saline isolates \( I_{KA} \) (A2). Application of 1 mM 4-AP partially blocked \( I_{KA} \) (A3). An 84% block was achieved following perfusion of 5 mM 4-AP (A4). All recordings were from the same cell. N.B. that the 2 concentrations of 4-AP were applied in the presence of 5 mM TEA. B: bar chart showing the mean % block of \( I_{KA} \) in response to 1 and 5 mM 4-AP (n = 33 cells).

Therefore \( Cl^- \) is unlikely to be the main charge carrier. Further confirmation of the \( K^+ \) selectivity of the channel would be obtained if we could show that \( I_{KA} \) could be reversed by elevating the levels of \( K^+ \) ions bathing the cell. In Fig. 8A2 \( I_{KA} \) is reduced dramatically in amplitude when the cell is bathed with a saline containing 100 mM \( K^+ \) when compared with the control currents evoked in Fig. 8A1. Elevating extracellular levels of \( K^+ \) ions to 100 mM caused a significant increase in the osmolality of the saline bathing the cells, and it was possible that this may have had an effect on the amplitude of \( I_{KA} \). However, the data presented are unlikely to be an artifact of the experimental procedure because all the other evidence presented earlier suggests that this current is a member of the A-type family of potassium currents.

Characterization of \( I_{KV} \)

We have used a variety of methods to isolate and characterize \( I_{KV} \). As we have shown previously (see Fig. 4), application of 1–5 mM TEA to type I cells leaves a transient current with properties that indicate that it is an A-type current. The current that is blocked by TEA is markedly different, and we believe that this current falls into the category of delayed rectifier \( K^+ \) currents that have previously been identified in a variety of cell types (Rudy 1988).

TEA sensitivity of \( I_{KV} \)

First, the current was markedly more sensitive to block by TEA than \( I_{KA} \), being completely blocked by 1 mM TEA, while a substantial proportion of the \( I_{KA} \) still remained active (see Fig. 4). The currents that remained after application of TEA \( I_{KA} \) are illustrated in Fig. 4, A2 and A3, whereas the TEA-sensitive current is illustrated in Fig. 4A4. This sensitivity to TEA is one of the characterizing properties of delayed rectifier type currents in a variety of cell types (Rudy 1988).

Second, the TEA-sensitive current activated at potentials that were significantly more positive than \( I_{KA} \) (\( P < 0.001 \)). Currents were first resolved at potentials between \(-30\) and \(-20\) mV \((-27 \pm 1\) mV) compared with \( I_{KV} \) that activated at potentials between \(-50\) and \(-40\) mV \((-46 \pm 1\) mV; Figs. 4B and 9B). This more depolarized activation threshold is again characteristic of the delayed rectifier type currents (Breznina et al. 1994a; Laurienti and Blankenship 1996a; Miyoshi et al. 1991; Yamamoto et al. 1989). Third, the activation and inactivation of the TEA-sensitive current were much slower than those seen for \( I_{KV} \). The time-to-peak for TEA-sensitive currents ranged between \(175 \pm 11.4\) ms at \(-10\) mV to \(74.1 \pm 3.9\) ms at \(+40\) mV. Because \( I_{KV} \) showed little time-dependent inactivation during the 200-ms voltage step, it was impossible to fit the declining phase of the current to an exponential. We have therefore calculated the % reduction in the amplitude of the current. With voltage steps to \(+40\) mV the TEA-sensitive current showed very little inactivation during \(6.2 \pm 2.1\%\) compared with the \(53.3 \pm 4.9\%\) inactivation seen with \( I_{KV} \) (\( P < 0.001 \)). We cannot exclude the possibility that the small amount of inactivation observed in the TEA-sensitive current was due to a residual \( I_{KA} \). Comparison with \( I_{KV} \) isolated from type I cells and type II cells showed there to be no significant difference in the activation threshold \((-46 \pm 1\) mV compared with \(-45 \pm 1\) mV; \( P > 0.05 \)), the time-to-peak \(74.1 \pm 3.9\) ms compared with \(70.1 \pm 4.5\) ms, \(+40\) mV; \( P > 0.05 \)) and the reduction in the amplitude of the current during the voltage step \(6.2 \pm 2.1\%\) compared with \(7.1 \pm 2.5\%\); \( P > 0.05 \)) for type I and type II cells, respectively.

Holding potential dependence of \( I_{KV} \)

A further property that separates the delayed rectifier type currents from the A-type currents is their holding potential dependence. Although A-type currents are completely inactivated by holding potentials in the range of \(-60\) to \(-40\) mV, the delayed rectifier currents are less sensitive to these low holding potentials. An example of the holding potential depen-
The current was isolated from a type I cell by perfusing the cell with 5 mM 4-AP to block $I_{K(A)}$. Currents were evoked from a variety of holding potentials (−90 to 0 mV; A1–A6) by short (200 ms) voltage steps to potentials between −70 and +50 mV. More depolarized holding potentials caused a reduction in the amplitude of the evoked currents.

**FIG. 9.** Holding potential dependence of $I_{K(V)}$. A: isolation of $I_{K(V)}$, was achieved in this type I cell by perfusing the cell with 5 mM 4-AP to block $I_{K(A)}$. Currents were evoked from a variety of holding potentials (−90 to 0 mV; A1–A6) by short (200 ms) voltage steps to potentials between −70 and +50 mV. More depolarized holding potentials caused a reduction in the amplitude of the evoked currents. B: $I-V$ plot of peak $I_{K(V)}$ currents evoked from 5 different holding potentials. C: holding potential dependence of $I_{K(V)}$ recorded from 3 different cells (different symbols). Data from all cells was fitted with a Boltzmann curve. Data in A are from the same cell.

**FIG. 10.** $K^+$ selectivity of $I_{K(V)}$. A: a series of tail currents evoked by holding the cell at −40 mV to inactivate $I_{K(A)}$, and then stepping the voltage to 0 mV for 200 ms and then down to a variety of potentials between −30 and −140 mV. Tail currents were recorded in normal saline (1.7K; A1) or a saline in which the $K^+$ concentration had been raised to 17 mM (17K saline; A2). B: reversal potentials for the tail currents are plotted against the log of the external $K^+$ concentration (solid line). Dotted line represents the predicted change in reversal potential with changing $K^+$ concentrations, for a current carried solely by $K^+$ calculated from the Goldman-Hodgkin-Katz (GHK) equation.
Hodgkin-Katz prediction for a purely K⁺-selective current of 58 mV (■), indicating that a significant proportion of the current carried through the delayed rectifier channels is carried by K⁺.

DISCUSSION

Dissociated Lymnaea heart ventricle cells are capable of generating regenerative action potentials

Current-clamp recordings were made from acutely dissociated Lymnaea heart ventricle cells. All cells were capable of generating two types of spontaneous action potentials. The first had a prepotential with fast rising and slow repolarizing phases and was the most common type seen. The second consisted of a prepotential and a fast spike that was followed by a plateau phase. The fast spikelike action potentials appear to be dependent on the influx of Ca²⁺ ions, whereas the repolarization phase is sensitive to TEA and is therefore presumably due to the efflux of K⁺ ions. The ionic basis of the plateau phase is unclear because we were unable to find a means of repeatedly activating this type of action potential. In the Mercenaria ventricle (Devlin 1993) the fast spikelike action potentials and the repolarization phase were also due to the influx of Ca²⁺ and efflux of K⁺ ions, respectively. The plateau phase, however, was shown to be the result of an influx of Na⁺ ions. This was a similar result to that found in other bivalve mollusks that had spike/plateau–like action potentials (Deaton and Greenberg 1980; Huddart and Hill 1996). Classical Na⁺ entry via voltage-gated channels can be ruled out as a possible mechanism of plateau generation in Lymnaea ventricle cells as previous work by Brezden and Gardner (1992) using a cell-attached patch technique and our work (see also Yeoman et al. 1999) failed to show the presence of any voltage-gated Na⁺ channels or currents, respectively. If Na⁺ is responsible for the plateau phase, the most likely routes of entry is via calcium-activated nonspecific cationic channels. Our observation that spike/plateau action potentials evoke relatively stronger muscle contractions compared with those evoked by the spike type potentials is suggestive that spike/plateau potentials are associated with a larger increase in intracellular Ca²⁺, which in turn may activate the nonspecific cation channels. The lack of voltage-gated Na⁺ currents in Lymnaea ventricle cells identifies these cells with those recorded in the sinoatrial and atrioventricular nodes of mammalian hearts in which the upstroke of the action potential is solely dependent on the influx of Ca²⁺ (Hagiwara et al. 1988; reviewed in Irisawa et al. 1993).

We believe that this is the first time that spontaneous action potentials have been recorded from dissociated ventricle cells of a mollusk. This general ability of all the Lymnaea ventricle cells to generate action potentials is significant, particularly as the rate of action potential generation (8.5 ± 1.2 spikes/min) is not dissimilar to beat rates recorded from intact isolated hearts (Buckett et al. 1990a–c). This observation is suggestive that the heart of Lymnaea may possess a distributed pacemaker system. However, further work characterizing the pacemaking properties of the auricle are required before this can be confirmed. Previous work on intact molluscan hearts have described three types of action potential, fast and slow spikelike action potentials, and spike and plateau action potentials (Jones 1983). Lymnaea ventricle cells appear to possess two of these action potential types and therefore appears to be most similar to the Mercenaria ventricle, which is also capable of generating the same two types of action potential (Devlin 1993). Both these types of action potential are seen in mammalian heart tissue, although not in the same region of the heart (Katz 1977). Both the sinoatrial node and atrioventricular node (pacemaker regions of the mammalian heart) show classical spikelike action potentials, whereas the auricle, ventricle, bundle of His and Purkinje network show the spike and plateau type of action potential.

Characterization of the potassium currents in Lymnaea ventricle cells

ABSENCE OF AN INWARD RECTIFIER CURRENT. Ramp protocols produced current profiles that were flat in the voltage region between −90 and −50 mV, indicating the absence of an inward rectifier potassium current. This type of current has been characterized in a noncardiac molluscan muscle, the ARC muscle of Aplysia (Breznia et al. 1994a), and similar currents have been reported in mammalian ventricle cells (Irisawa et al. 1993). However, inward rectifying currents are absent from the sinoatrial node pacemaker in the mammalian heart (Giles and Van Ginneken 1985; Irisawa 1987). The absence of the inward rectifier in this region results in a high-input resistance compared with other cardiac cells, and this is thought to be important in increasing the sensitivity of the cell to small current fluxes. Lymnaea ventricle cells also have high-input resistances (939 ± 73.9 MΩ) presumably for the same reason, and this is consistent with their proposed role as pacemaker cells in the heart.

CHARACTERIZATION OF OUTWARD RECTIFYING POTASSIUM CURRENTS. The large outward current seen in both the ramp and voltage-step protocols activated between −50 and −40 mV and increased in magnitude as the membrane potential became more positive. Based on our voltage-clamp analysis, we have classified Lymnaea ventricle cells into two groups, termed type I and type II cells. We have shown that in type I cells the outward current consists primarily of two types of current; an A-type current [I_{K(A)}] and a delayed rectifier current [I_{K(V)}]. Type II cells also contain I_{K(A)} and I_{K(V)} as well as an outward Ca²⁺-dependent current that was not studied in detail in this paper. We found no quantitative differences between the I_{K(A)} and I_{K(V)} currents recorded in the two different cell types. I_{K(A)} activated between −50 and −40 mV and was fully available for activation at holding potentials more negative than −90 mV. As with all A-type currents, I_{K(A)} showed progressive steady-state inactivation because the holding potential from which it was evoked was made more positive. I_{K(A)} was half inactivated at −75 mV and completely inactivated at holding potentials more positive than −40 mV. I_{K(A)} unlike a number of other A-type currents (Connor and Stevens 1971; Neher 1971) was relatively slow to activate (13.7 ± 0.7 ms to peak amplitude at +40 mV), but inactivated fairly rapidly with currents reduced in amplitude by 53.3 ± 4.9% by the end of the 200-ms voltage step. We were able to fit the declining phase of the current to a single exponential with a time constant τ = 102.6 ± 10.6 ms with voltage steps to +40 mV. I_{K(A)} was blocked by low concentrations of 4-AP (1–5 mM) and was relatively insensitive to TEA (<10 mM). These are two further criteria that classify I_{K(A)} as an A-type current (Thompson...
1977). A-type channels are also highly selective for K⁺ ions (Thompson 1977). \(I_{K(A)}\) current amplitude was markedly reduced in muscle cells bathed with salines containing elevated levels of K⁺ ions. More indirect evidence of K⁺ selectivity was obtained by demonstrating that the properties of \(I_{K(A)}\) were unaltered by lowering the concentration of Cl⁻ ions in the saline bathing the muscle cell. Cl⁻ is the only other ion besides K⁺ that could be responsible for \(I_{K(A)}\). The second component of the large outward current seen in steady-state I-V curves is \(I_{K(V)}\). This current had properties that characterized it as a member of the delayed rectifier family of K⁺ currents. It activated −20 mV more positive than \(I_{K(A)}\) between −30 and −20 mV. The amplitude of its currents was also holding potential dependent. Currents were fully available at holding potentials more negative than −90 mV; however, unlike \(I_{K(A)}\) a significant proportion of the current (≈15%) was still available for activation at holding potentials as positive as 0 mV. The time-to-peak of the current was characteristically slower with \(I_{K(A)}\), taking on average 74.1 ± 3.9 ms at +40 mV. \(I_{K(V)}\) was also extremely slow to inactivate, showing little or no inactivation during standard 200-ms voltage steps (6.2 ± 2.1%).

The detailed properties of both the A-type and delayed rectifier currents in different cells are highly variable (Rudy 1988). These differing properties are what gives a particular cell its electrophysiological fingerprint. In particular, the slow activation rates and the relatively depolarized activation thresholds of both \(I_{K(A)}\) and \(I_{K(V)}\) currents in dissociated Lymnaea ventricle cells compared with other A-type and delayed rectifier currents in other muscles and neurons (Rudy 1988) probably help explain the ability of these cells to fire action potentials. It is interesting to note that the activation range for the delayed rectifier current (−30 to −20 mV) in Lymnaea ventricle cells is comparable with that recorded for the delayed rectifier currents present in mammalian ventricular muscle cells (Clapham and Logothetis 1988; Tohse et al. 1987). This activation range differs from that classically recorded in cells from the sinoatrial node (−60 to −50 mV) (Nakayama et al. 1984; Shibasaki 1987). The delayed rectifier current has previously been shown to be important in determining the rate of pacemaking in mammalian hearts (Giles et al. 1982, 1986), and its delayed activation may partially explain the increased duration of Lymnaea ventricular action potentials (2–4 s) compared with that seen in the sinoatrial node (200–300 ms) and the relatively slow rate of the Lymnaea heart in vivo (≈20 beats/min) compared with the (60–80 beats/min) seen in mammalian hearts. A-type currents have been shown to regulate the frequency of firing of action potentials in neurons (Connor and Stevens 1971). However, the relatively depolarized activation threshold and slow activation kinetics suggests that this is not its main role in Lymnaea ventricle cells. The properties of \(I_{K(A)}\) suggest that it is involved in the repolarization of the action potential. Transient A-type currents can be recorded in the Purkinje and ventricular cells of mammalian hearts where they are responsible for the transient repolarization following the phase 0 depolarization (Callewaert et al. 1984; Coraboeuf and Carmeliet 1982). However, they are characteristically absent from pacemaker cells in the sinoatrial node (Irisawa et al. 1993).

Thus while Lymnaea ventricle cells share some properties in common with the pacemaker cells of the sinoatrial node (high-input resistance, lack of inward rectifier, absence of voltage-gated Na⁺ current, and simple spikelike action potentials), they also have a number of properties in common with mammalian ventricle cells (presence of an A-type current and an inward rectifier current, and a high-threshold delayed rectifier current).

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Address for reprint requests: M. S. Yeoman, School of Pharmacy and Biomolecular Sciences, Cockroft Building, University of Brighton, Moulsecoomb, Brighton, East Sussex BN2 4GJ, UK.

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