Transient outward potassium current in ICC

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Interstitial cells of Cajal (ICC) are the pacemakers of the gut, initiating slow-wave activity. Several ion channels have been identified that contribute to the depolarization phase of the slow wave. Our aim was to contribute to knowledge about the identity and role of ICC potassium channels in pacemaking. Here we describe a transient outward potassium current in cell-attached patches of ICC. This current was activated almost instantaneously at potentials positive of the resting membrane potential and inactivated as a single exponential or biexponential with time constants that varied widely from patch to patch. Averaged traces gave a biexponential inactivation with time constants of ~40 and ~500 ms, with no clear voltage dependence. Analysis of single-channel openings and closings indicated a channel conductance of 5 pS and permeability sequence of K⁺ (111) > Na⁺ (1) > N-methyl-D-glucamine⁺ (0.11). The current was completely blocked by 20 μM clotrimazole but was unaffected by 20 μM ketoconazole, 10 μM E4031, or 20 μM clofilium; 5 mM 4-aminopyridine slowed the activation of the current. The transient outward current may be important in moderating the upstroke of the pacemaker potential.

Knowledge of this background current, apart from reasons in and of itself, was important so that experimentally induced or transient currents could be better distinguished or isolated. We found that the background current was carried by potassium and was of the “transient outward” type as characterized in cardiac cells (7). This furthers our previous study that was the first to record single potassium channels in ICC (36).

MATERIALS AND METHODS

Solutions

The names and constituents of HEPES-buffered saline (HS) solutions are given in Table 1. N-methyl-D-glucamine (NMDG) chloride solutions were made by titrating NMDG with HCl to pH 5.2. Culture solution (CS) consisted of Clonetics SmGM-2 bullet kit (basal media, fetal bovine serum, hEGF, insulin, hFGF-B, GA-1000; Lomax, supplied by Cedarlane) with 2% (vol/vol) antibiotic-antimycotic (10,000 U/ml penicillin, 10 mg/ml streptomycin, 25 μg/ml amphotericin B; Sigma).

Cell Culture

All procedures were approved by the Animal Ethics Board of McMaster University. CD-1 mice of 5–15 days of age were euthanized by chloroform inhalation followed by exsanguination. The intestines were removed into cold HS, the jejenum pinned out along the mesentery, cut open along the mesenteric border and the mucosa pulled off. Pieces of muscle were incubated for 15 min at 36°C in HS with the addition of 0.5 mM CaCl₂, 0.3 mM MgCl₂, 1 mg/ml type F collagenase, 1 mg/ml bovine serum albumin, 0.5 mg/ml papain, 0.5 mg/ml soybean trypsin inhibitor and 0.2 mg/ml (−)-1,4-dithio-L-threitol (all from Sigma). The muscle was then washed with HS and triturated with glass Pasteur pipettes (fire-polished bore) to give a cell suspension. Cells were settled on collagen-coated coverslips for an hour before being washed into CS and cultured in a humidified, 5–95% CO₂–O₂, 36°C incubator for 3–4 days before use. ICC were identified as small, smooth, roughly triangular cells with processes at each apex, often occurring in clusters (Fig. 1).

Patch Clamp

The patch-clamp apparatus consisted of an Eclipse FN1 microscope (Nikon, Mississauga, ON, Canada), Axon MultiClamp 700B amplifier (Molecular Devices, Sunnyvale, CA), Axon Digidata 1440A, Axon CV-7B headstage, and PCS-6000 micromanipulator (Burleigh/EXFO, Mississauga, ON, Canada). Acquisition software was Clampex 10.2 (Molecular Devices). Currents were filtered by the amplifier with a 2.8-kHz cutoff, low-pass, 8-pole Bessel filter and digitized at 5 kHz. Pipettes had resistances of 5–10 MΩ and gigaseals were in the range of 5–20 GΩ. Voltage-clamp protocols were adjusted according to the junction potential between the pipette and bath solutions (Table 1), with $E_{app} = E_j - E_m$ where $E_{app}$ is the applied (command) voltage, $E_j$ is the junction potential, and $E_m$ is the transmembrane potential. As all experiments were in the cell attached configuration potentials are expressed as relative to resting membrane potential (rrmp) throughout the study.

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Table 1. **HS solutions**

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<th>K⁺</th>
<th>NMDG⁺</th>
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<th>Mg⁺</th>
<th>Cl⁻</th>
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HS, HEPES buffered saline All concentrations are in mM. *Junction potential (Ei) of named solution in pipette with HS(physiological) in bath, calculated in Clampex. †For HS(high Na) with 5 mM 4-aminopyridine, pH was adjusted with HCl. NMDG, N-methyl-d-glucamine.

**Computation and Analysis**

Except where stated all computation was carried out with programs written in C++ by using the wxDev-C++ IDE. Origin (Northampton, MA) was used for regression (fitting exponentials) and plotting of data.

**Crossfile Statistics**

For any set of files recorded from one patch, cross-file current mean and variance (m and σ², respectively) were calculated as

\[
m_{\text{crossfile}}(s_j, t_k) = \frac{1}{N_{\text{files}}} \sum_{i=0}^{N_{\text{files}}} I(f_i, s_j, t_k)
\]

\[
\sigma_{\text{crossfile}}^2(s_j, t_k) = \frac{1}{N_{\text{files}}} \sum_{i=0}^{N_{\text{files}}} \left( I(f_i, s_j, t_k) - m_{\text{crossfile}}(s_j, t_k) \right)^2
\]

where \( I \) is current as a function of file \( (f_i) \), sweep \( (s_j) \), and time \( (t_k) \) and \( N_{\text{files}} \) is the number of files in the set.

**Leak and Capacitance Subtraction and Filtering**

After capacitance subtraction by the MultiClamp software, residual capacitive transients remained with most patches. These transients could not be fitted by a sum of exponentials (explaining why they could not be subtracted by MultiClamp) and so a subtraction algorithm was written based on the P/4 approach (1). Note that this was applied only to voltage-step data, not voltage-ramp data. Briefly,

1) For any set of files recorded from one patch, a voltage step response was chosen where \( \sigma_{\text{crossfile}}^2 \) was low (i.e., with little channel activity; Supplementary Fig. S1B).

2) The \( m_{\text{crossfile}} \) of this response was smoothed using a “graded” boxcar,

\[
I_{\text{capacitance}}(t_0) = \frac{1}{1 + 2F \alpha} \sum_{i=a}^{b} m_{\text{crossfile}}(s_j = \beta, t_k)
\]

where \( \alpha = 0 \) is the start of the voltage step, \( \beta \) is a particular sweep, and \( 1 > F > 0 \) (but was always set to 0.4). In this way the length of the boxcar was equal to 1 at the start of the voltage step and then gradually increased (according to \( F \)). This avoided both edge effects and rounding off of the top of the capacitance transient.

3) Leak current, \( I_{\text{leak}} \), was defined as the mean of \( I_{\text{capacitance}} \) after it had fully decayed.

4) \( I_{\text{leak}} \) and \( I_{\text{capacitance}} \) were scaled to the absolute and change in potential, respectively:

\[
\tilde{I}_{\text{capacitance}} = (I_{\text{capacitance}} - I_{\text{leak}})/\Delta E
\]

\[
\tilde{I}_{\text{leak}} = I_{\text{leak}}/E
\]

5) A “zero-channel” file was constructed by rescaling \( \tilde{I}_{\text{leak}} \) and \( \tilde{I}_{\text{capacitance}} \) according to the entire voltage protocol of the file. This zero-channel file was then subtracted from all data files (Supplementary Fig. S1B, C and D).

Subsequent to leak and capacitance subtraction files (again only voltage-step data) were filtered with the electrical interference filter in Clampfit, with a reference frequency of 60 Hz, harmonics 1–10 and 20 cycles to average (Supplementary Fig. S1E). This was automated for batches of files with a macro written in AutoHotKey. Files were then passed through an eight-pole Bessel low-pass filter with a cutoff of 200 Hz (Supplementary Fig. S1F).

**Jump Analysis**

An algorithm was written to measure the size of single channel openings and closings, generically termed “jumps.” Any such analysis consists of two steps: first detecting the jumps and secondly measuring their size. Briefly,

**Jump detection.** The most common method for detecting jumps is the “half-amplitude method” whereby jumps are detected when the current crosses a threshold that is half the size of the estimated open-channel current. As such this method is of limited use if determining open-channel current is itself the object of the analysis. The method also depends on knowing the closed-current level, which may be problematic if leak currents are comparable in size. This problem has been solved by using a statistical measure, the sum of the squared deviations (\( \chi^2 \)), to detect jumps (23); \( \chi^2 \) will peak during a jump, independently of the absolute current level. It is then just a matter of setting a variance threshold. \( \chi^2 \) was calculated.

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Fig. 1. Appearance of cultured interstitial cells of Cajal (ICC). A and B are differential interference contrast and phase contrast images, respectively, of the same field of view showing a pipette cell-attached to an ICC. C and D are differential interference contrast and phase contrast images, respectively, of 2 separate fields showing a cluster of ICC and 2 ICC, respectively. The scale bar in A applies to all images.
where $W$ is the window width (the number of data points that $\chi^2$ is calculated from). Jumps were then detected with the threshold $T_{\text{jump}}$.

**Jump measurement.** Once jumps have been detected, jump size is typically measured as the absolute open-channel current. However, this is a problem where the leak current (which varies with time and from patch to patch) is of a similar magnitude to the jump size. Therefore we measured the change in current with the jump. For each peak in $\chi^2$ that crossed the threshold $T_{\text{jump}}$,

\[
\chi^2(t_a) = \alpha + 0.5W \sum_{k=\alpha}^{\alpha + 0.5W} \left[ I(t_k) - \bar{I}(t_a) \right]^2
\]

\[
\bar{I}(t_a) = \sum_{k=\alpha}^{\alpha + 0.5W} I(t_k) \frac{1}{W}
\]

where $W$ is the window width (the number of data points that $\chi^2$ is calculated from). Jumps were then detected with the threshold $T_{\text{jump}}$.

\[
J = I(t_\beta) - I(t_\alpha)
\]

where $J$ is the jump magnitude and $\alpha$ and $\beta$ are the points either side of the peak (before and after, respectively), where

\[
\chi^2(t_\alpha) = \chi^2(t_\beta) = T_{\text{fits}} < T_{\text{jump}}
\]

\[
t_\beta - t_\alpha < W \cdot F_{\text{noise}}
\]

In this way the values used to calculate the jump are either side of that jump in regions of low variance ($T_{\text{fits}}$), but not too far from the jump itself (according to $F_{\text{noise}}$) as may occur in regions where the changes in current are complicated, leading to compound peaks in $\chi^2$.

For analysis of the data in this study, $W$ was 10 ms, $T_{\text{jump}}$ was 2 pA$^2$, $T_{\text{fits}}$ was 0.5 pA$^2$, and $F_{\text{noise}}$ was 5. Simulated single-channel

**Fig. 2.** Jump analysis of simulated single-channel data. Single channels were modeled as step changes (“jumps”) in current (1 pA in A; 0.25 pA in B) sampled at 5 kHz with open and close times applied randomly according to exponential distributions (0.05 s mean open, 0.1 s mean closed; both A and B). To this was added Gaussian noise (0.5 pA$^2$ standard deviation), and the data were then filtered as for the real data (8-pole Bessel low-pass filter with a cutoff of 200 Hz). For both conductance sizes (1 or 0.25 pA), 100 s of data were analyzed by the jump algorithm with the same parameters as used for the real data (see METHODS). In Aa and Ba jumps detected by the algorithm are shown as black, nonzero lines against the current trace in gray. Ab and Bb, jump magnitudes ($J$) are plotted against random values (with a uniform distribution between $-5$ and $+5$) to visualize the distribution of $J$. The central band of values in Ab results from noise and jumps shorter than the window width ($W$) of the algorithm. The upper band consists of openings and the lower band, closings.

**Fig. 3.** Two distinct currents are apparent in voltage ramps of cell-attached patches of ICC. All ramps have been filtered with a 200-Hz cutoff low-pass Bessel. All ramps are sequential from left to right. Aa and Ab show examples of $I_{\text{hump}}$ current from 2 different patches (ramps 6 s apart), characterized by a hump in outward current rising from $-0$ mV relative to resting membrane potential (rmp) and the variable appearance of an outward saw tooth pattern (Ab). Ac, loss of $I_{\text{hump}}$ with patch excision (ramps 12 s apart). Excision occurred between the second and third ramp. B: example of $I_{\text{flicker}}$ at variable intervals. Note in some ramps the presence of a clear inward conductance at the base of the flicker current.
data was analyzed with these values to test the validity of the algorithm (Fig. 2).

To calculate conductances and reversal potentials, distributions of the absolute value of $J$ ($|J|$) were plotted as histograms with bins of 0.05 pA, and Gaussian functions were fitted to the nonzero peaks (i.e., nonnoise peaks). The center of the peaks as a function of potential, $U_{\text{CENT}}(E)$, was fitted with

$$|J|_{\text{CENT}}(E) = \left| G \times 0.001 \times (E - E_{\text{rev}}) \right|$$

where $G$ is the conductance (in pS) and $E_{\text{rev}}$ is the reversal potential (in mV).

RESULTS

Voltage ramps were used to assess single-channel activity in cell-attached patches of ICC, to obtain a quick “signature” of the currents expressed by the patch. With a HS(high Na) pipette solution and HS(physiological) bath solution, two patterns were seen. In over 90% of patches the response to a ramp from $-80$ to $+80$ mV rrmp (see below for discussion of resting membrane potential in ICC) consisted of a rounded “hump” of outward current, rising at $>0$ mV rrmp (Fig. 3A). The smoothness of this hump varied from patch to patch and to a lesser extent within patches, with the less smooth humps having noticeable “saw tooth” rises (Fig. 3Ab). These currents were collectively termed $I_{\text{hump}}$. Upon excising the patch, $I_{\text{hump}}$ was lost, the current response going flat (Fig. 3Ac). In more than half of patches a second type of response occurred, either transiently or consistently after a period of time (from 5 to 20 min) and superimposed on $I_{\text{hump}}$. This consisted of a flicker current of 5–20 pA magnitude at negative potentials rrmp only (Fig. 3B). In some cases clear step changes in conductance could be seen, particularly at lower conductances (Fig. 3B; see also Fig. 10). These currents were termed $I_{\text{flicker}}$.

Voltage steps of cell-attached patches were used to further assess the voltage dependence of $I_{\text{hump}}$. Note that $I_{\text{hump}}$ will be used to designate the currents recorded under voltage step, if the initially observed ramp current was humped. Two complementary protocols were used to assess responses of $I_{\text{hump}}$ to depolarization and repolarization (Fig. 4, B and C, respectively). $I_{\text{hump}}$ showed activation upon depolarization followed by partial inactivation. The magnitude of activation or inactivation varied from patch to patch, from a short peak with shallow decay to a strong outward transient (Fig. 4Bb). Throughout, the current was typically noisy and clear single-channel current levels were difficult to identify (Fig. 4Ba).

Fig. 4. $I_{\text{hump}}$ is a transient outward current with step depolarization. All data with a HS-(high Na) pipette. [See Table 1 for names and constituents of HEPES-buffered saline (HS) solutions.] A: voltage-ramp responses. The far left is the ramp protocol, the middle 4 traces are examples from different patches, and the far right trace is the mean of 18 patches (20–80 ramps each). B and C: responses to step depolarization and repolarization, respectively. $B_0$ and $C_0$: traces from single patches with the protocol used shown above the traces. Ellipses indicate the region of the protocol used to determine capacitance and leak subtraction; see METHODS. The traces have been separated by 4 pA to improve clarity. $B_b$ and $C_b$: patch-to-patch variation. Cross-file means ($m_{\text{crossfile}}$) of the response at $+100$ mV for 9 patches ($B_b; N_{\text{files}} = 97$) and $+40$ mV for 5 patches ($C_b; N_{\text{files}} = 83$). $B_c$ and $C_c$: right: average response of all patches. $B_c$ and $C_c$: left: current values indicated by the symbols in the traces at the right, plotted against potential relative to resting membrane potential (rrmp).
Responses to repolarization were only apparent at positive potentials \( \text{rrmp} \) (Fig. 4C). At these potentials there was activation over 200–300 ms (Fig. 4, Ca and Cb). As with the response to depolarization, clear single-channel current levels were difficult to identify (Fig. 4Ca).

To further characterize the channel or channels underlying \( I_{\text{hump}} \), one of two different cations were substituted for Na\(^+\) in the pipette (K\(^+\) or NMDG\(^+\)) and responses to the same voltage-clamp protocols were recorded in cell-attached patches. Throughout, the bath solution was HS(physiological), as before. Qualitatively the responses with HS(NMDG) (Fig. 4) were indistinguishable from those with HS(high Na) (Fig. 4). Single-channel conductance levels were most easily distinguished around the resting membrane potential.

With HS(high K) in the pipette (Fig. 6), currents were markedly different from the \( I_{\text{hump}} \) seen with either HS(high Na) or HS(NMDG). Rather than a hump, the ramp response was characterized by sudden inward hook [channel opening(s)] at negative potentials \( \text{rrmp} \), followed by a linear response thereafter (Fig. 6A). Step depolarization produced almost no current (Fig. 6B), whereas repolarization produced steady inward current at positive potentials \( \text{rrmp} \) (Fig. 6C) and often produced an inward tail current at negative potentials \( \text{rrmp} \) (Fig. 6Ca, lower three traces). Single-channel conductance levels were very clear at potentials around 0 mV \( \text{rrmp} \) (the resting membrane potential) and as tails at negative potentials \( \text{rrmp} \). Partial substitution of HS(high Na) with HS(high K) (up to 20% by molarity) had no qualitative effect (not shown).

Despite the different current response obtained with a HS-(high K) pipette solution, it is unlikely that different channels were involved from those of \( I_{\text{hump}} \). The same response was seen in >90% of patches with HS(high K), similar to the frequency of \( I_{\text{hump}} \) with HS(high Na) and HS(NMDG), and no patches with HS(high K) showed \( I_{\text{hump}} \). Therefore from this point on we will use “\( I_{\text{hump}} \)” to refer to both the channel that actually gave a humped ramp current in HS(high Na) or HS(NMDG) and the channel that gave the inward hook ramp current in HS(high K).

The kinetics of the transient response of \( I_{\text{hump}} \) to depolarization, with HS(Na) or HS(NMDG) in the pipette, were quantified by fitting exponentials (Fig. 7). When the responses of all patches were averaged, the sum of two exponentials was found to adequately fit responses at all potentials (Fig. 7, A and B) with time constants of 30–40 ms (\( \tau_1 \)) and 400–1,200 ms (\( \tau_2 \)), with the faster decaying exponential (\( \tau_1 \)) of larger amplitude. However, in most cases, averaged responses of indi-
Individual patches could be fitted with a single exponential, or two exponentials with the same decay constant (see METHODS; Fig. 7C). In at least two patches, with HS(NMDG) or HS(high Na) in the pipette, outward transients “ran down” with time (Fig. 8).

To measure single-channel conductance of \(I_{\text{hump}}\), a program was written to measure the size of single channel closings and openings or current jumps (see METHODS). The output of this program for step protocols of cell-attached patches recorded with HS(high Na), HS(NMDG), or HS(high K) in the pipette is shown in Fig. 9, \(A, B,\) and \(C\), respectively. Jump size \(J\) is plotted against the potential \(E\). To aid visualization of the distribution of \(J\) at each potential, the values of \(E\) have been spread out by adding a random number (uniformly distributed between \(+100\) mV and \(-100\) mV) to the actual value associated with each jump. A central band at \(J = 0\) pA reflects noise (Fig. 2) whereas bands at either side of this reflect channel openings and closings (a \(+J\) is either an outward opening or inward closing; \(-J\) is either an inward opening or outward closing).

As might be expected from visual inspection of traces (Fig. 4), there were no distinct conductance levels for \(I_{\text{hump}}\) with a HS(high Na) pipette, except at potentials around 0 mV rmp (Fig. 9A). However, this was enough to calculate conductance. The conductance of the jumps with HS(high Na) was calculated as 5 pS, with a reversal potential of \(-75\) mV rmp (dotted line; Table 2). Peaks in \(J\) were better resolved with HS(NMDG) (Fig. 9B), but again these were limited to potentials \(\pm 20\) mV rmp. Fitting of these peaks gave a conductance of 3.3 pS and a reversal potential of \(-132\) mV rmp (dotted line; Table 2). At around 0 mV rmp a “double” conductance was visible (Fig. 9B, arrowhead), perhaps indicating that the more usual (3.3 pS) conductance was actually a subconductance or that channels could gate synchronously. With a HS(high K) pipette the peaks were better resolved at a larger range of potentials (Fig. 9C), giving a conductance of 29.2 pS and a reversal potential of 43 mV rmp (dotted line; Table 2). The distributions with all three ions were largely symmetrical about \(J = 0\), indicating that there were on average no more clear openings than closings from the start of a voltage step to the end.

The values of \(E_{\text{rev}}\) calculated with HS(high Na), HS(NMDG), and HS(high K) were used to calculate relative permeabilities of \(I_{\text{hump}}\) according to the Goldman-Hodgkin-Katz equation.
The permeability sequence \((P_K/P_{Na})\) was \(K^+/(111) > Na^+/(1) > NMDG^+/(0.1)\) (Table 2).

Because the current is highly selective for potassium, if we assume that the potassium concentration of HS(high K) is equal to that of the cytosol, then there is no concentration driving force with HS(high K) in the pipette and at the reversal potential the electrical driving force is also zero (0 mV absolute membrane potential). Therefore if the reversal potential is on average 43.27 mV rmp with HS(high K), it follows that the membrane potential of the ICC must be on average \(-43.27\) mV. This is consistent with values measured previously by perforated-patch current clamp (18).

In contrast to \(I_{hump}\), \(I_{flicker}\) appeared unchanged by substituting the pipette cation. With a HS(high Na), HS(NMDG), or HS(high K) pipette, it remained an inward, flickery current (Fig. 10). This and the fact that \(I_{flicker}\) is inward with HS(NMDG) (i.e., no external permeable cations) confirms that \(I_{flicker}\) is a chloride current.

Five channel blockers [4-aminopyridine (4-AP), E4031, clofilium, clotrimazole, and ketoconazole] were tested for their effect on \(I_{hump}\) in cell-attached patches. All the blockers were applied in a HS(high Na) pipette. With 5 mM 4-AP the transient was blunted (Fig. 11A; \(N_{cell} = 7\)). Instead of an almost infinitely fast rise followed by exponential decay, as with controls (Fig. 4Bc), there was a hyperbolic rise followed by a monoexponential decay (Fig. 11E). This effect was consistent from patch to patch (Fig. 11F). Applied together, 10 \(\mu\)M E4031 and 20 \(\mu\)M clofilium had no effect on \(I_{hump}\) or the transient outward current (Fig. 11, B and E; \(N_{cell} = 6\)); 20 \(\mu\)M clotrimazole almost completely blocked \(I_{hump}\) and the transient outward current (Fig. 11C; \(N_{cell} = 5\)). At all potentials only very brief channel openings occurred. This effect could not be obtained at lower concentrations (10 \(\mu\)M or less; data not shown), and 20 \(\mu\)M ketoconazole had no effect on \(I_{hump}\) or the transient outward current (Fig. 11, D and E; \(N_{cell} = 2\)).

**DISCUSSION**

In cell-attached recordings of ICC we commonly recorded a current, \(I_{hump}\), which had similar characteristics to the transient outward current \((I_{to})\) of the heart or the “A-type” current \((I_{A})\) of neurons and other cells in that it was carried by potassium, inactivated exponentially over a period of hundreds of milli-
conduction system, it has been found that \( I_{\text{to1}} \) has at least two decay constants, up to four in some cases (32). These are each given names such as \( I_{\text{fast,1}}, I_{\text{to,slow}}, \) etc. The correspondence of these kinetic entities to separable, distinct channels is then based on either pharmacology (can the number of constants be reduced by a drug?), anatomy (can the same constants be observed in different cells but with different relative amplitudes?), biophysical first principles (the envelope of tails test), or molecular (can the number of constants be reduced by knockout of a channel isoform?).

Applying the first of these criteria to the \( I_{\text{to}} \) in ICC would seem to give a clear answer: there were two components and the faster of these was blocked by 4-AP. However, considering the actual distribution of constants from one patch to another (the second criterion), it may not be that straightforward. Although in many cells a single exponential was satisfactory to fit \( I_{\text{to}} \) (making the third criterion irrelevant), the distribution of these decay constants was not clearly divisible into two populations, which corresponded in value to those patches with biexponentials or the averaged data. This may be just a matter of \( N \) value; with more data some pattern may emerge. But for now it is not plausible to generalize \( I_{\text{to}} \) as two concrete kinetic entities.

The fourth, molecular, criterion has been used over the last few years to show that kinetic components of \( I_{\text{to1}} \) can be correlated with members of the Kv1, Kv2, and Kv4 families of voltage-gated potassium channels (7). The time constants that individual members are correlated with are not always consistent between studies, suggesting that there is more to the story than gene = time constant. Indeed it has been shown that kinetics can vary because of heteromer channels (Kv channels are tetramers), alternative splicing, and accessory (beta) subunits (21). Any one of these may explain the diversity of kinetics of the ICC \( I_{\text{to}} \). Only one study has examined Kv expression in ICC. From immunohistochemical data, Hatton et al. (8) suggested that Kv1.1 and Kv1.5 may form heteromers in ICC.

Another explanation for heterogeneous kinetics is modulation of inactivation by the intracellular environment. The use of imidazole drugs in this study was prompted by Zhu et al. (36), who showed that clotrimazole could inhibit outward potassium current in cultured jejunal ICC. We cannot be sure about

### Table 2. Conductance and permeability of \( I_{\text{hump}} \) based on jump algorithm data

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</tr>
<tr>
<td>( N_{\text{jump}} )</td>
<td>21959</td>
<td>31310</td>
<td>31063</td>
</tr>
<tr>
<td>( N_{\text{peak}} )</td>
<td>3</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>( G, \mu S )</td>
<td>4.99 ± 0.27</td>
<td>3.32 ± 0.96</td>
<td>29.22 ± 1.47</td>
</tr>
<tr>
<td>( E_{\text{rev}}, \text{mV} )</td>
<td>−75.74 ± 4.48</td>
<td>−131.7 ± 37.9</td>
<td>43.27 ± 1.86</td>
</tr>
<tr>
<td>( P_{\text{G}}/P_{\text{Na}} )</td>
<td>1.00 ± 0.25</td>
<td>0.11 ± 0.16</td>
<td>111.2 ± 23.5</td>
</tr>
<tr>
<td>( P_{\text{G}}/P_{\text{NMDG}} )</td>
<td>9.17 ± 13.86</td>
<td>1.00 ± 2.12</td>
<td>1019.8 ± 1532.8</td>
</tr>
<tr>
<td>( P_{\text{G}}/P_{\text{K}} )</td>
<td>0.009 ± 0.002</td>
<td>0.001 ± 0.000</td>
<td>1.000 ± 0.040</td>
</tr>
</tbody>
</table>

Values are means ±SE. \( N_{\text{file}} \), number of files (repeats of protocol) in data set; \( N_{\text{jump}} \), number of step changes in current detected by jump algorithm; \( N_{\text{peak}} \), number of Gaussians fitted to jump data, used to calculate conductance (G) and reversal potential (\( E_{\text{rev}} \)) (see METHODS). Permeability ratios (\( P_{\text{G}}/P_{\text{Na}} \)) were calculated from \( E_{\text{rev}} \) values as described in Parsons and Sanders (22).
identifying the present single channel currents with the whole-cell current in their study, because they could block at a much lower concentration of clotrimazole (1 μM). However, they did show that inactivation could be speeded by increasing extracellular calcium to 10 mM, which they suggest acts by increasing intracellular calcium. The same or a similar mechanism, for instance involving phosphorylation, might fit with the fact that the fast component of ICC $I_{c1}$ ran down in some patches. However, it has been shown that the kinetics of cardiac $I_{c1}$ can be effected directly by extracellular calcium (11).

Fig. 10. $I_{\text{flicker}}$ is a chloride current. Voltage-ramp response of $I_{\text{flicker}}$ with HS(high Na) pipette (A), HS(NMDG) pipette (B), and HS(high K) pipette (C). All traces are from different patches. The fact that $I_{\text{flicker}}$ is inward with HS(NMDG) indicates it is carried by chloride.

Fig. 11. $I_{\text{hump}}$ is 4-aminopyridine (4-AP) and clotrimazole sensitive. Pharmacology of $I_{\text{hump}}$. All blockers were applied in a HS(high Na) pipette. The lower traces of each panel are the response to the depolarization protocol (see Fig. 4A) averaged across all patches. Current is in gray and biexponential functions fitted to the current are in black. Top traces of each panel are the ramp responses (see Fig. 4A), an example from a single patch (top) and averaged across all patches (bottom). A: 5 mM 4-AP ($N_{\text{cell}} = 7, N_{\text{file}} = 101$). B: 10 μM E4031 and 20 μM clotrimazole ($N_{\text{cell}} = 6, N_{\text{file}} = 65$). C: 20 μM clotrimazole ($N_{\text{cell}} = 5, N_{\text{file}} = 37$). D: 20 μM ketoconazole ($N_{\text{cell}} = 2, N_{\text{file}} = 14$). E: biexponential time constants for the response to depolarization to +100 mV for each blocker. F: responses to depolarization to +70 mV for individual patches with natural log current axis. Left: HS(Na) pipette. Right: HS(Na) pipette with 5 mM 4-AP.
A fifth criterion to distinguish different channels is single-channel conductance. This approach was limited by the “noise” of the recordings. Although we could clearly distinguish a conductance at potentials around the resting membrane potential, this itself may represent a smeared average of closely spaced conductances, and there may have been other conductances at higher or lower potentials. The noise likely reflects two processes. Firstly, extracellular Na\(^+\) can block Kv channels (25), with the result that most single-channel studies of Kv are done with high extracellular K\(^+\). This is consistent with the change in conductance visibility between HS(high Na) and HS(NMDG) on the one hand and HS(high K) on the other. Secondly, the conductances were best distinguished at potentials around 0 mV rmp, suggesting sensitivity to the applied potential rather than absolute electrical driving potential. A possible explanation could be mechanosensitivity. It is known that applied potential causes patch “creep” along the pipette, changing the membrane shape and membrane stress, and this may affect the conformation of the channel (28).

Whatever molecular heterogeneity there may be in the \(I_{\text{to}}\) of ICC, there is homogeneity in the sensitivity to extracellular cations (potassium, 4-AP, and clotrimazole), suggesting that this molecular heterogeneity is limited. The effect of extracellular potassium on gating (vs. just reversal potential) is an established phenomenon for all Kv channels (12). Two types of inactivation mechanism occur in Kv channels: N-type (“ball and chain”) and C-type (involving conformational changes around the channel mouth). Extracellular potassium slows the latter and, depending on the relative importance and timing of N-type vs. C-type inactivation (which are themselves coupled) and the potassium concentration, the kinetics are altered. The slowing of activation of \(I_{\text{to}}\) by 4-AP has been characterized as the “crossover effect” in ventricular myocytes (2). Slowing of both activation and inactivation means that, despite a reduced amplitude, the current trace with 4-AP will cross over the trace without 4-AP. This appears to occur with ICC \(I_{\text{to}}\) (Fig. 11, A and F). A study of canine colonic ICC showed whole-cell transient outward currents to be 4-AP insensitive (17).

In cardiac cells, changes in the expression of \(I_{\text{to}}\) and its kinetics, from the pacemaking nodes to conducting fibers, ventricles to atria, can be correlated with changes in the shape of the action potential. Such changes do not occur across the different organs of the gut and between different classes of ICC and smooth muscle cells. For instance, a recent study of the mouse small intestine (14) showed that 5 mM 4-AP increased the rate of the depolarization upstroke of the slow wave but decreased this in pacemaker potentials. Hence the \(I_{\text{to}}\) revealed in this study may have an important function in establishing the rate of depolarization of the ICC pacemaker potential.

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**DISCLOSURES**

No conflicts of interest are declared by the author(s).

**REFERENCES**


