Characterisation of the transient outward K⁺ current in rabbit sinoatrial node cells

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Received 17 June 1999; accepted 31 January 2000

Abstract

Objective: To (i) characterise the electrophysiological and pharmacological properties of the transient outward K⁺ current, Iₒ, (ii) determine the relationship between the density of Iₒ and cell size, and (iii) determine the role of Iₒ in electrical activity in rabbit sinoatrial node cells at 35°C. Methods: Rabbit sinoatrial node cells were studied using whole-cell voltage and current clamp techniques. Results: Iₒ showed half activation and inactivation at +11 and −49 mV, respectively. Iₒ was blocked by 4-aminopyridine (4-AP) as well as the class I agents, quinidine and flecainide, with EC₅₀ values of 326, 21 and 19 μM, respectively. The densities of the transient and sustained components of 4-AP-sensitive current were significantly correlated with cell capacitance, a measure of cell size, and were greater in cells with a larger capacitance. Block of Iₒ by 4-AP affected both the action potential and pacemaker activity of sinoatrial node cells and the effects were greater in cells with a larger capacitance. Conclusions: Iₒ in sinoatrial node cells shows similar electrophysiological and pharmacological properties to Iₒ in atrial and ventricular cells. The expression of Iₒ in sinoatrial node cells is heterogeneous and differs in large and small cells (likely to be from the periphery and centre of the sinoatrial node, respectively). Iₒ plays an important role in action potential configuration and pacemaker activity in sinoatrial node cells, especially in larger cells. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: K⁺ channels; Sinus nodes

1. Introduction

Transient outward K⁺ current, Iₒ, characterised by rapid activation and inactivation and sensitivity to 4-aminopyridine (4-AP), has been recorded in cells from various regions of the heart from many species [1]. Iₒ plays an important role in action potential repolarization and contributes to cardiac electrical heterogeneity [1]. Iₒ is also affected by antiarrhythmic agents [1].

The sinoatrial (SA) node is known to be a heterogeneous tissue and the electrophysiological properties of SA node cells vary in different regions of the SA node. In the SA node, the action potential is usually first initiated in the centre of the SA node (distant from the surrounding atrial muscle). Normally, the function of the periphery of the SA node is to conduct the action potential from the centre to the surrounding atrial muscle, although it can take over the role as the leading pacemaker site [2]. Action potentials recorded from the centre of the rabbit SA node have a more positive take-off potential, slower upstroke, longer duration and less negative maximum diastolic potential than action potentials recorded from the periphery; the intrinsic pacemaker activity of the centre is also slower than that of the periphery [3]. We have suggested that the regional differences in electrical activity are mainly the result of regional differences in the intrinsic properties of the cells (rather than electrotonic influences), because similar heterogeneity of action potential characteristics are observed in single SA node cells from the rabbit [4]. Furthermore, cell size increases from the centre to the periphery of the rabbit SA node [5] and small cells (presumably from centre) show electrical activity charac-
teristic of the centre, whereas large cells (presumably from the periphery) show electrical activity characteristic of the periphery [4]. We have suggested that cell size-dependent differences in the density of ionic currents underlie regional differences in electrical activity within the SA node [4]. The lack of TTX-sensitive Na⁺ current, INa, and a low density of the hyperpolarization-activated current, IK, in small cells presumably from the centre may be responsible, at least in part, for the slower upstroke and slower intrinsic pacemaker activity of these cells [4]. Low densities of the rapid and slow delayed rectifier K⁺ currents, IKr, and IKs, in small cells may also contribute to regional differences in electrical activity in the SA node (M. Lei, unpublished observations).

Recently, we have shown that 4-AP affects the rabbit SA node and the effects are greater in the periphery than the centre [6]. We have also described a 4-AP-sensitive transient outward current in rabbit SA node cells [7]; this study was carried out at room temperature. The present study was designed to: (i) characterise the electrophysiological and pharmacological properties of I\textsubscript{m} in rabbit SA node cells at 35°C, (ii) determine whether there is heterogeneous expression of I\textsubscript{m} within SA node cells at 35°C, and (iii) assess whether I\textsubscript{m} plays a significant role during the action potential and pacemaker potential at 35°C.

2. Methods

2.1. Cell isolation

New Zealand white rabbits (500–1000 g) were killed by cervical dislocation and the heart removed quickly and placed in oxygenated Tyrode’s solution at 37°C (procedures were covered by UK Home Office licence and conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996)). Thin strips of SA node tissue (~3×0.5 mm) were placed for 5 min in Ca\textsuperscript{2+}-free Tyrode’s solution at 37°C, incubated for 30–40 min in collagenase (type I, Sigma Chemical Co., Poole, UK), 230 U/ml, and elastase (type IIA, Sigma), 15 U/ml, in Ca\textsuperscript{2+}-free Tyrode’s solution at 37°C, and then stored for at least 1 h in KB medium at 4°C. Single cells were released from the strips by glass pipette suction. During experiments, cells were superfused with Tyrode’s solution (plus appropriate drugs or blockers) at ~1 ml/min at 35°C.

2.2. Whole cell current and voltage clamp

The whole cell patch clamp technique was used for electrical recording from single SA node cells with amphotericin-permeabilised patches (after formation of a giga-seal, 10–15 min was allowed for amphotericin to act). Amphotericin (200 μg/ml) was added to the pipette solution just before use. Pipettes (tip diameter ~1–2 μm, resistance 3–8 MΩ) were made from 1 mm diameter glass (Clark Electromedical Instruments, Reading, UK) using a Narishige pipette puller (PP-83, Narishige Scientific Instruments Laboratory, Tokyo, Japan). An Axopatch-1C patch clamp amplifier (Axon Instruments Inc., Foster, USA) was used for current and voltage clamping. Cell capacitance (C\textsubscript{m}) was obtained from the capacity compensation control of the amplifier after the whole cell capacity current (in response to 5-ms pulses to ~70 mV at 100 Hz from a holding potential of ~60 mV) was eliminated. In a previous study [7], the accuracy of this method was checked. The series resistance (>80%) was electronically compensated, and the current signal was filtered by a low pass Bessel filter with a cut-off frequency of 10 kHz (~3 dB). Electrical signals were displayed during an experiment on an oscilloscope (5111A, Tektronix, Holland) and a chart recorder (2007, Gould, France). Data were digitised using an AD/DA converter (Digidata1200A, Axon Instruments Inc.) and stored on computer (sample rate, 1–2 kHz) for later analysis using pCLAMP version 6.2 software (Axon Instruments Inc.).

2.3. Solutions

Tyrode’s solution contained (in mM): 140 NaCl, 5.4 KCl, 1.8 Ca\textsubscript{2+}, 1 MgCl\textsubscript{2}, 10 glucose, 5 HEPES, titrated with NaOH to pH 7.4. Ca\textsuperscript{2+}-free Tyrode’s solution: as normal Tyrode’s solution, but without CaCl\textsubscript{2}, KB solution contained (in mM): 25 KCl, 80 L-glutamic acid, 20 taurine, 10 KH\textsubscript{2}PO\textsubscript{4}, 3 MgCl\textsubscript{2}, 10 glucose, 10 HEPES, 0.5 EGTA, titrated with KOH to pH 7.4. Pipette solution contained (in mM): 140 KCl, 1.8 MgSO\textsubscript{4}, 5 HEPES, 1 EGTA, titrated with KOH to pH 7.4; prior to use amphotericin was added as described above. In all experiments, except those in which action potentials were recorded, 300 μM CdCl\textsubscript{2} was present in the Tyrode’s solution to block I\textsubscript{Ca}. This concentration of Cd\textsuperscript{2+} does not affect the activation curve for I\textsubscript{m} in rabbit SA node cells (although, it is not known whether it affects inactivation) [7]. CdCl\textsubscript{2} was made as a 100 mM stock solution in deionized waer and stored at 4°C. I\textsubscript{m} was blocked in the present study and I\textsubscript{Na} can be seen in some of the traces (e.g. Fig. 1A). All chemicals were from Sigma. In some experiments, quinidine and flecainide were also added to Tyrode’s solution. A stock solution of 1 M 4-AP was prepared in deionized water, titrated with HCl to pH 7.4 and stored at 4°C. Quinidine and flecainide were made up as 100 μM stock solutions in deionized water and frozen until needed.

2.4. Statistics

All results are presented as means±S.E.M. (number of cells). Statistical significance was determined by Student’s t test for paired or unpaired observations. Linear regression
Fig. 1. Relationship between \( I_{\text{to}} \) and \( C_m \). (A) Example of \( I_{\text{to}} \) (protocol shown in inset). Pulse rate: 0.5 Hz. (B) Current–voltage relationship for \( I_{\text{to}} \). Means ± S.E.M. (\( n=9 \)) shown. (C) Relationship between density of \( I_{\text{to}} \) at +40 mV and \( C_m \). Data fitted with a straight line (\( n=32, r^2=0.58, P<0.001 \)). In this and other figures, \( C_m \) of cell or range of \( C_m \) values of group of cells given adjacent to plots.

3. Results

3.1. Heterogeneous expression of \( I_{\text{to}} \) in SA node cells

Experiments were only carried out on cells showing spontaneous activity — they were spindle and/or spider shaped, with no obvious or faint striations. Fig. 1A shows an example of transient outward current. It shows membrane current evoked by 200 ms pulses to −40 to +60 mV from a holding potential of −80 mV. During the pulses, outward current activated rapidly within 10 ms and then inactivated within 200 ms. Fig. 1B shows the current–voltage relationship for transient outward current (measured as the difference between the peak outward current at the start of the pulse and the current at the end of the pulse) in nine cells. Fig. 1C shows the relationship between the density of transient outward current (current amplitude divided by \( C_m \)) and cell size (as measured by \( C_m \)) in 32 cells with capacitances ranging from 15 to 71 pF. There is a significant correlation between the current density and \( C_m \).

The transient outward current shown in Fig. 1 is assumed to be \( I_{\text{to}} \). To confirm that the density of \( I_{\text{to}} \) is dependent on \( C_m \), in some experiments, 4-AP was applied to isolate \( I_{\text{to}} \) from other ionic currents. Fig. 2A shows an example of 4-AP-sensitive current, which was obtained by subtracting current in the presence of 10 mM 4-AP from current under control conditions, during 200 ms pulses to −40 to +60 mV from a holding potential of −80 mV. In some cases, 500 nM atropine was applied to block muscarinic receptors, because 4-AP has been reported to activate muscarinic receptors and, thus, the muscarinic K⁺ current [8] — there was no observable difference between results in the absence and presence of atropine. 4-AP-sensitive current was made up of two components: a transient component with rapid activation and inactivation (\( I_{\text{trans}} \)) and a time-independent sustained component (\( I_{\text{sust}} \); seen at the end of the pulse). Fig. 2B shows the relationship between the density of \( I_{\text{trans}} \) and \( C_m \) and Fig. 2C shows the relationship between the density of \( I_{\text{sust}} \) and \( C_m \) — there is a significant correlation between the density of

\[ r^2=0.65, P=0.011 \]

\[ r^2=0.62, P<0.01 \]
both $I_{\text{trans}}$ and $I_{\text{sus}}$ and $C_m$. The results for $I_{\text{trans}}$ in Fig. 2B are in good agreement with the results in Fig. 1C.

### 3.2. Activation and inactivation of $I_{\text{to}}$

The activation curve for $I_{\text{to}}$ shown in Fig. 3B was obtained by holding a cell at $-80$ mV and applying 40-ms conditioning pulses to potentials ranging from $-30$ to $+60$ mV; the prepulse was followed by a test pulse to $-40$ mV for 100 ms (Fig. 3A). The amplitude of the outward tail current during the test pulse was normalized to the largest tail current (after the conditioning pulse to $+60$ mV). In Fig. 3B, the open symbols show mean data from six cells plotted as a function of the conditioning pulse potential and fitted by the Boltzmann equation. A double-pulse protocol was used to measure the voltage-dependence of inactivation of $I_{\text{to}}$: from a holding potential of $-80$ mV, a 500-ms conditioning pulse to potentials ranging from $-100$ to $0$ mV was followed by a 150-ms test pulse to $+60$ mV (Fig. 3A). The amplitude of $I_{\text{to}}$ (measured as the difference between peak outward current at the start of the pulse and current at the end of the pulse) during the test pulse was normalized to the maximal amplitude of $I_{\text{to}}$ (after the conditioning pulse to $-100$ mV). In Fig. 3B, the filled symbols show mean data from six cells plotted as function of the conditioning pulse potential and fitted with the Boltzmann equation. From Fig. 3B it can be seen that $I_{\text{to}}$ displayed half-maximal activation at $+11$ mV and half-maximal inactivation at $-49$ mV.

The time-dependence of inactivation of $I_{\text{to}}$ (measured as 4-AP sensitive current) was assessed in seven cells during a 200-ms pulse to $+60$ mV from a holding potential of $-80$ mV (current in the presence of 10 mM 4-AP was subtracted from current under control conditions). The decay of 4-AP sensitive current was best fitted by a double exponential function with fast and slow decaying components. The time constants for these two components are $10\pm3$ and $107\pm17$ ms.

Recovery from inactivation was determined by a standard double-pulse protocol: a 100-ms test pulse to $+40$ mV was applied at different intervals after a 100-ms conditioning pulse to $+40$ mV (Fig. 4A). The holding potential was $-80$ mV. The amplitude of $I_{\text{to}}$ (measured as the difference between the peak outward current at the start of the pulse and the current at the end of the pulse) during the test pulse was normalized to the amplitude of $I_{\text{to}}$ during the conditioning pulse. In Fig. 4B, mean data from seven cells are plotted as a function of the test interval. The time course of recovery of $I_{\text{to}}$ from inactivation was best described by a double exponential function with time constants of 43 and 1434 ms.
3.3. Effects of 4-AP, quinidine and flecainide on $I_{\text{to}}$

The effects of 4-AP and two class I antiarrhythmic agents, quinidine and flecainide, on $I_{\text{to}}$ were investigated. Fig. 5 shows the effect of 4-AP on $I_{\text{to}}$. Fig. 5A shows currents evoked by 200-ms pulses to $-40$ to $+60$ mV from a holding potential of $-80$ mV under control conditions and in the presence of 10 mM 4-AP. In the presence of 10 mM 4-AP the inactivating component of outward current and some of the sustained outward current was abolished. Fig. 5A also shows 4-AP-sensitive current (obtained by subtracting current in the presence of 4-AP from that under control conditions) — this is similar to that shown in Fig. 2A. Fig. 5B shows activation curves for $I_{\text{to}}$ from five to nine cells under control conditions and in the presence of 100 μM, 1 and 10 mM 4-AP. The activation curves were constructed by plotting the conductance for $I_{\text{to}}$, measured from current during the pulse rather than the tail current as in Fig. 3B, against the pulse potential. The conductance was calculated by dividing the difference between the peak outward current at the start of the pulse and the current at the end of the pulse by the driving force (the reversal potential was assumed to be $-80$ mV under all conditions). The conductance has been normalised to the conductance at $+60$ mV under control conditions. Fig. 5B shows a concentration-dependent decrease in conductance. The data in Fig. 5B have been fitted with the Boltzmann equation — there is no significant change of $V_h$ (potential at which half-maximal activation occurred) or $k$ (slope factor) by 4-AP.

Fig. 6 shows the effect of quinidine on $I_{\text{to}}$. Fig. 6A shows current under control conditions, in the presence of 100 μM quinidine and the quinidine-sensitive current. Quinidine-sensitive current, like 4-AP-sensitive current, was made up of two components: a transient component showing inactivation and a time-independent sustained component at the end of the pulse. Fig. 6B shows activation curves for $I_{\text{to}}$ (constructed as in experiments on 4-AP) from five to nine cells under control conditions and in the presence of 30 and 100 μM quinidine. Fig. 7 shows

![Fig. 5. Effect of 4-AP on $I_{\text{to}}$. (A) Membrane potential, membrane current under control conditions, membrane current in presence of 10 mM 4-AP and 4-AP-sensitive current. Pulse rate: 0.5 Hz. (B) Activation curves for $I_{\text{to}}$ under control conditions and in presence of 100 μM, 1, and 10 mM 4-AP. Means±S.E.M. ($n=5–9$) shown. Data fitted with Boltzmann equation.](https://academic.oup.com/cardiovascres/article-abstract/46/3/433/288749)

![Fig. 6. Effect of quinidine on $I_{\text{to}}$. (A) Membrane potential, membrane current under control conditions, membrane current in presence of 100 μM quinidine and quinidine-sensitive current. Pulse rate: 0.5 Hz. (B) Activation curves for $I_{\text{to}}$ under control conditions and in presence of 30 and 100 μM quinidine. Means±S.E.M. ($n=5–9$) shown. Data fitted with Boltzmann equation.](https://academic.oup.com/cardiovascres/article-abstract/46/3/433/288749)
the effect of flecainide on $I_{\text{to}}$. Fig. 7A shows block of $I_{\text{to}}$ by 100 μM flecainide. Once again, the flecainide-sensitive current was made up of transient and sustained components (although the sustained component was small in this example) (Fig. 7A). Fig. 7B shows activation curves for $I_{\text{to}}$ (constructed as in experiments on 4-AP) from five to nine cells under control conditions and in the presence of 10 and 100 μM flecainide. Figs. 6B and 7B show a concentration-dependent decrease in conductance by quinidine and flecainide. The data in Figs. 6B and 7B have been fitted with the Boltzmann equation — there is no significant change of $V_h$ or $k$ by either drug.

Fig. 8 shows dose–response curves for the block of $I_{\text{to}}$ (measured as the difference between the peak outward current at the start of the pulse and the current at the end of the pulse) by 4-AP, quinidine and flecainide. Mean data have been fitted with a typical dose–response relation with a Hill coefficient of 1. Block of $I_{\text{to}}$ by 4-AP, quinidine and flecainide occurred with an EC$_{50}$ (calculated concentration that gives half-maximal block) of 326, 21 and 19 μM, respectively.

3.4. Effect of 4-AP on spontaneous activity of SA node cells

To help determine the role of $I_{\text{to}}$ in the action potential, the effect of block of $I_{\text{to}}$ by 1 mM 4-AP on the action potential was examined. Because the density of $I_{\text{to}}$ varies in cells of different size (Figs. 1 and 2), the effects of 4-AP
4. Discussion

In rabbit SA node cells at 35°C, the present study has (i) shown that the density of $I_{to}$ is correlated with $C_m$ and is greater in larger cells (presumably from the periphery of the SA node) than in smaller cells (presumably from the centre), (ii) characterised the electrophysiological and pharmacological properties of $I_{to}$, and (iii) shown that block of $I_{to}$ affects action potential configuration and pacemaker activity and the effects are greater in larger cells than in smaller cells.

4.1. Activation and inactivation of $I_{to}$

In the present study, in rabbit SA node cells at 35°C (i) activation of $I_{to}$ was voltage-dependent and half-maximal activation occurred at $+11$ mV, (ii) $I_{to}$ activated in $<10$ ms and at $+60$ mV inactivated with time constants of $10\pm3$ and $107\pm17$ ms, (iii) inactivation of $I_{to}$ was voltage-dependent and half-maximal inactivation occurred at $-49$ mV and (iv), at $-80$ mV, recovery of $I_{to}$ from inactivation occurred with time constants of 43 and 1434 ms.

Half-maximal activation of $I_{to}$ in rabbit SA node cells at 35°C (this study) occurred at a similar potential ($+11$ mV) to that in rabbit SA node cells at 24–26°C ($+19$ mV) [7] and rabbit ventricular cells at 33–35°C ($+12$ mV) [9], but at a more positive potential than in rabbit atrial cells at 20–22°C ($-12$ mV) [10]. Half-maximal inactivation of $I_{to}$ in rabbit SA node cells at 35°C (this study) occurred at a substantially more negative potential ($-49$ mV) than in rabbit SA node cells at 24–26°C ($-32$ mV) [7] and rabbit atrial cells at 22°C ($-32$ mV) [10]. Half-maximal inactivation of $I_{to}$ in rabbit SA node cells at 35°C (this study) occurred at a more similar potential to half-maximal inactivation of $I_{to}$ in rabbit atrial (−41 mV) [11] and ventricular cells (−38 mV) [9] at 33–35°C. The slope factors for activation and inactivation curves are comparable in different studies.

The inactivation kinetics of $I_{to}$ in the present study are comparable to those reported for rabbit SA node cells at 24–26°C by Honjo et al. [7] and rabbit atrial and ventricular cells at 33–35°C by Yamashita et al. [11] and Hiraoka and Kawano [9], but faster than those reported for rabbit atrial cells at 22°C reported by Clark et al. [10].

4.2. Pharmacological properties of $I_{to}$

In the present study, $I_{to}$ in rabbit SA node cells was inhibited by 4-AP, quinidine and flecainide with EC$_{50}$ values of 326, 21 and 19 μM, respectively. The findings with quinidine and flecainide are potentially of clinical importance, because inhibition of $I_{to}$ by clinically relevant concentrations of the antiarrhythmic agents (quinidine: ~19 μM [12]; flecainide: ~2 μM [13]) could affect action potential repolarization and pacemaker activity in SA node cells and, thus, modulate heart rate. Similar effects of
4-AP, quinidine and flecainide on $I_{to}$ have been reported previously in atrial and ventricular cells. Inhibition of $I_{to}$ has been reported by quinidine in rabbit atrial and ventricular cells (EC$_{50}$: 7 µM [14]), by flecainide in rabbit atrial cells (EC$_{50}$: ~17 µM [15]), by quinidine and flecainide in rat ventricular cells (EC$_{50}$: 3.9 and 3.7 µM, respectively [16]) and by 4-AP, quinidine and flecainide in human atrial cells (EC$_{50}$: ~2 mM, 5–10, ~10 µM, respectively [17]).

4.3. Identity of the $I_{to}$ channel in rabbit SA node

In the heart, it has been suggested that the Kv1.4 channel [18] or Kv4.2 and Kv4.3 channels [19–21] are responsible for $I_{to}$. A characteristic feature of Kv1.4 is slow recovery from inactivation, whereas Kv4.2 recovers from inactivation more rapidly [19]. For example, at ~80 mV the time constant for recovery from inactivation is 3400–8000 and <200 ms for Kv1.4 and Kv4.2, respectively [19]. The time constant of recovery from inactivation of Kv4.3 is similar to that of Kv4.2 [21]. In the present study, recovery of $I_{to}$ from inactivation at ~80 mV was best fitted with a double exponential with time constants of 43 and 1434 ms (Fig. 4). On this basis it is possible that Kv4.2 and/or Kv4.3 is responsible for at least the fraction of $I_{to}$ that recovers rapidly in rabbit SA node cells. We have recently cloned a Kv4.2 channel from the rabbit SA node [22].

4-AP-inhibits Kv1.4, Kv4.2 and Kv4.3 currents — half-maximal inhibition of Kv1.4, Kv4.2 and Kv4.3 is obtained at 0.2–0.5, ~2–4, and 1.54 mM, respectively [19,21,20,18]. Also, 10 and 20 µM quinidine cause approximately half-maximal inhibition of Kv1.4 and Kv4.2 currents, respectively [19,20]. In contrast, whereas 10 µM flecainide causes an approximate half-maximal inhibition of Kv4.2, it has minimal effects on Kv1.4 [19]. A concentration of 26 µM flecainide causes half-maximal block of Kv4.3 [23]. We have shown that 4-AP, quinidine and flecainide inhibit the Kv4.2 channel from the rabbit SA node [22]. These results suggest that Kv1.4 is at least unlikely to be responsible for the majority of $I_{to}$.

In the present study, 4-AP, quinidine and flecainide blocked a sustained outward current as well as a transient outward current. Although the sustained outward current may be a non-inactivating component of $I_{to}$, it may also be a separate current, such as an ultra-rapid delayed rectifier K$^+$ current, $I_{Kur}$ [7]. In human atrial myocytes, whereas 4-AP and quinidine inhibited $I_{Kur}$ (EC$_{50}$: 50 and 5 µM, respectively, at +40 mV), flecainide had no effect [17]. In contrast, in rat ventricular cells flecainide, as well as 4-AP and quinidine, inhibited an $I_{Kur}$-like current (EC$_{50}$: 15 µM, >1, and 14 µM, respectively) [16].

4.4. Heterogeneity and physiological role of $I_{to}$

As explained in the Introduction, the SA node is a heterogeneous tissue. The present study shows that the density of $I_{to}$ (measured either as the difference between the peak outward current at the start of a pulse and the current at the end of the pulse or 4-AP-sensitive current) is significantly correlated with $C_m$ and is greater in larger cells (Figs. 1 and 2). In the case of 4-AP-sensitive current, both the densities of the transient and sustained components ($I_{trans}$ and $I_{sus}$) are significantly correlated with $C_m$ and are greater in larger cells (Fig. 2). In our previous study of $I_{to}$ in rabbit SA node cells at room temperature [7], whereas the sustained component of 4-AP-sensitive current ($I_{sus}$) was correlated with $C_m$, the density of the transient component ($I_{trans}$) was not. $I_{to}$ is highly temperature-sensitive [24] and it is possible that the lack of correlation between the density of $I_{trans}$ and $C_m$ in our former study was the result of the lower temperature.

Because of the cell size-dependent variation in the density of $I_{to}$ (at 35°C), $I_{to}$ is expected to play a more important role in larger cells. To test this, the effects of 1 mM 4-AP (blocks 70±10% of $I_{to}$ — Fig. 8) on the action potential in cells of different size were investigated (Fig. 9). Consistent with the prediction, the effect of block of $I_{to}$ by 4-AP was greater in larger cells: whereas it abolished spontaneous activity in large cells, it did not abolish the activity of small cells (although in small cells, 4-AP significantly decreased action potential amplitude, increased action potential duration, decreased maximum diastolic potential and increased cycle length (Fig. 9). The increase in action potential duration and the decrease of maximum diastolic potential are consistent with the loss of an outward current. It is likely that the decrease in action potential amplitude and slowing or abolition of spontaneous activity were the result of the decrease or failure of repolarization.

In an earlier study of small ball-like tissue preparations of SA node tissue from different regions of the rabbit SA node [6], we observed that the effects of 4-AP on the action potential were greater in tissue from the periphery than from the centre. The results from the present study are consistent with this, because larger cells (in which the effect of 4-AP on the action potential was greater) are likely to be from the periphery of the SA node, whereas smaller cells (in which the effect of 4-AP on the action potential was less) are likely to be from the centre. In the previous study on small balls of SA node tissue [6], the effects of 4-AP on tissue from the centre of the SA node were similar to the effects on small SA node cells in the present study (Fig. 9): the action potential was prolonged and the maximum diastolic potential could be reduced. In the present study, 4-AP abolished the spontaneous activity of large cells (Fig. 9). However, in the study of small balls of SA node tissue [6], although the effects of 4-AP on peripheral tissue were greater than on central tissue, spontaneous activity was not abolished. The reason for this difference is not known. If the effects of 4-AP on the action potential are primarily the effect of block of $I_{trans}$
and \( I_{\text{sus}} \) (although 4-AP has been observed to block a small fraction of \( I_{\text{Kr}} \) — J. Hancox, unpublished observations), the greater effect of 4-AP on large cells (and also small balls of tissue from the periphery of the SA node) are consistent with the densities of \( I_{\text{trans}} \) and \( I_{\text{sus}} \) being greater in large cells and the periphery of the SA node.

It is interesting that there is a significant decrease in the density of a number of currents from large SA node cells (presumably from the periphery) to small SA node cells (presumably from the centre): \( I_{\text{to}} \) (this study), \( I_{\text{Na}} \) [4], \( I_{\text{K_{r}}/\text{K_{s}}} \) (M. Lei, unpublished observations), \( I_{\text{K_{s}}} \) (M. Lei, unpublished observations) and \( I_{\text{t}} \) [4]. A decrease in the density of \( I_{\text{to}} \) from the periphery to the centre of the SA node may help to explain the prolongation of the action potential from the periphery to the centre [25,3]; this in turn may be a protective mechanism to help prevent reentrant arrhythmias [25]. Regional differences in the density of \( I_{\text{to}} \) are involved in regional differences in the action potential in other regions of the heart as well.

Recently Verheijck et al. [26] suggested that the gradual change in the electrical activity from the periphery to the centre of the SA node is not the result of a gradual change in the intrinsic electrical properties of SA node cells from the periphery to the centre (gradient model). Instead they suggested that atrial cells together with SA node cells with uniform properties are found throughout the SA node and the regional differences in electrical activity are the result of a gradual decrease in the number of atrial cells from the periphery to the centre (mosaic model). The results from the present study as well as our previous studies are consistent with the gradient model rather than the mosaic model (see Ref. [5] for fuller consideration).

References

[22] Conley E, O’Beirne H, Owen JM, Hopkins PM, Boyett MR, Hancock JC. Cloning and expression of a \( \text{Kv4.2} \) channel from the rabbit sinoatrial node. J Physiol 1998;511:149P–150P.