Journal of Physiology (1991), **442**, *pp.* 191–209
With 8 figures
Printed in Great Britain

REGIONAL VARIATIONS IN ACTION POTENTIALS AND TRANSIENT OUTWARD CURRENT IN MYOCYTES ISOLATED FROM RABBIT LEFT VENTRICLE

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(*Received 7 January 1991*)

SUMMARY

1. Regional variations in the shape of early repolarization of the action potential have been correlated to differences in transient outward K^+ current, I_t, in myocytes isolated from the epicardial surface, the endocardial trabeculae and the papillary muscles of rabbit left ventricles. Temperature was 35 °C during whole-cell, and 22–23 °C during cell-attached experiments.

2. Membrane resting potentials were very similar regionally. At 0·1 Hz stimulation the action potential plateau amplitude in papillary muscle cells was significantly higher (104·7 mV) than in epicardial cells (96·47 mV). Exposure to 4-aminopyridine or increases in the rate of stimulation from 0·1 Hz to 3.3 Hz increased plateau height and diminished the initial notch on repolarization. These effects were correlated to the magnitude of I_t in these cells. At low rates of stimulation I_t caused a 'spike and dome' morphology of the action potential.

3. Voltage clamp experiments confirmed a higher current density of I_t in epicardial cells (7·66 pA/pF at +20 mV) than in endocardial (6·45 pA/pF) or papillary muscle cells (3·69 pA/pF). I_t at 35 °C was faster and larger than previously reported and individual currents inactivated almost completely during 100 ms pulses to plateau potentials. No differences in the kinetics or voltage dependence of whole-cell currents were found. Thus, the half-inactivation potential was −37·8 mV in cells from all three regions.

4. Cell-attached recordings from endocardial and epicardial cells showed very similar single-channel amplitudes, burst open probabilities and ensemble averages. The peak channel open probability soon after the start of depolarizing voltage clamp pulses did not change between cell types ($P \sim 0{\cdot}8$). The slope conductance of I_t channels was 13·0 pS with an intercept near the resting potential of the cell.

5. We conclude that regional variations in the shape of initial repolarization in cells from rabbit left ventricle are caused by variations in the magnitude of the transient outward K^+ current, I_t. Epicardial cells have the largest, and papillary

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muscle cells the smallest I_t. The differences are not explained by alterations in the whole-cell kinetics or single-channel kinetics and conductance. The most likely explanation for variations in whole-cell current density is therefore a decrease in channel density in endocardium and papillary muscle compared with epicardial tissue. We estimate the density of I_t channels per cell to be 1495 (one per 3–4 μm^2) in epicardium, 1175 (one per 4–5 μm^2) in endocardium, and 875 (one per 6 μm^2) in papillary muscle cells.

INTRODUCTION

The transient outward K^+ current, I_t, is an important repolarizing current during the early phase of the cardiac action potential of many species. It is prominent in atrial tissues of the rabbit (Giles & Van Ginneken, 1985; Giles & Imaizumi, 1988) and humans (Escande, Coulombe, Faivre, Deroubaix & Coraboeuf, 1987; Shibata, Drury, Refsum, Aldrete & Giles, 1989). A similar K^+ current is also present in tissues of the conducting system and can be divided clearly into a voltage-activated component (I_{to}, Boyett, 1981*a*; Nakayama & Irisawa, 1984) which is sensitive to 4-aminopyridine (4-AP) and a calcium-dependent component (Siegelbaum & Tsien, 1980). These may co-exist in sheep (Coraboeuf & Carmeliet, 1982) and calf Purkinje fibres (Kenyon & Sutko, 1987). The role of these currents during normal repolarization in ventricular muscle cells is much less clear. Some tissues, such as rat ventricular muscle are known to possess a large transient outward current (Payet, Schanne & Ruiz-Ceretti, 1981; Josephson, Sanchez-Chapula & Brown, 1984). Action potential studies have suggested the presence of a transient outward current in rabbit ventricle (Kukushkin, Gainullin & Sosunov, 1983) and this was shown by Giles & Imaizumi (1988) to be much smaller in ventricle than in rabbit atrium and to be mainly of the time- and voltage-dependent type (I_t), although a Ca^{2+}-activated component has been described (Hiraoka & Kawano, 1989). Tseng, Robinson & Hoffman (1987) have also demonstrated these two types of transient outward current in canine ventricular cells, but noted that only $\sim 50\%$ of their cells possessed significant 4-AP-sensitive current. There seems little doubt that it is the 4-AP-sensitive component, in atrial cells and Purkinje fibres at least, that can account for many of the physiological variations in action potential shape with rate (Boyett, 1981*b*). This conclusion is given support by mammalian action potential studies on the heterogeneity of the action potential in different regions of the heart. A 'spike and dome' morphology in endocardial, but not epicardial, preparations has been reported in the canine heart *in vivo* (Levine, Spear, Guarnieri, Weisfeldt, DeLangen, Becker & Moore, 1985). In canine tissue strip preparations Litovsky & Antzelevitch (1988) have shown clear differences in the rate dependence and pharmacological properties of endocardial and epicardial action potentials that have been attributed to the virtual absence of a transient outward K^+ current in endocardium as opposed to epicardium. Their studies report an absence of 'spike and dome' action potentials in cells from endocardium. Thus, the presence of this action potential morphology is correlated with the presence of a 4-AP-sensitive transient outward current. Regional differences have also been observed in tissue and isolated cells from rat ventricular muscle and have been attributed to variations in the large voltage-activated transient outward current in this tissue (Watanabe, Delbridge, Bustamante & McDonald, 1983). In cat

myocytes Furukawa, Myerburg, Furukawa, Bassett & Kimura (1990) have shown the presence of a notch in the epicardial action potential and have correlated this with the presence of a transient outward current in this species, which was not previously thought to possess this current (Boyett & Jewell, 1980).

In this study we have analysed action potentials and transient outward current in cells isolated from rabbit left ventricular muscle. Comparing the results from epicardial cells with endocardial and papillary muscle cells we found that there is a progressive decrease in the mean size of transient outward current (I_t) and that this is well correlated with the amplitude of the action potential plateau. Single-channel recordings of transient outward currents made in the cell-attached mode were very similar irrespective of the site of origin of cells.

METHODS

Many of the methods used in this study have been previously described in recent papers from this laboratory that have dealt with α-adrenergic effects on rabbit atrial cells (Braun, Fedida, Clark & Giles, 1990; Fedida, Shimoni & Giles, 1990). Cell dissociation and storage was carried out using these standard methods (Fedida *et al.* 1990). However, some changes were required to isolate cells from different regions of the ventricles. After the entire heart had been perfused for a ten minute period with the enzyme-containing solution (Fedida *et al.* 1990), tissue samples from the three regions under study were obtained. Samples were taken from the left ventricle in the following manner. A razor blade was used to obtain shavings from the epicardium from apex to base. The ventricle was then opened and one, or more usually two, papillary muscles were excised. Finally, superficial trabeculae or surface endocardial layers were excised from the internal ventricular surface with the razor. These tissue samples were then incubated separately in a more concentrated enzyme solution which contained collagenase (Yakult, Toyko, Japan) 250 i.u./ml and protease (P5147, Sigma) 20 i.u./ml. The solution also contained bovine serum albumin, 10 mg/ml, and taurine, 20 mM. Once cells appeared in the incubation supernatant aliquots were transferred to a storage medium as previously described (Fedida *et al.* 1990). Cells were stored at room temperature until use. The rabbits used in these experiments were 1·5–2·0 kg in size ($\sim$ 3 months of age). We also obtained results from twelve cells isolated from adult rabbit endocardium or epicardium which were qualitatively similar to those results obtained from younger rabbits.

The experimental solution was the modified Tyrode solution as used in our previous experiments. It consisted of (in mM): NaCl, 121; KCl, 5·0; sodium acetate, 2·8; $NaHCO_3$, 24; $MgCl_2$, 1·0; Na_2HPO_4, 1·0; glucose, 5·49; $CaCl_2$, 2·0; pH 7·4 after equilibration with 95% O_2–5% CO_2. Experiments were carried out at $35 \pm 1·0$ °C using whole-cell voltage clamp recording techniques previously described. A heated microscope stage was used to control and vary bath temperature which was continuously monitored via a thermistor probe incorporated in the control loop (Narashige Instruments, Japan). The micropipette filling solution had the following composition (in mM): potassium aspartate, 120; KCl, 30; adenosine 5′-triphosphate, 4; Na^+, 8; HEPES, 5; $MgCl_2$, 1·0; ethyleneglycol-bis-(β-aminoethylether)*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 0·2; adjusted to pH 7·2 with KOH. The small amount of EGTA in the filling solution did not alter cell contractions but was added to slow 'run down' of currents. A liquid junction potential of approximately 10 mV (pipette solution negative) arose from the use of potassium aspartate in microelectrodes. All results have therefore been corrected by this amount.

Cell-attached patch clamp experiments were performed as described by Braun *et al.* (1990). Both the bath and the recording pipette contained a HEPES-buffered solution consisting of (in mM): NaCl, 121; KCl, 5·0; sodium acetate, 2·8; $MgCl_2$, 1·0; glucose, 10·0; HEPES, 10·0; $CaCl_2$, 2·0. Single-channel current events were analysed using the program IPROC-2 (Axon Instruments, CA, USA). Initially we attempted to make single-channel recordings at 35 °C but the rapidity of channel openings and closings exceeded the resolution of our recording equipment. For this reason, cell-attached recordings were made at room temperature (22–23 °C).

RESULTS

Rate dependence of the early phase of repolarization of rabbit ventricular action potentials

The action potentials in Fig. 1 illustrate the range of early plateau shapes observed in cells isolated from rabbit ventricular myocardium. In *A* and *B* the rate dependence

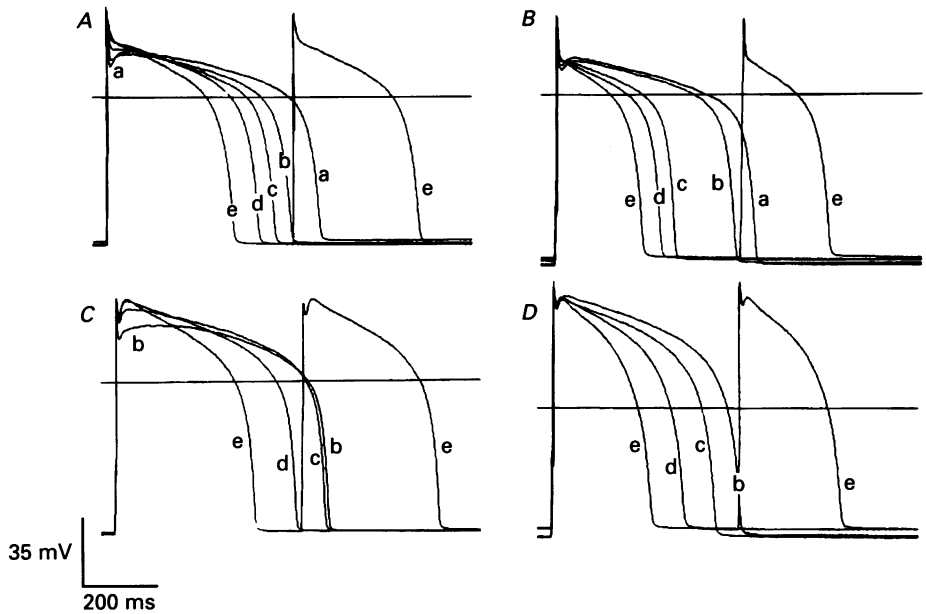

Fig. 1. Rabbit ventricular action potentials at different stimulus frequencies. Epicardial (*A*) and papillary muscle cell (*B*) from the same heart. Epicardial (*C*) and endocardial (*D*) cells from another heart. Rates were: a, 0·1 Hz; b, 0·2 Hz; c, 0·5 Hz; d, 1 Hz; e, 2 Hz. In this and all subsequent figures, action potentials were elicited using 2 ms suprathreshold current pulses. The horizontal line in each panel denotes −10 mV. Note that stimulation at 2 Hz causes the appearance of a second action potential on this time base. Temperature 35 °C in this and all subsequent figures unless otherwise stated.

of action potentials is shown in an epicardial cell and a papillary muscle cell from the same heart. As the stimulus frequency was increased from 0·1 to 2 Hz there was an obvious shortening of the action potential. Of more interest are the changes in the initial phase of repolarization. At 0·1 Hz in the epicardial cell (*A*) there is a prominent 'notch' denoting rapid repolarization. As the stimulus frequency was increased this notch disappeared and only a small effect can be seen at stimulus frequencies higher than 1 Hz. In contrast the papillary muscle cell showed much smaller changes in the initial rate of repolarization as the stimulus frequency was increased. In *C* and *D* are data from an epicardial cell and an endocardial cell from an adult rabbit heart. Very similar results were observed to those described in *A* and *B*. In the epicardial cell changes of 10–20 mV occurred in the initial phase of repolarization during an increase in the stimulus frequency. Most of this change occurred when the stimulus frequency was increased from 0·1–1 Hz. However, in the endocardial cell no similar

pattern of changes occurred in the initial rapid phase of repolarization throughout this range of stimulus frequencies.

Boyett demonstrated that in sheep Purkinje fibres, changes in the rate of early repolarization correlated well with the magnitude of the voltage-activated transient

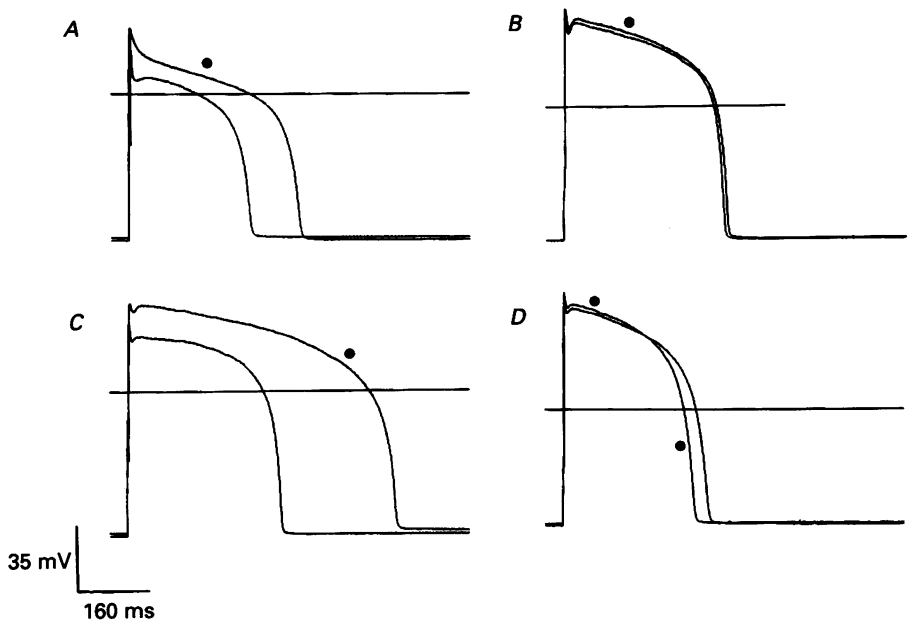

Fig. 2. Effect of 4-AP on rabbit ventricular action potentials in same cells as in Fig. 1. *A* and *C* from epicardium; *B*, papillary muscle; *D*, endocardium. Action potentials shown at 0·1 Hz in control and after exposure to 1 mM-4-AP (●). Note that cells which showed very small changes in the early phase of repolarization upon changes in the stimulus frequency (Fig. 1*B* and *D*) are also little affected by 4-AP. The horizontal line in each panel denotes −10 mV.

outward current (Boyett, 1981*a*, *b*). The rate dependence of the effects described in Fig. 1 also correlate well with the known rate dependence of the transient outward current in rabbit heart cells (Giles & Imaizumi, 1988; Fedida *et al.* 1990). The results in Fig. 1 therefore suggest that a transient outward current is present to a much greater extent in epicardial cells than in endocardial or papillary muscle cells. A more direct test of the contribution of the transient outward current to the action potential waveform is shown in Fig. 2. Here, during low-rate stimulation at 0·1 Hz to maximize I_t, cells were exposed to 1 mM-4-AP, a concentration which blocks $\sim 80\%$ of I_t. All data in this figure were obtained from the same cells as Fig. 1. As expected, administration of 4-AP to epicardial cells during stimulation at 0·1 Hz resulted in significant plateau elevation, action potential prolongation and reduction of the notch during early repolarization. 4-Aminopyridine exerted much smaller effects on the papillary and endocardial cells (*B* and *D*), although there was reduction of the initial phase of repolarization in these cells, and some plateau elevation which suggested the presence of a smaller voltage-activated transient outward current in these cells. It is interesting to note that in three of the four examples in Fig. 2,

1 mM-4-AP did not completely abolish the repolarization notch of the action potential. Thus 1 mM-4-AP is probably not sufficient to completely block I_t (Giles & Imaizumi, 1988; Furukawa *et al.* 1990; see Fig. 5) but we cannot completely exclude the presence of a calcium-activated component of transient outward current in these cells at 35 °C (Hiraoka & Kawano, 1989). Generally we were unable to observe

TABLE 1. Action potential properties

	Cell type		
Parameter	Epicardium	Endocardium	Papillary muscle
Resting potential (mV)	−81·3 ± 3·9(15)	−78·9 ± 6·0(11)	−81·0 ± 2·4(12)
Amplitude (mV)	96·5 ± 9·3(15)	102·2 ± 8·2(9)	104·8 ± 6·5(9)
Capacitance*(pF)	55·9 ± 13·4(10)	72·3 ± 22(9)	80·1 ± 16·6(10)
Mean rating	4·1 ± 1·1(15)	2·6 ± 1·1(9)	2·4 ± 1·1(11)

* Only measured in cells used for voltage clamp. Resting potential measurements are corrected for liquid junction potential (see Methods). Amplitude refers to maximum action potential plateau height reached after initial repolarization notch. Cell 'rating', see text. All values are means ± S.D. Numbers in parentheses are number of cells used.

current components attributable to a Ca^{2+}-activated transient outward current (Siegelbaum & Tsien, 1980). It is possible that this may have been related to the use of 0·2 mM-EGTA in pipettes, although cell contraction was not reduced significantly. The Ca^{2+}-activated transient outward currents are rather small in our cells as they are in dog ventricle (Tseng & Hoffman, 1989). It is of note that the latter authors were still able to observe a Ca^{2+}-activated transient outward current in canine ventricular cells when they included 1 mM-EGTA in their pipette solutions.

Table 1 describes the action potential properties we have deemed relevant to the presence or absence of transient outward current in these three groups of ventricular cells. Changes in the resting potential in different regions of the left ventricle may alter availability or reactivation time for the transient outward current and thus give rise to apparent regional differences in the properties of the current. However, the mean resting potential was near −80 mV in all groups of cells studied. The maximum action potential height was higher in the endocardial and papillary muscle cells than in cells from the epicardium (cf. Fig. 1). This difference was significant between the papillary muscle cells and epicardial cells ($P < 0·02$) and may be explained by a small depressant effect of the transient outward current on the epicardial action potential plateau. In order to illustrate the spectrum of action potential behaviour and the effect of 4-AP we have given the various types of ventricular cells a rating between 1 and 5; higher values denote larger rate-dependent changes in the initial rate of repolarization, greater effects of 4-AP and larger transient outward currents observed under voltage clamp. The mean rating of 4·1 for epicardial cells reflects the fact that a majority of these cells exhibited significant changes in plateau shape and elevation with rate. On the other hand, the values between 2 and 3 for endocardial and papillary muscle cells reflect that, although many of these cells did not show large transient outward currents or changes in plateau height, nevertheless some cells did have large currents (Fig. 5). The mean cell capacitance varied somewhat between the cell types for those that were voltage clamped, although the mean value for all cells (66·2 ± 23 pF, S.D.) is close to that reported by Giles & Imaizumi (1988).

Litovsky & Antzelevitch (1988) have shown that premature action potentials from canine ventricular epicardium and endocardium show plateau elevation when the 'control' action potential possesses a prominent 'spike and dome' morphology. We observed that in rabbit ventricle, even cellular action potentials which exhibited

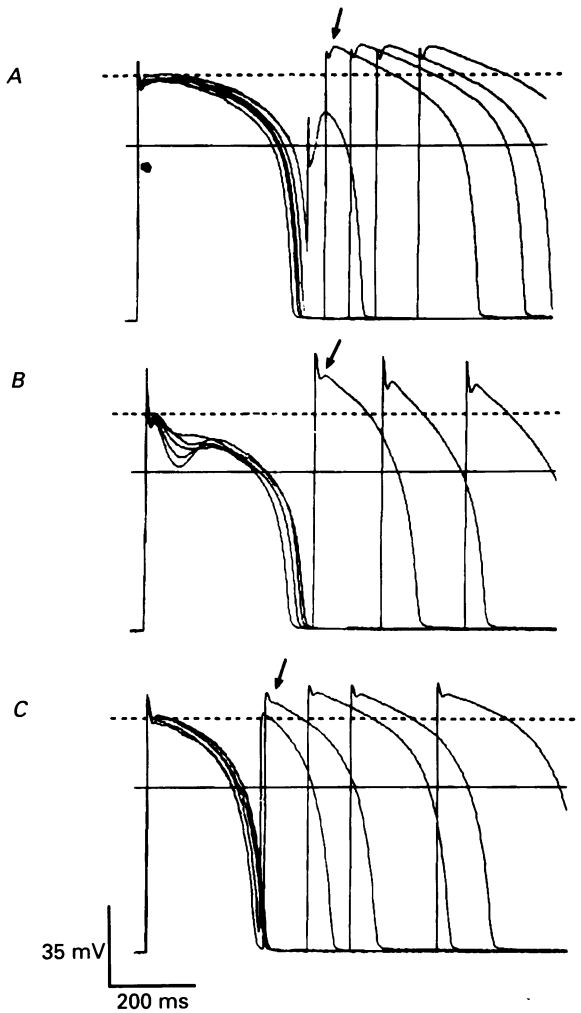

Fig. 3. Effect of premature stimuli on the action potential plateau in rabbit ventricular myocytes. Cells were from epicardium (*A*), endocardium (*B*) and papillary muscle (*C*). Cells from three different hearts. Action potentials were elicited at a basic rate of 0·2 Hz and premature stimuli (2 ms duration) at 1–2 times the basic intensity were used to generate extra action potentials at varying intervals after control action potentials. The continuous horizontal line in each panel denotes −10 mV. The dashed lines illustrate the plateau level of control action potentials. The arrows indicate a decreased initial rate of repolarization of the premature action potential.

very small initial notches on repolarization could show significant plateau elevation in response to premature stimuli. This finding is illustrated in Fig. 3. Control action potentials were generated at 0·2 Hz from epicardial, endocardial and papillary

muscle cells. Note that none of the three control action potentials has a very prominent initial phase of repolarization. However, when premature stimuli were applied, significant plateau elevation was seen in all action potentials elicited soon after complete repolarization of the control action potentials. The arrows in panels

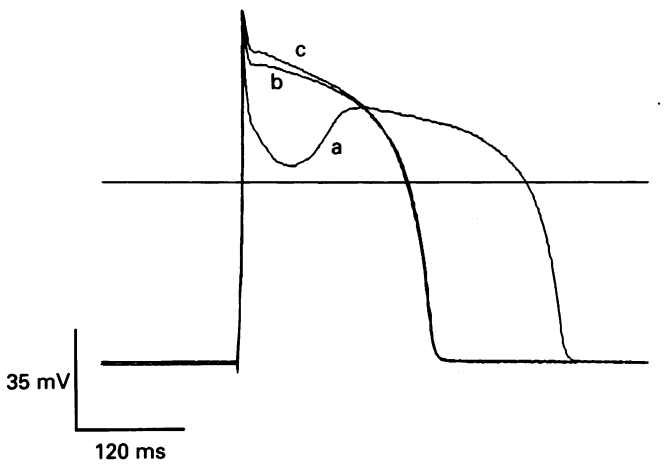

Fig. 4. The 'spike and dome' action potential morphology in rabbit ventricle demonstrated by changes in stimulus rate in an endocardial cell. a, 0·1 Hz; b, 1 Hz; c, 2 Hz. This morphology was seen only at low stimulus rates < 1 Hz and in three left ventricular cells altogether. The horizontal line indicates -10 mV.

A–C denote loss of the notch on repolarization in all three examples. At longer test intervals the initial phase of repolarization gradually recovered, although full recovery of the notch to control levels required rests of up to 5 s (data not shown).

The 'spike and dome' morphology of the rabbit ventricular action potential was not observed at physiological driving rates of 1 Hz and above at 35 °C. However, at low stimulus rates of 0·5 Hz and below action potentials were occasionally observed which had a deep notch on initial repolarization followed by a regenerative plateau phase. An example of such a result is shown in Fig. 4. At rates of 1 and 2 Hz a typical ventricular action potential was observed. However, at 0·1 Hz (a) a 'spike and dome' type of action potential was recorded. These action potentials were recorded from an endocardial cell and in total three examples of a clear 'spike and dome' shape were observed at low stimulus rates, one from each regional cell type. The explanation given by Litovsky & Antzelevitch (1988) that the 'spike and dome' morphology of an action potential is related to the amount of transient outward current present is borne out by the observations in Fig. 4 where an increase in the stimulus frequency, which will inactivate the transient outward current, abolished the 'spike and dome' shape of the action potential.

The action potential data presented in Figs 1–4 support the idea that in rabbit ventricular muscle, as in other mammalian species, there is a prominent transient outward current that is responsible for the rapid initial phase of repolarization of the cardiac action potential. However these results also suggest that the regional differences in plateau shape between the inside and outside surfaces of the ventricle

are more subtle than those described previously in dog ventricle (although see Tseng *et al.* 1987). A number of possible explanations exist for this. One is that the transient outward current is somewhat smaller in rabbit ventricle (Giles & Imaizumi, 1988) than in other mammalian preparations and another is that inactivation is extremely

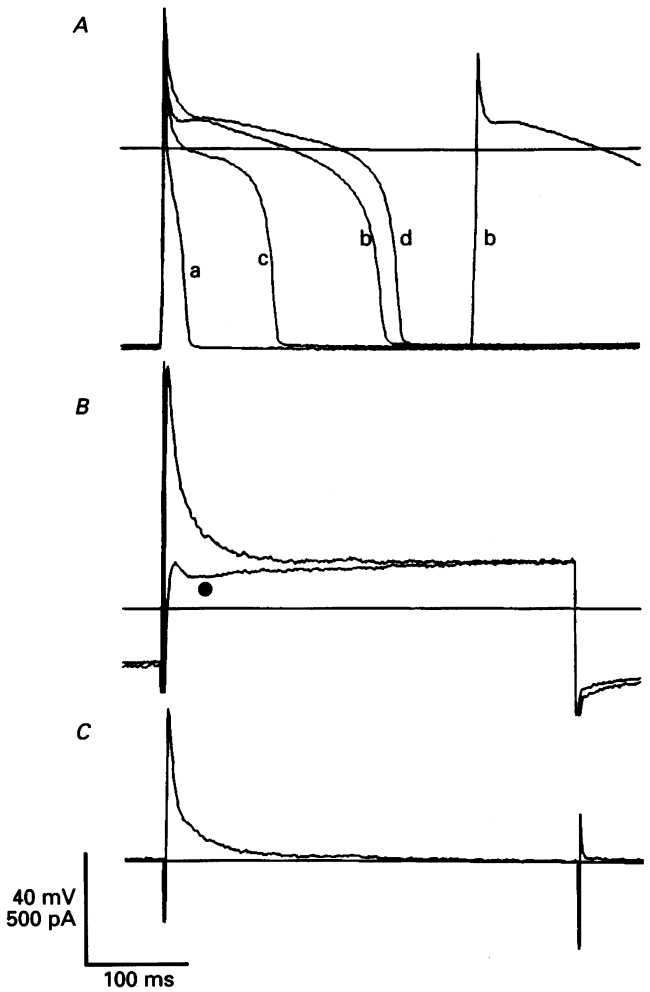

Fig. 5. Effect of transient outward current on action potential duration in cells studied in presence of 0·3 mM-$CdCl_2$. *A*, epicardial cell action potentials at: a, 0·1 Hz; b, 3·3 Hz; c (2 min) and d (steady-state), 0·1 Hz after addition of 1 mM-4-AP. Note that increase in rate or 4-AP, both of which inhibit transient outward current can increase duration even when I_{Ca} is blocked almost completely. Horizontal line depicts −10 mV. (Transient outward currents from same cell are shown in Fig. 6*A*.) *B*, voltage clamp currents in another cell. Pulses from −80 to +15 mV for 400 ms in control and presence of 1 mM-4-AP (●). *C*, difference current from data in *B*. Note transient nature of 4-AP-sensitive current. Electrode resistance 1·5 MΩ, measured series resistance 2·5 MΩ.

rapid. In the remaining figures in this paper we further investigate the properties of the transient outward K^+ current, I_t, in rabbit ventricular cells.

The transient outward current is a relatively large current in rabbit ventricular myocytes at 35 °C. The ability of this current to initiate repolarization is illustrated in Fig. 5. This cell was treated with 0·3 mM-$CdCl_2$ which abolishes much of the calcium current, I_{Ca}. The action potential at 0·1 Hz under these conditions is extremely brief ($\sim$ 50 ms). However, stimulation at 3·3 Hz, which inactivates I_t, caused the action potential to be restored to its normal shape. Administration of 1 mM-4-AP to the same cell during low-rate stimulation has a very similar effect (Fig. 5*A*, c and d). Figure 5*B* shows a voltage clamp measurement of I_t from another cell. The membrane current trace is shown before and after (●) exposure to 4-AP. The large current transient in the control situation can be abolished almost completely by 4-AP at this concentration. The small time-dependent current transient still seen in the presence of 4-AP may represent residual I_t or I_{Ca} not entirely blocked by $CdCl_2$. Subtraction of the records in *B* gave the difference current plotted in Fig. 5*C*. This procedure shows the relatively large (almost 1 nA in amplitude), but transient nature of I_t in rabbit ventricular cells at 35 °C. The current is almost completely inactivated 50 ms after being activated. The implications of the transient nature of this current are considered further in the Discussion.

The amplitude of transient outward current in the three different regions of the rabbit ventricle which we have studied are shown in Fig. 6. Representative transient outward currents from an epicardial cell and a papillary muscle cell are shown in Fig. 6*A*. These current traces were obtained during 400 ms step voltage clamp pulses from a holding potential of −80 mV, which was very close to the cell resting potentials. These traces depict the range of amplitudes of transient outward currents recorded. Very large and fast transient outward currents were consistently recorded from epicardial and sometimes endocardial cells, but only rarely from papillary muscle cells. Small transient currents were almost always recorded from papillary muscle cells, but sometimes were observed in endocardial cells. The mean peak current–voltage relations for I_t from these three regions of rabbit ventricle are shown in Fig. 6*B*, where the data has been normalized to cell capacitance. The findings show clearly that the highest current density was observed from epicardial cells, a somewhat lower density from endocardial cells and an even smaller current was recorded from papillary muscle cells. These differences were significant for papillary muscle cells over the entire potential range: but when epicardium and endocardium were compared, the differences were significant only at the most positive potential compared (+40 mV), as indicated.

The smaller transient outward current in endocardium and papillary muscle than in epicardium could be caused by a number of factors. Conceivably, the threshold for activation of this current could be shifted to more positive potentials in the endocardial and papillary muscle cells. This does not seem a very likely explanation since the peak current–voltage relations do not appear shifted along the voltage axis, but are simply scaled down from one group of cells to the next. Another possibility is that the inactivation kinetics of the currents in the three groups of cells are different, so that when activated from a holding potential of −80 mV, the steady-state inactivation of the current in papillary muscle cells would result in smaller peak

outward currents on depolarization. This hypothesis was tested and the results are illustrated in Fig. 7. Figure 7*A* illustrates the protocol used to measure steady-state inactivation in the ventricular muscle cells. From a holding potential of −80 mV the cell was clamped at various potentials for 0·5 s. Currents recorded at the end of such

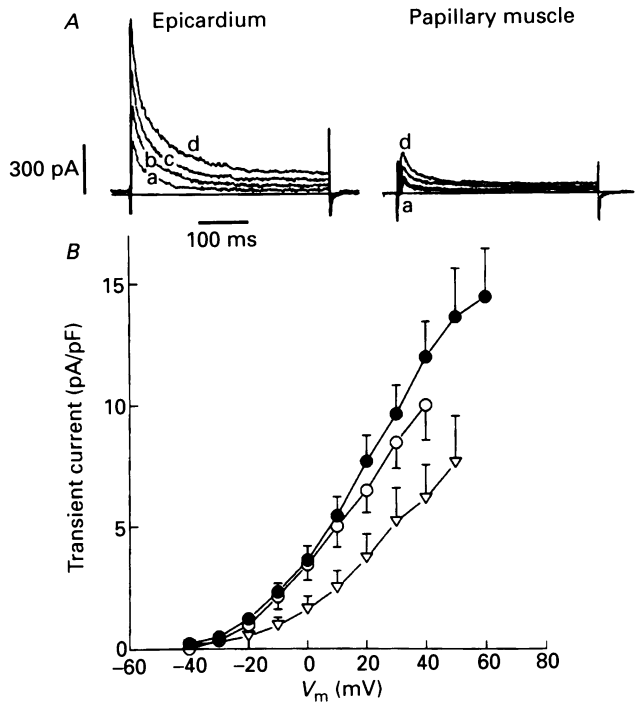

Fig. 6. Cumulative data on magnitude of transient outward current in cells from different regions of the left ventricle at different membrane potentials (V_m). *A*, examples of the transient currents recorded from epicardial cells (left) and papillary muscle cells (right). These data are representative of the range of current magnitudes that were recorded. Cells were held at −80 mV and clamped to a range of potentials at 0·1 Hz: a, −10 mV; b, 0 mV; c, +10 mV; d, +20 mV. *B*, mean magnitude of peak outward transient current in ten epicardial cells (●), nine endocardial cells (○) and ten papillary muscle cells (▽). Data were generated from a holding potential of −80 mV at 0·1 Hz and normalized to the capacitances of the individual cells (values ± S.E.M.). Mean values from papillary muscle cells were significantly less than data from endocardial cells or epicardial cells at all potentials studied ($P < 0·05$). Differences between epicardial and endocardial data, other than at +40 mV were not significant ($P < 0·02$).

pre-pulses are shown to the left of Fig. 7*A*. Subsequently the cell was pulsed to a positive test potential of +30 mV to elicit residual I_t. As the pre-pulse potential became more positive, less I_t was elicited until, at about −20 mV, the pre-pulse completely inactivated the current. The currents during the test pulses were normalized to the maximum current obtained when inactivation was completely removed at a holding potential of −110 mV. Normalized data from one representative cell from each of the three regions of the heart under study are shown in Fig. 7*B*. Data from all three cell types were very similar and none of the cells shows

significant inactivation at the resting potential (~ -80 mV). Thus, data from all three cells were fitted to a Boltzmann function (see figure legend) which gave a half-inactivation potential of $-37{\cdot}8$ mV and which predicts complete reactivation negative to -60 mV. Clearly, regional variations in the amount of I_t cannot be explained by differences in the steady-state inactivation of the current.

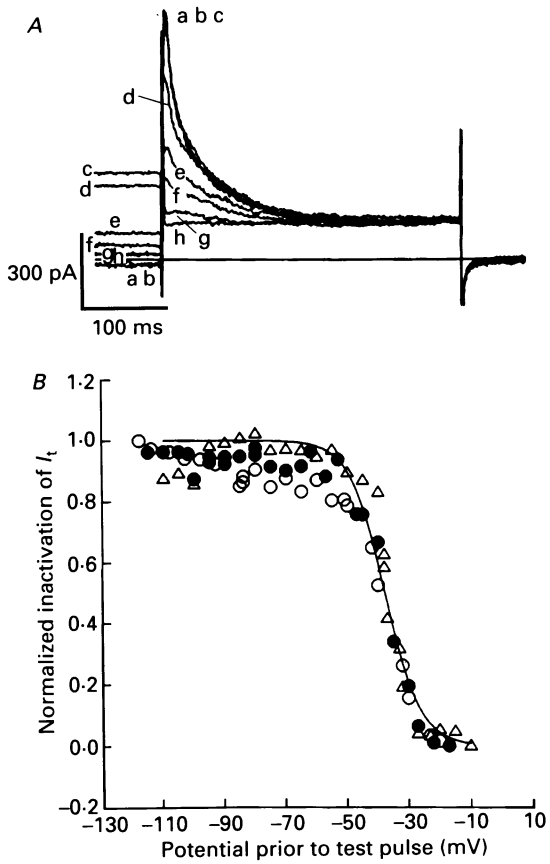

Fig. 7. Steady-state inactivation of transient current in cells from rabbit ventricle. *A*, two-step voltage clamp protocol was employed to measure inactivation. Representative data from an epicardial cell. Cell was held at -80 mV and a 500 ms pre-pulse was given to a range of potentials between -120 and -10 mV to partially inactivate the transient current. The traces at the extreme left of panel *A* show the current levels at the end of a number of such pre-pulses. Subsequently the cell was clamped at a constant test potential ($+30$ mV) to elicit the remaining transient current as shown in the main part of the current records in *A*. The pre-pulse potentials were, in mV: a, -90; b, -80; c, -62; d, -53; e, -40; f, -35; g, -27; h, -22. *B*, normalized data from an epicardial cell (●), an endocardial cell (○) and a papillary muscle cell (△) all lie on a similar line. An equation of the form: $f = 1/(1+\exp((x+V_{0{\cdot}5})/S))$ was fitted to the data as the continuous line in *B*. The half-inactivation potential ($V_{0{\cdot}5}$) was $-37{\cdot}8$ mV and the slope factor (S) 5·7. Similar data were recorded from eleven other cells.

A second explanation for the reduction in transient outward current from epicardium to endocardium and papillary muscle cells is a difference in the properties of the single transient outward current channels. We have therefore made recordings of the single-channel events which underlie the transient outward current in rabbit epicardial and endocardial ventricular cells, using the cell-attached configuration of the patch clamp technique. We were unable to detect single I_t channels in four patches on papillary muscle myocytes. In epicardial and endocardial myocytes, single-channel events were evoked by holding the patch membrane potential at 0 or -50 mV with respect to cell resting potential and depolarizing the patch for 150 ms at 5 s intervals to $+75$, $+100$ and $+125$ mV with respect to the resting potential. Outward currents between 1 and 2 pA in amplitude were elicited over this potential range. Representative data from one heart are shown in Fig. 8. In this heart single I_t channels were recorded from three of ten patches from endocardial cells and three of six patches from epicardial cells. Data from one epicardial cell and one endocardial cell are shown in Fig. 8. Panels *A* and *B* show single sweeps of channel activity elicited by repetitive depolarizations at a rate of 0·2 Hz. Note that only one channel is active in each of these two patches. Patches containing more than one channel were only observed in one of nineteen cell-attached patch recordings from epicardial rabbit ventricular cells. This can be compared to rabbit atrial cells (Clark, Giles & Imaizumi, 1988) where multi-channel patches accounted for three of sixteen active patches. Channel activity occurs in bursts, mainly at the start of depolarizations and is quite variable in duration. Numerous rapid partial closings occur during each burst, however these could not be fully resolved at the filter frequency used in the experiments. Re-opening of channels was also commonly seen during sustained depolarizations. Kinetic analysis of such data revealed an open probability of channels during bursts of 0·8–1·0 in both epicardial and endocardial cells. This behaviour is very similar to that observed previously in rabbit atrial cells (Braun *et al.* 1990). For depolarizations of 100 mV with respect to rest, the mean single-channel amplitude was $1{\cdot}45 \pm 0{\cdot}15$ pA in endocardial and $1{\cdot}37 \pm 0{\cdot}02$ pA (mean $\pm$ S.D., $n = 3$) in epicardial cells. Data on single-channel current amplitudes from all cells from this heart were pooled at the three potentials studied and a least-squares fit to a straight line gave a mean conductance of the single I_t channels from epicardium and endocardium of 13·0 pS, which is in close agreement with previous recordings of I_t channels in rabbit atrial cells (13·9 pS, Clark *et al.* 1988). The intercept of the line through data points was $-0{\cdot}8$ mV with respect to the resting potentials of the ventricular cells. Single-channel currents recorded from rabbit ventricle cells were sensitive to 1 mM-4-AP and this activity was suppressed when the depolarizing clamp steps were applied at rates greater than 1 Hz. Figure 8*C* and *D* shows ensemble averages for complete data sets from which the examples in *A* and *B* were taken. Most striking is the transient nature of the I_t ensemble current which decays almost to the zero current level within the first 50 ms. No apparent maintained current level was observed. The averages from the epicardial and endocardial cells are very similar and the conclusion we draw from this data is that single I_t channels in rabbit endocardium and epicardium have the same amplitude and kinetics.

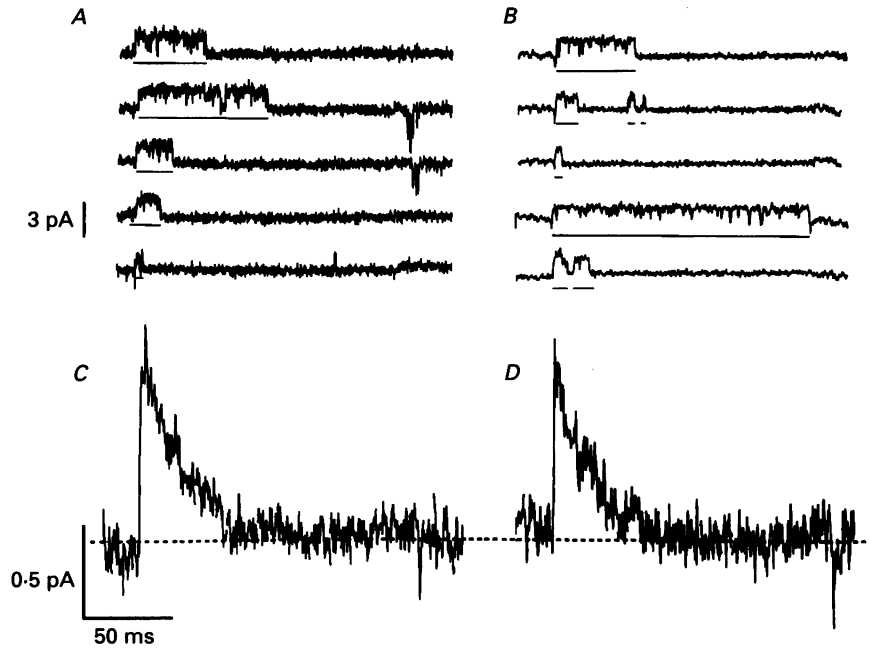

Fig. 8. Examples of single-channel currents recorded from cell-attached patches on an epicardial cell (*A*) and an endocardial cell (*B*) from the same heart. In both cases the patch was held at −50 mV with respect to the cell's resting potential (V_{rest}). Depolarizing pulses with respect to V_{rest} of +125 mV in *A* and +100 mV in *B* were applied. Capacity and 'leak' currents were removed from the traces by applying depolarizing steps at 2 Hz and averaging sweeps during which no channel openings were observed. These averages were then subtracted from individual sweeps to give the records in *A* and *B*. Currents were filtered at 2 kHz in *A* and 1 kHz in *B* and sampled at 10 kHz. The horizontal lines under bursts of channel openings indicate event detection by the analysis program. *C* and *D*, the ensemble averages for data in *A* and *B* were obtained from fifty-one traces in *C* (epicardial patch) and twenty-five traces in *D* (endocardial). The dashed lines represent 0 pA.

DISCUSSION

Action potentials from rabbit ventricular myocytes

Indirect evidence for regional variations in the amount of transient outward current has been obtained previously from action potential experiments on rat ventricular myocytes (Watanabe *et al.* 1983) and on canine ventricular tissue (Litovsky & Antzelevitch, 1988). It is of interest that in both of these studies, papillary muscle tissue was the one in which the transient outward current was least apparent. In the study on rat muscle, Watanabe *et al.* showed that the largest-amplitude action potentials and those with the slowest rate of initial repolarization came from left ventricular papillary muscles. Litovsky & Antzelevitch (1988, 1990) did not find a significant electrophysiological distinction between endocardial trabeculae and papillary muscle preparations and suggested that the absence of the 'spike and dome' type of action potentials might be attributable to a lack of

transient outward currents in these cells. Earlier studies suggested that it may be possible to identify action potential changes attributable to I_t in canine endocardium but not papillary muscles. Greenspan, Edmands & Fisch (1967) studied surface endocardial cells from canine right ventricle and showed that premature action potentials are associated with plateau elevation and diminution of the 'notch', while action potentials recorded after rest are short and have a depressed plateau phase. Similarly, Iinuma & Kato (1979) demonstrated augmented premature responses from canine right ventricular trabeculae. On the other hand, canine papillary muscle action potentials show little change in shape or duration during, or immediately after pauses in stimulation or rate changes (Hoffman & Suckling, 1954; Miller, Wallace & Feezor, 1971). In a more recent study, Tseng *et al.* (1987) noted that 50% of isolated canine ventricular myocytes exhibited fast repolarization and a 'notch' during the initial phase of repolarization of the action potential. These cells had a large transient outward current that was sensitive to 4-aminopyridine.

Action potentials from rabbit papillary muscles do not show a rapid phase of repolarization (Wohlfart, 1982) unlike those from other regions of the rabbit ventricle (Gibbs & Johnson, 1961). Our results are in keeping with all the observations described above. Epicardial cell action potentials almost invariably showed a notch on initial repolarization that disappeared at rates of stimulation above 1 Hz (Figs 1, 3 and 4) and was sensitive to 4-AP (Fig. 2). However, in endocardial cells and papillary muscle cells (Figs 3 and 4), although the action potential plateau was higher, a shallow notch was often apparent on repolarization and the notch was suppressed as the stimulus frequency was increased. We have identified a spectrum of action potential shape changes from epicardium to papillary muscle cells that suggested a gradual diminution of the effects of a transient outward current on the action potential.

Transient outward current

When we measured the transient outward current in these cells at 35 °C we were somewhat surprised by the magnitude and rapidity of its kinetics. Activation of the current was too fast to resolve during the depolarizing voltage clamp pulses, and at 35 °C the density of I_t in epicardial cells was $\sim$ 5 pA/pF at 0 mV. This level is similar to that observed in rabbit atrial cells at room temperature (Fedida *et al.* 1990) and is in keeping with the 2- to 3-fold increase in I_t observed by Giles & Imaizumi (1988) when the temperature was increased from 23 to 33 °C. Inactivation of large transient currents occurred within 100 ms and no steady current level was observed that could be attributed to 4-AP-sensitive transient outward currents (Fig. 5). The effect of this current on early repolarization of the action potential and the production of a 'notch' depends on the balance or interaction of this transient current with the inward currents activated at the same time. In a few cells we observed a 'spike and dome' morphology of the action potential (Fig. 4). This has been reported to be typical of epicardial action potentials in canine myocardium (Litovsky & Antzelevitch, 1988), but we observed these action potentials at low stimulus rates in all three cell types. In our cells this notch may be a non-physiological phenomenon related to delayed inactivation of the transient outward current or delayed activation of I_{Ca} or Ca^{2+}-activated currents. When calcium current is blocked by

$CdCl_2$, I_t can produce 'all or none' repolarization, as illustrated in Fig. 5. Under some conditions involving hypoxia or metabolic blockade, I_{Ca} can be reduced (Carmeliet, 1978) and the transient outward current may lead to premature termination of the action potential (Lukas & Antzelevitch, 1988).

Regional variation in the transient outward current

Our results have demonstrated a difference in the density of transient outward current in different regions of the left ventricle. There is an approximately 50% decrease in I_t from epicardium to endocardium and papillary muscle cells (Fig. 6). Despite this, in only very few cells was the transient outward current actually absent. The observed decrease in this current supports the action potential data described above and is consistent with the previous indirect evidence of others (Watanabe *et al.* 1983; Litovsky & Antzelevitch, 1988). In cat myocytes, Furukawa *et al.* (1990) have demonstrated the virtual absence of I_t in endocardial cells while recording a current of ~ 40 pA/pF at +20 mV in epicardial cells. Our results do not show these striking differences, and we would emphasize that we observed a spectrum of behaviour in cells from different regions. We cannot attribute the decrease in I_t in endocardial cells to a shift in the activation curve since the peak current–voltage relations did not suggest this (Fig. 6) and nor was there a shift in the steady-state inactivation properties of the current. The resting potentials of cells isolated from all three regions of the heart are very similar (~ −80 mV, Table 1) and the steady-state inactivation curve reaches a steady level at −50 mV (Fig. 7). The half-inactivation potential of −37·8 mV is similar to a value of −32 mV reported by Clark *et al.* (1988) using an identical protocol in rabbit atrium.

The single-channel events underlying transient outward currents have been previously described in mouse ventricular myocardium (Benndorf, Markwardt & Nilius, 1987). A 12 pS channel observed in three of ninety patches has the same conductance and reversal potential as the 13 pS channel we have described, but slower kinetics with long open times extending up to 200 ms. Transient outward current channels have also been reported in rabbit atrioventricular node cells (Nakayama & Irisawa, 1984; 19·9 pS, 5·4 mM-external K^+, 32–36 °C) and a large-conductance Ca^{2+}-activated channel in Purkinje cells (Callewaert, Vereecke & Carmeliet, 1986). The properties of the channels that we have observed in ventricular cells are, however, more similar to those described previously in rabbit atrium (Clark *et al.* 1988: Braun *et al.* 1990). Clark *et al.* (1988) identified a channel of this type with a slope conductance of 13·9 pS and a reversal potential at the cell resting potential. Channel openings appear in bursts at the start of depolarizing clamp pulses and show numerous, rapid, partial closings during a burst. Despite these frequent closings, kinetic analysis of bursts reveals an opening probability during the bursts of between 0·8 and 1·0 (cf. Braun *et al.* 1990). Ensemble averages of currents obtained from patches containing only a single channel (Fig. 8) allow an estimate to be made of the peak open probability of the channel. The peak of the ensemble average occurs in Fig. 8*C* very soon after the start of the depolarizing pulses. The peak open probability, calculated by taking the peak value of the ensemble average divided by the single-channel amplitude, was 0·776 for the endocardial cell and 0·851 for the epicardial cell data in Fig. 8. The slope conductances for whole-cell I_t can be obtained from linear least-squares fits to peak current–voltage relations (data in Fig. 6) over

a range of potentials from +10 to +50 mV. These were 216 pS/pF in epicardium, 169·6 pS/pF in endocardium and 126·4 pS/pF in papillary muscle myocytes. If the peak open probability over this potential range is taken as 0·8, and the average capacitance of rabbit ventricular cell is 72 pF with a surface area of 5200 μm^2 (Giles & Imaizumi, 1988), the density of I_t channels in each of the three cell types can be calculated. In epicardial cells this amounts to 1495 channels/cell (one per 3·48 μm^2); in endocardial cells, 1174 channels/cell (one per 4·43 μm^2) and in papillary myocytes, 875 channels/cell (one per 5·94 μm^2). An important caveat must be applied to these calculations. This is that single-channel data were obtained at 22–23 °C whereas whole-cell currents were recorded at 35 °C. It is likely that the single-channel conductance will increase at higher temperatures (perhaps by as much as 6 pS, or 50%; cf. Nakayama & Irisawa, 1984). Thus, the calculations of I_t channel density must be considered as upper limits and may be up to one-third lower.

The calculated single-channel density of one per 3–4 μm^2, is very similar to that reported in rabbit atrial cells by Clark *et al.* (1988) for whole-cell data collected at 22–23 °C. Taken together, our findings support the observations of Giles & Imaizumi (1988) that the density of whole-cell transient outward current is lower in rabbit ventricular than atrial myocytes. Further, our data suggest that the smaller current arises from the lower density of I_t channels in cells rather than any changes in the intrinsic channel properties. Regionally, within the rabbit myocardium, our results indicate a progressive decrease in I_t channel numbers from epicardium to papillary muscle cells. An estimate of one channel per 6 μm^2 on papillary myocytes suggests that I_t channels can be few in number and explains why patches containing I_t channels may be scarce (cf. Results and Benndorf *et al.* 1987).

Implications of regional differences in I_t

It has been suggested that large epicardial–endocardial differences in the density of transient outward current may account for the heterogeneous rate dependence of the action potential and the T-wave of the electrocardiogram (cf. Burgess, 1979; Watanabe, Rautaharju & McDonald, 1985; Litovsky & Antzelevitch, 1989). In unphysiological situations in which I_t has an important role, regional differences may also be important (Burgess, 1979). For example quinidine, an agent widely used in the treatment of cardiac arrhythmias, can substantially block I_t (Imaizumi & Giles, 1987), and therefore may be expected to have somewhat different electrophysiological effects in the epicardium compared with endocardium and papillary muscles.

We would like to thank Dr R. B. Clark for his help in the collection and analysis of the single-channel data in Fig. 8. In addition we thank Mrs Ewa Dulski for her help in the isolation of rabbit ventricular cells. This work was supported by operating grants from the Medical Research Council of Canada and the Canadian Heart Foundation and by a postdoctoral fellowship (D.F.) and Medical Scientist Award (W.R.G.) from the Alberta Heritage Foundation for Medical Research.

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