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ELECTROPHYSIOLOGY OF SINGLE HEART CELLS FROM THE RABBIT TRICUSPID VALVE

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SUMMARY

1. The electrophysiology of single myocytes isolated from the rabbit tricuspid valve was studied using the patch-clamp method (whole-cell configuration). Cell dispersion was achieved by collagenase treatment, using the Langendorff retrograde perfusion procedure.

2. After isolation, and while incubating in the recovery (Kraftbrühe) solution, cells had clear striations and were mostly spindle-shaped, or rod-like (< 10%), with length varying from 35 μm to over 150 μm, and diameter from 3 to 10 μm.

3. Upon exposure to Tyrode solution, the calcium-tolerant cells were mostly rounded with smooth surfaces and well-defined borders. The mean diameter of these cells was 15 ± 5 μm (S.D., $n = 9$). A smaller percentage (about 30%) retained the original elongated shape.

4. Patch pipette recordings showed the presence of spontaneous activity in about 30% of round cells, and less frequently in elongated cells. Maximum diastolic potentials (MDPs) in the round cells averaged -82 ± 6 mV, with a take-off potential of -56 ± 3 mV ($n = 9$), and an average maximum upstroke velocity ($\dot{V}_{max}$) value of $6{\cdot}3 \pm 0{\cdot}6$ V/s ($n = 4$). In quiescent cells, the mean resting potential was 69 ± 12 mV ($n = 43$).

5. Voltage clamp ramps revealed a steady-state I–V relation with a negative slope region. The mean input resistance value was 25 ± 9 MΩ ($n = 16$) for the elongated, and 883 ± 481 MΩ ($n = 8$) for the round cells.

6. Hyperpolarizing 5 s pulses (holding potential = −50 mV) occasionally revealed a slow, time-dependent inward current whose peak increased progressively as a function of clamp potential. The slowly activating current was sensitive to caesium (2 mM), indicating its similarity to the so-called 'pacemaker current' (i_F). In alternate voltage- and current-clamp experiments, blocking of i_F did not stop pacemaker activity, but there was up to a fourfold increase in pacemaker cycle length.

7. In some cells, 5 s hyperpolarizing steps from a holding potential of −40 or −50 mV produced large, inwardly directed and voltage-dependent current surges that decayed rapidly with time, similar to the inactivation described for the inward rectifier current, i_{K1}. The current was very prominent at voltages more negative than −100 mV, and its decay process was best fitted by two time constants, one fast and

one slow. For example, at −150 mV the time constants were 61 and 634 ms. The inward current was blocked by barium (1 mM).

8. The current inactivation process was also shown to occur at a less negative potential, at −90 mV; however, one time constant, with a value of 270 ms, was sufficient to fit the current decay process at this potential.

9. Depolarizing voltage-clamp steps (200 ms duration) from a holding potential of −50 or −60 mV revealed a cobalt (2 mM)-sensitive transient inward current that peaked around 0 mV and that had a reversal potential of +50 to +60 mV. Longer duration (3–5 s) depolarizing pulses produced, first, the cobalt-sensitive current and then an outward current with a time course and activation kinetics similar to those of the delayed rectifying outward current (i_K).

10. In one set of experiments, we could record in six out of eight cells a tetrodotoxin (30 μM)-sensitive transient inward current, which had a relatively small amplitude; the peak current value ranged from 3 to 12 nA. No such current was present in the remaining two cells.

11. It is concluded that there are spontaneously active cells in the rabbit tricuspid valve. The cells have membrane ionic currents similar to those described for other cardiac cells and a complex interplay of these currents may be involved in the generation of the pacemaker potential. The i_F significantly *modulates* pacemaker activity, but it appears that it does not by itself *generate* the activity in the cells. Furthermore, the electrophysiological properties of the cells are such that they can generate ectopic impulses, with a potential for generating supraventricular arrhythmias.

INTRODUCTION

Atrioventricular (AV) valves in several mammalian species, including man, contain cardiac fibres which have been described as originating from the atrium (Gross & Kugel, 1931; Ellison & Hibbs, 1973). The fibres encircle the outer ring of the valve, and some may form 'islands' of cells within the leaflets (Rozanski & Jalife, 1986). Occasionally, some cells can be seen terminating singly on the valve leaflets (J. M. B. Anumonwo, M. Delmar & J. Jalife, unpublished). Electrophysiological studies on isolated AV valve tissue have shown that the fibres therein are capable of rhythmic activity (Wit, Fenoglio, Wagner & Bassett, 1973; Makarychev, Kosharskaya & Ul'Yaniskii, 1976; Wit, Fenoglio, Hordoff & Reemtsma, 1979; Rozanski & Jalife, 1986). In addition it has been shown that when the simian mitral valve is externally driven in the presence of catecholamines, delayed after-depolarizations are developed resulting in sustained triggered activity (Wit & Cranefield, 1976).

In one of the studies (Rozanski & Jalife, 1986), the properties of automatic fibres in the rabbit tricuspid and mitral valves were determined and the effects of intrinsic neurotransmitters on the valve cell impulse initiation and conduction to the atrium investigated. It was shown that in normal Tyrode solution slow impulse conduction and exit block prevented the activation of atrial myocardium by the valve automatic focus. However, in the presence of noradrenaline (liberated by intrinsic autonomic nerves) valve automaticity and conduction properties were modified with a consequent manifestation of the valve pacemaker activity in the atrial tissue. It was

therefore suggested that the rhythmically active cells on the AV valves could act as a site for ectopic impulses which could lead to supraventricular arrhythmias.

Rozanski & Jalife (1986) compared the AV valve electrophysiology with that of the sinoatrial node (SAN) and showed some qualitative similarities between the two. For example, similar to the SAN, rhythmic activity in the valve tissue is tetrodotoxin (TTX)-insensitive, but can be blocked by calcium channel antagonists such as cobalt or verapamil. Also, Rozanski (1987) suggested that current systems similar to those in the SAN might be responsible for automatic activity in the rabbit tricuspid valve. Recent voltage-clamp experiments (Anumonwo, Delmar, Angeles-Alvarez & Jalife, 1988) in small tissue strips from the rabbit tricuspid valve showed that the so-called 'pacemaker' current, i_F, is present in this tissue. However, rhythmic activity continued in the presence of a low concentration (2 mM) of caesium which blocks the channel (DiFrancesco, 1982). They therefore concluded that the i_F was not the major factor initiating the electrical activity in the valve tissue.

The whole-tissue studies of Rozanski & Jalife (1986) did not investigate transmembrane ionic currents of the valve. Moreover, the voltage-clamp studies by Anumonwo *et al.* (1988) were limited by the inherent problems of voltage clamping multicellular preparations (Johnson & Lieberman, 1971; Beeler & McGuigan, 1978). Furthermore, other transmembrane ionic currents were not investigated. The implications of the presence of spontaneous and triggered activity in the valve make it imperative that electrophysiology of the cardiac fibres contained therein be more quantitatively investigated. In this study we present for the first time electrophysiological data from single myocytes freshly dissociated from the rabbit tricuspid valve. Our results show that, indeed, there are cells with spontaneous electrical activity in the tricuspid valve, and that they have properties comparable to those present in nodal cells. Some of the results presented here have appeared previously in an abstract form (Anumonwo *et al.* 1988).

METHODS

Isolation of single valve cells

Adult rabbits weighing 1·5–2·5 kg were injected with heparin (500 U, I.V.) and after 5 min were anaesthetized with pentobarbitone sodium (35 mg/kg, I.V.). A mid-line thoracotomy was performed and the hearts were quickly removed and cannulated through the aorta, for Langendorff retrograde perfusion. Residual blood in the heart chambers was washed out by perfusing for 5 min with normal oxygenated (100%) HEPES Tyrode solution maintained at 37 °C. Subsequently, a low-Ca^{2+} Tyrode solution was perfused for 10 min. The low-Ca^{2+} solution was finally replaced by a collagenase-containing medium, which was perfused for 30–40 min, depending on the enzyme batch. At the end of the enzyme perfusion, a recovery solution (Kraftbrühe (KB) medium; Isenberg & Klöckner, 1982) was perfused for 15 min. Immediately after this, the atrium was removed to reveal the tricuspid valve inside the right ventricle. Under a dissecting microscope, an incision was made on the right ventricle and as the flap of ventricular muscle was lifted, the papillary muscles were cut, thereby freeing the valves. Non-septal valve leaflets were removed, gently rinsed in a beaker containing warm KB medium (to shake off other contaminating cells), and finally transferred to a small beaker, also containing warm KB medium (1 ml). Using a fire-polished Pasteur pipette, cells were freed from the leaflets by gentle agitation and a small volume (200 μl) was then transferred to the recording chamber where cells were kept in KB medium for another 20 min. At a later stage of the study, a perfusion system (open perfusion micro-incubator; OPMI-2, Medical Systems Corp., Greenvale, NY, USA) was acquired. Recording started after an additional 30 min equilibration period in normal Tyrode solution. All experiments were carried out at room temperature (20–23 °C), except where otherwise indicated.

Solutions

Chemicals were obtained from Sigma Chemical Co., St Louis, MO, USA.

Normal Tyrode solution. The normal Tyrode solution contained (mM): 130 NaCl; 4 KCl; 1·2 NaH_2PO_4; 1·0 $MgCl_2$; 1·8 $CaCl_2$; 5·6 glucose; 5 HEPES; pH was adjusted to 7·4 with NaOH.

Low-Ca^{2+} Tyrode solution. The composition (mM) of the low-Ca^{2+} solution was: 148 NaCl; 5·4 KCl; 5·8 $NaHCO_3$; 0·4 NaH_2PO_4; 1·0 $MgCl_2$; 5·5 glucose; 5·0 HEPES; 1 mg/ml bovine serum albumin.

Enzyme solution. This contained the same solution as the low-Ca^{2+} solution, plus 0·8 mg/ml collagenase (Worthington Biochem. Corp. Freehold, NJ, USA).

Recovery solution. The recovery solution contained (mM): 85 KCl; 5 $MgSO_4$; 30 KH_2PO_4; 20 glucose; 5 β-hydroxy-butryic acid; 5 creatine; 5 ATP; 20 taurine; 0·25 EGTA; 5 pyruvic acid; 25 mg/ml polyvinylpyrollidone (PVP-40).

Patch pipette solution. The patch pipette solution contained (mM): 150 KCl; 1·0 $MgCl_2$; 2 β-hydroxy-butyrate; 5 ATP; 5 phosphocreatinine; 5 HEPES; 1 EGTA; pH 7·2.

Stock solutions (100 mM) of Cs^+, Ba^{2+} and Co^{2+} were made and appropriate amounts were added to the superfusate as needed just before an experiment. Tetrodotoxin (TTX; Sigma Chemical Co.) stock solutions were made by dissolving the contents of a vial in 1 ml of distilled water to give a concentration of 1 mg/ml, from which aliquots were taken to give the desired final concentration of 30 μM in the Tyrode solution.

Data acquisition and analysis

The recording chamber containing isolated cells was placed on the stage of an inverted microscope (Olympus IMT-2), and membrane potential and current recordings were made using patch pipettes in the whole-cell configuration (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Patch pipettes were fabricated with borosilicate glass using a two-stage programmable puller (Flaming–Brown, model P.80/PC, Sutter Instruments, San Rafael, CA, USA) and then filled with filtered patch pipette solution. Using a Ag–AgCl bridge, the pipettes were connected to the input stage of an amplifier (Axoclamp-2A, Axon Instruments, Burlingame, CA, USA). Pipette resistance (R_e) values ranged between 2·5 and 5 MΩ. Pipette tip potential was determined as +8 mV and was corrected for in all the experiments.

In the initial stages of the study, voltage-clamp protocols were generated by using appropriate pulses applied from a Fredrik Haer stimulator. Voltage and current traces were photographed using a Grass kymographic camera (Grass Instrument Corp., Quincy, MA, USA: model CR 4), and subsequently projected onto graph paper for analysis. Later, data acquisition and analysis were carried out using the pClamp system (Axon Instruments, Burlingame, CA, USA) connected to an IBM-AT computer. Data were plotted using the Hewlett–Packard plotter (7475A).

The time course of current inactivation was determined by the use of a generalized non-linear curve-fitting computer program, based on the least-squares criterion for determining convergence. The software was written in PASCAL and it runs on an IBM-PC computer (Armonk, NY, USA) using double precision. It was developed by Mr Bill Goolsby at Emory University, Atlanta, GA, USA, and was kindly given to us by Dr Richard Veenstra of the Department of Pharmacology, SUNY Health Science Center at Syracuse, NY, USA.

Morphological studies

Cell measurements were made on the stage of the inverted microscope using Hoffman optics. Pictures were taken using a Nikon camera attached to the camera port of the microscope. The method of tissue fixation involved a 24–48 h treatment with 2·5% glutaraldehyde, and washing three times in 0·1 M-sodium cacodylate buffer (pH = 7), followed by an overnight infiltration in 10% (v/v) dimethylsulphoxide (DMSO). The tissue was subsequently immersed in cool 15% (w/v) gelatin solution containing 0·8% (v/v) DMSO and then left to harden. The embedded tissue was quick-frozen for 15–30 min after which 6–8 μm thick sections were made using a microtome. Sections were stained with Toluidine Blue for 5 min, rinsed and then covered with a cover-slip. Tissue photographs were taken from a light microscope (Nikon) with Nomarski optics with a Bausch and Lomb L-type camera using Kodak Ektapan film.

RESULTS

Morphology of tricuspid valve cells

In the recovery (KB) solution dissociated cells were elongated with very well-defined striations. These cells were mostly spindle-shaped, but a few (< 10%) were rod-like in shape. Upon exposure to normal Tyrode solution, while a majority of cells (about 70%) became rounded a few retained their elongated shape. In one experiment for example, immediately after cell dissociation and with cells still in KB, there were thirty-two elongated cells. When subsequently exposed to normal Tyrode solution, while ten of these cells retained their original elongated shape (E cells), the remaining cells became rounded. A number of the rounded (R) cells had well-defined cell boundaries as well as smooth surfaces (shown in Fig. 1). Calcium-intolerant cells could easily be identified by a lack of these features (see Fig. 1). In a majority of cells with well-defined cell boundaries, rhythmic contractions could be visualized, and usually continued for up to an hour. We also visualized contractions in E cells (spindle and rod-like); however, this occurred much less frequently.

The light micrographs presented in Fig. 1 show morphological features of isolated tricuspid valve cells. Panel *A* shows a section of a fixed valve tissue, with several bundles of intertwining cells which encircle the upper one-third of the valve. Notice several red blood cells in a blood vessel running within the bundles of myocytes. Panel *B* shows a cluster of such intertwining cells, which was obtained by the enzymatic dissociation procedure. A single cell from one such cluster is shown in panel *C*. Morphometric analysis was carried out on cells in Tyrode solution. Cell dimensions, especially the length, were very variable ranging from very short (30–40 μm) to very long (> 150 μm). In one experiment, the determination of cell dimensions in the E cells showed an average length of 85 ± 27 μm and a diameter of 8 ± 3 μm ($n = 45$). In R cells, the average cell diameter was 13 ± 5 μm ($n = 9$). Electrophysiological studies were carried out on single E or R cells (panel *D*). The micrograph in panel *D* was taken following exposure to normal Tyrode solution. Several calcium-intolerant cells can be seen (e.g. the top arrow). The lower arrow shows a round cell with a well-defined membrane.

Electrophysiology of isolated tricuspid valve cells

Input resistance determination

The membrane potential determined in quiescent E cells had a mean value of $-68{\cdot}5 \pm 12$ mV ($n = 43$); mean input resistance (R_{in}) was $25{\cdot}0 \pm 3{\cdot}4$ MΩ ($n = 16$). The input resistance was calculated from the linear part (negative to the resting potential) of *I–V* plots generated by voltage-clamp ramps (−100 mV to +20 or +50 mV at 15 mV/s). Tetrodotoxin (30 μM) and cobalt (2 mM) were present in the Tyrode solution. The steady-state *I–V* relations were characterized by a negative slope region, which was flattened in the presence of 1 mM-Ba^{2+}, increasing R_{in} by almost thirtyfold to 640 MΩ (not shown). Input resistance values in R cells were very high, ranging from 266 MΩ to 1·8 GΩ, with a mean value of 883 ± 481 MΩ ($n = 8$). We also observed negative slope regions in the *I–V* relations of R cells; however, the slope was less than that found in the E cells.

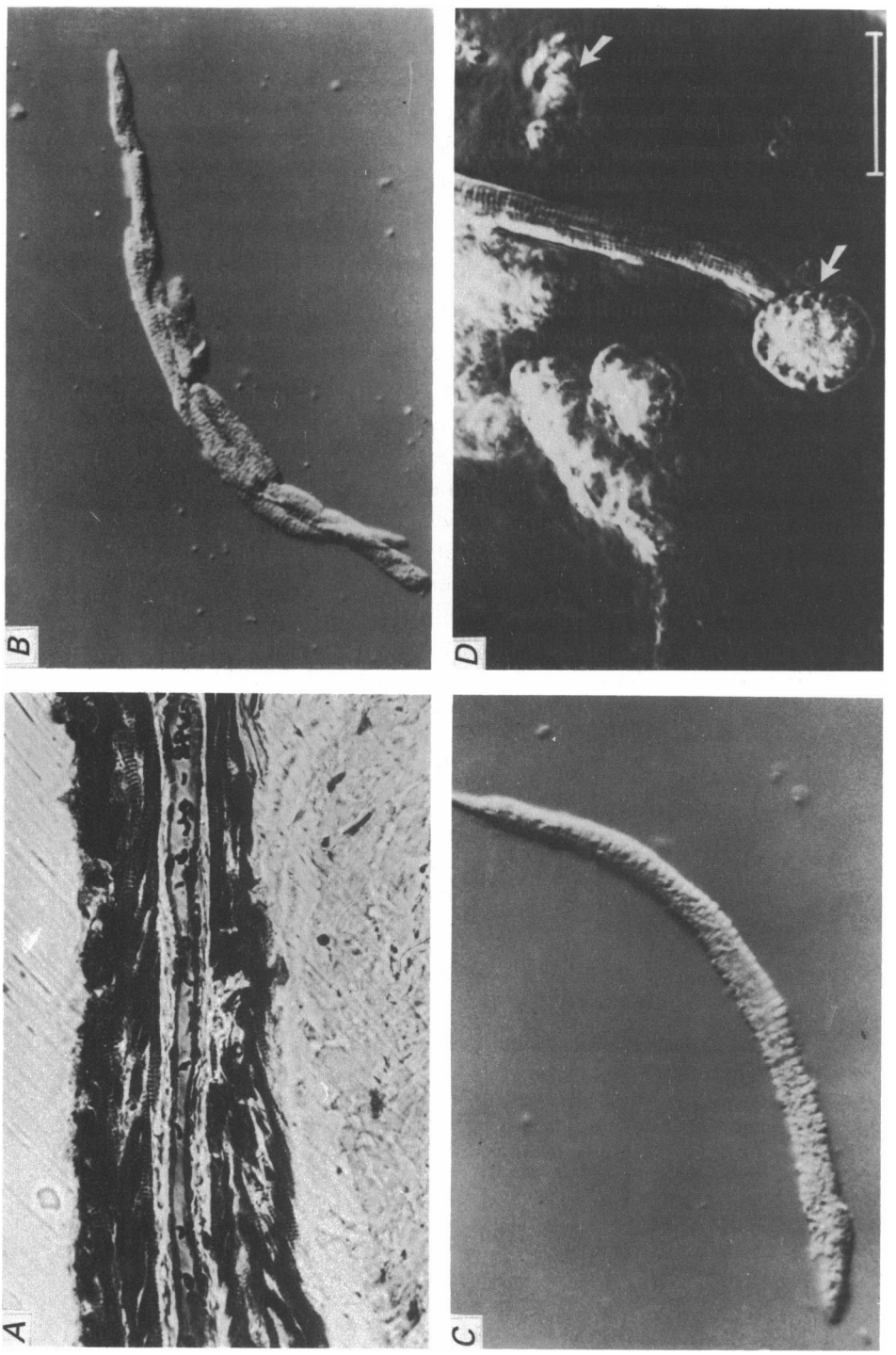

Fig. 1. Morphology of tricuspid valve cells. *A*, valve leaflet sectioned to show bundles of cardiac myocytes at the rim of the valve, next to the atrioventricular ring. Notice red blood cells in a vessel running within the bundles. *B*, a bundle of cells following enzymatic dissociation, with similar intertwining of cells as shown on panel *A*. *C*, a single spindle-shaped valve cell. Cells in *B* and *C* were stored in KB medium. *D*, an elongated cell with clear striations, and a round cell with a well-defined membrane (lower arrow) in normal Tyrode solution. Upper arrow shows a shrivelled cell following exposure to normal Tyrode solution. Horizontal bar: *A*, *C* and $D = 40\ \mu m$; $B = 80\ \mu m$.

Spontaneous activity in single valve cells

Although spontaneous electrical activity has been previously demonstrated in AV valve tissue preparations (Rozanski & Jalife, 1986), it is possible that such activity is not normally present but is induced artificially, for example by stretching the leaflets during the isolation procedure. Moreover, it is also possible that this activity is mediated by the neurotransmitters present in the intrinsic nerves of the AV valves. It has been shown, for instance, that rhythmic activity in the subsidiary atrial pacemaker (as well as the triggered activity of the AV valve) requires the presence of catecholamines (Wit & Cranefield, 1976; Rozanski, Lipsius & Randall, 1983). Following cell isolation, therefore, we determined whether the spontaneous activity was present in the cells in normal Tyrode solution.

Recording of spontaneous activity was carried out only in cells in which we initially could visualize rhythmic contractions. This ensured that any recorded spontaneous activity was not induced by the impaling process as a result of membrane depolarization (see Giles & Van Ginneken, 1985). The ability to record action potentials for prolonged periods, up to an hour or more, was also taken to indicate the spontaneous nature of the electrical activity.

The spontaneous electrical activity recorded from an isolated tricuspid valve cell shows a characteristic multiphasic diastolic depolarization (see Fig. 2*A–C*, top traces). The mean value of maximum diastolic potential (MDP) recorded from R cells was -82 ± 6 mV, with a take-off potential of -56 ± 3 mV ($n = 9$). The take-off potential was determined at the intersection of two lines: one line drawn through the secondary phase of depolarization (D_2; Rozanski, 1987), and the other through the steepest portion of the upstroke. Maximum diastolic potential values recorded in two spontaneously active E cells were -75 and -79 mV, with take-off potentials respectively of -52 and -55 mV. In four R cells, maximum upstroke velocity ($\dot{V}_{max}$) ranged between 5·7 and 6·8 V/s with a mean value of $6{\cdot}3 \pm 0{\cdot}6$ V/s. In the whole-tissue experiments of Rozanski & Jalife (1986), MDP and take-off potential values were, respectively, $-80{\cdot}6$ and $-59{\cdot}4$ mV. Also, $\dot{V}_{max}$ values of 11·2 and 11·6 V/s were reported, respectively, for the tricuspid and mitral valves. The value of the $\dot{V}_{max}$ was significantly lower (3·6 V/s) for the sinus node.

Transmembrane ionic currents in single valve cells

In a preliminary study (Anumonwo *et al.* 1988) we investigated the presence as well as the participation of the hyperpolarization-activated current i_F in pacemaker activity of the tricuspid valve. Although we demonstrated the presence of i_F in the tissue, and showed that it was not the major current responsible for pacemaking, we did not investigate any other current systems, such as the inward calcium current (i_{Ca}) or the delayed rectifier current (i_K), that are thought to play a role in generating diastolic depolarization in cardiac pacemaker cells. Therefore, following the dissociation of the tricuspid valve into single cells, we carried out a preliminary study to identify and characterize transmembrane ionic currents present in the cells.

Hyperpolarization-activated current, i_F

Figure 2 shows the results of alternate voltage- and current-clamp experiments in which the presence, as well as the involvement, of the hyperpolarization-activated current (i_F) in pacemaker activity of the valve cell was investigated. A rhythmically contracting R cell was impaled, and under current-clamp conditions its spontaneous

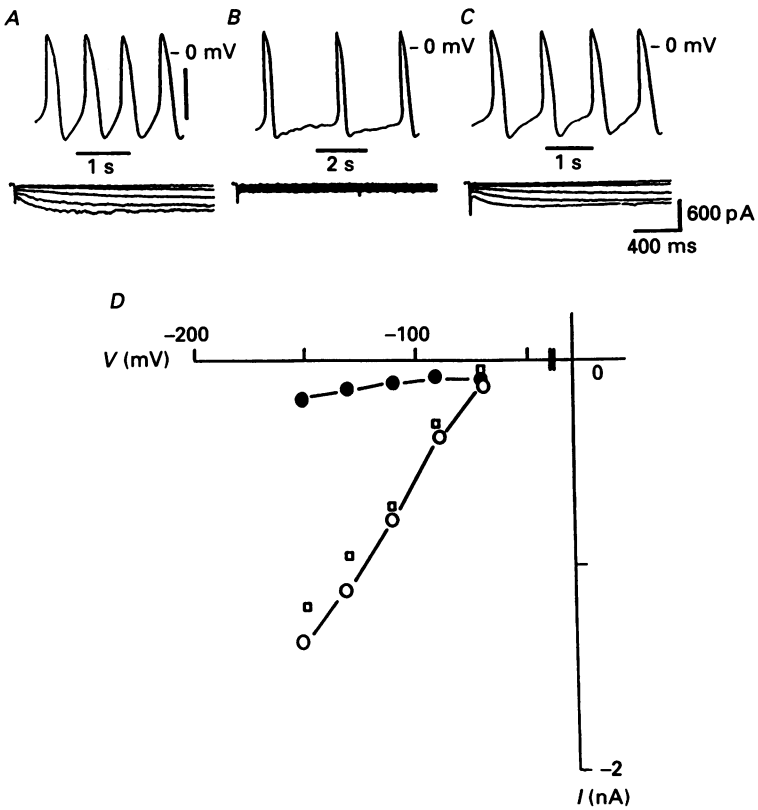

Fig. 2. Effect of 2 mM-caesium on the hyperpolarization-activated current, i_F, and on the spontaneous electrical activity of a single valve cell in an alternate current- and voltage-clamp experiment. Control spontaneous cycle (top trace in panel *A*) was recorded under current-clamp conditions from a rhythmically contracting R cell. The amplifier was switched to voltage clamp, and 5 s hyperpolarizing pulses (HP = −50 mV) were applied to elicit the i_F (bottom trace in panel *A*). Following the caesium-induced blockade of i_F (bottom trace in panel *B*), the frequency of spontaneous activity was reduced (top trace, panel *B*; notice a different horizontal calibration for this trace). Panel *C* represents spontaneous activity (top trace) and the activation of i_F (bottom trace) after the onset of caesium wash-out. Panel *D* shows *I–V* relation of the i_F in another cell. Open circles represent control conditions while closed circles show currents in the presence of caesium. Open squares are currents after wash-out.

activity was recorded (panel *A*, top trace). The amplifier was then switched to voltage clamp and 5 s hyperpolarizing pulses (holding potential, HP = −50 mV) were applied to elicit the i_F (bottom trace). The i_F was then blocked by 5 min superfusion with 2 mM-caesium (panel *B*, bottom trace). The amplifier was returned to current-

clamp conditions (top trace). It can be seen that although the cell was still spontaneously active, there was almost a fourfold prolongation of cycle length (notice that the time calibration for the action potentials in panel *B* is twice that in panel *A*). The spontaneous activity shown in panel *B* was obtained 12 min after switching to the current-clamp mode. Recordings shown in panel *C* were obtained 8 min after the onset of caesium wash-out, and show the frequency of spontaneous activity (top trace) as well as the i_F magnitude (bottom trace), returning towards control conditions. A qualitatively similar effect of caesium on spontaneous cycle length was observed in two other cells investigated. Nevertheless, it must be noted that caesium also blocks the i_K, and will therefore have a similar effect of slowing discharge rate (Brown, 1982). It is unlikely that such a blocking effect on i_K was responsible for our observation of a marked reduction in rate, because only a relatively small proportion of i_K would be blocked at the caesium concentration used. Moreover, the caesium effect on potassium channels is also associated with membrane depolarization (reduction in the MDP value), which is clearly not the case in our study (see Fig. 2).

In another experiment (panel *D*), the *I–V* relations for the i_F were determined under control conditions, as well as in the presence of 2 mM-caesium in the Tyrode solution. The i_F was elicited by 5 s hyperpolarizing pulses from a holding potential of −50 mV. This plot shows the effectiveness of the low caesium concentration in blocking the current. Also, the rectification of the *I–V* curve in the presence of caesium shows that the block is voltage dependent. Low concentrations of caesium have been shown to block the i_F in multicellular preparations (DiFrancesco, 1982; Callewaert, Carmeliet & Vereecke, 1984: Anumonwo *et al.* 1988), as well as in single cells (DiFrancesco, Ferroni, Mazzanti & Tromba, 1986).

The i_F was present in a majority of the R cells investigated; however, its magnitude was variable and in some cells barely detectable. Similar experiments performed on E cells showed the presence of the current in only about 10% of the cells.

Inward rectifier current, i_{K1}

The inward rectifier current can be studied by determining the steady-state *I–V* relation of a cell in control conditions and following channel blockade (see Shah, Cohen & Datyner, 1988). The difference current under these conditions represents the *I–V* relation of i_{K1}. In a set of experiments, we analysed the inward rectifier current in the valve cells, the results of which are shown in Fig. 3. The inward rectifier current of the valve cell was investigated by the use of caesium, which has been shown to block the channel (Biermens, Vereecke & Carmeliet, 1987). A voltage-clamp ramp was applied to an E cell in TTX (30 μM)- and cobalt (2 mM)-containing Tyrode solution (panel *A*), and after superfusion of 8 mM-caesium (panel *B*). The difference current is shown in panel *C*. We found that in both E and R cells, voltage-clamp ramps produced *I–V* relations that showed inward-going rectification, with distinct negative slope regions that were more prominent in E than in R cells.

As previously stated, the i_F magnitude in R cells was variable and in some instances undetectable. Also, we could record little or no i_F in a majority of the E cells studied, even when pulsing to very negative (e.g. −150 mV) potentials. On the

contrary, we recorded large (up to 10 nA) voltage-dependent, inwardly directed biphasic current surges, for test pulses beyond −90 mV. We further investigated this current, as shown in Fig. 4. The data points in panel *C* were obtained from the same experiment as that in panel *A*; traces in panel *B* were obtained from a different experiment. Application of 100 ms hyperpolarizing pulses from a holding potential of

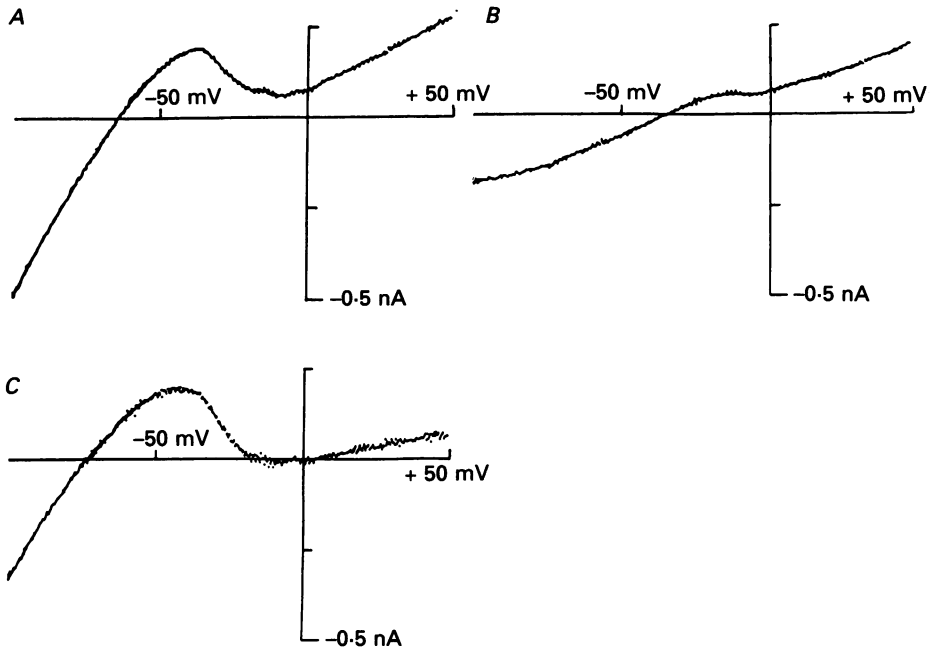

Fig. 3. Analysis of the inward rectifier current in the valve cells. Panel *A* shows the current due to a voltage-clamp ramp applied to an E cell in TTX (30 μM) and cobalt (2 mM)-containing Tyrode solution. Following the superfusion of 8 mM-caesium in the Tyrode solution, a second ramp was applied. Membrane current under this condition is shown in panel *B*. The difference current from the two ramps is shown in panel *C*. Ramps were applied from −100 to +50 mV, at a rate of 15 mV/s.

−50 mV elicited at each voltage large multiphasic current surges with peak currents that rapidly relaxed to a steady-state value (panel *A*; see also Tourneur, Mitra, Morad & Rougier, 1987). The current surge, especially its time-dependent component, was particularly sensitive to barium (Fig. 4*B*). In the experiment shown in Fig. 4*B*, an E cell was voltage clamped (HP = −50 mV) and a 5 s hyperpolarizing pulse was applied to −100 mV in control (*C*), and in the presence of barium (0·5 mM). In another set of experiments, peak and steady-state currents were plotted as a function of voltage, an example of which is shown in Fig. 4*C*, from which the time-dependent decay of the current is further demonstrated. The current decay process such as shown above has been previously attributed to potassium depletion (Baumgarten & Isenberg, 1977) although, recently, properties similar to these have also been reported for the i_{K1} in cardiac cells (Sakmann & Trube, 1984; Kurachi, 1985; Biermans *et al.* 1987; Harvey & Ten Eick, 1988), and have been attributed to a channel inactivation process. One interesting observation was that in some cells

from which we could record the large inward current surges, a sizeable amount of i_F could be recorded if barium was present in the superfusion medium.

The inactivation process of the i_{K1} has been shown to occur when hyperpolarizing to very negative potentials. For example, in the guinea-pig ventricular myocyte, it was reported that the decay of current was observed when hyperpolarizing pulses

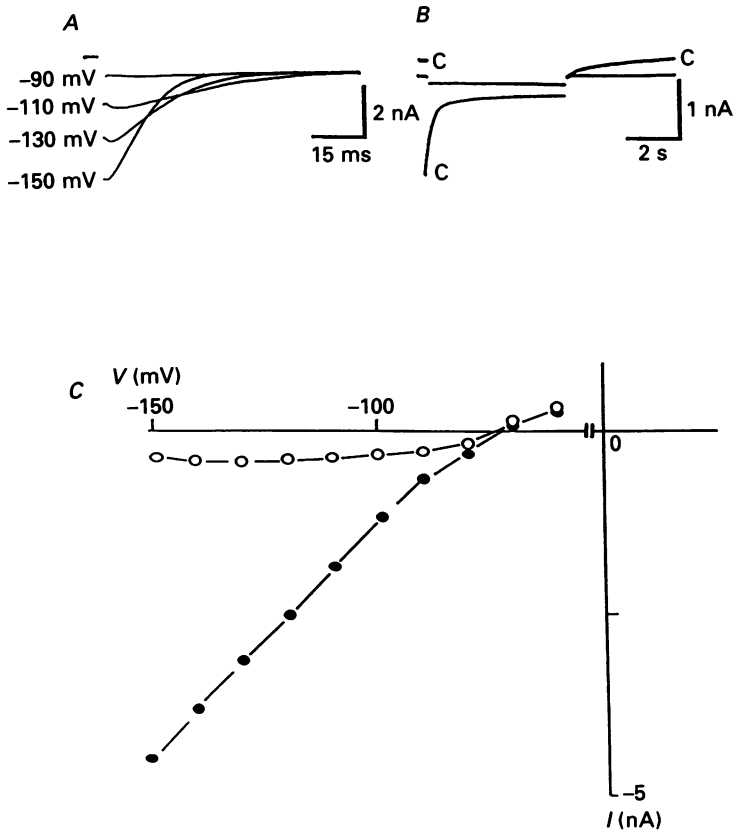

Fig. 4. Voltage-dependent inactivation of i_{K1} in a single valve cell. Inwardly directed multiphasic current surges (for test pulses beyond −90 mV) were elicited by hyperpolarizing pulses of 100 ms duration from a holding potential of −50 mV (panel *A*). In panel *B*, another cell was voltage clamped and a 5 s hyperpolarizing pulse (HP = −50 mV) was applied to −100 mV in both control (trace marked C) and in the presence of barium, 0·5 mM. In panel *C*, peak (●) and steady-state (○) currents were plotted as a function of voltage (data points obtained from the same experiment as shown on panel *A*). TTX (30 μM) and cobalt (2 mM) present in Tyrode solution.

more negative than −100 mV were applied (Biermans *et al.* 1987). Similar results were also obtained in cat ventricular myocytes (Harvey & Ten Eick, 1988). However, in some of our initial experiments, a sizeable current inactivation was noticeable at less negative potentials in the valve cells (Fig. 4*C*). We therefore considered the possibility that there could be channel inactivation at these less negative potentials, e.g. at −90 mV, but because the potentials are close to the potassium equilibrium potential, the lack of a driving force will make undiscernible any such inactivation.

We investigated this possibility by applying an appropriate voltage-clamp protocol as illustrated in Fig. 5*A*. The protocol allows the activation of the i_{K1} channel at −90 mV (HP = −50 mV), by the application of conditioning pulses (CP) of variable duration. At the end of each CP, a test pulse (TP) is applied to −150 mV, a potential at which a sizeable i_{K1} is elicited (TC; panel *B*). If channel inactivation

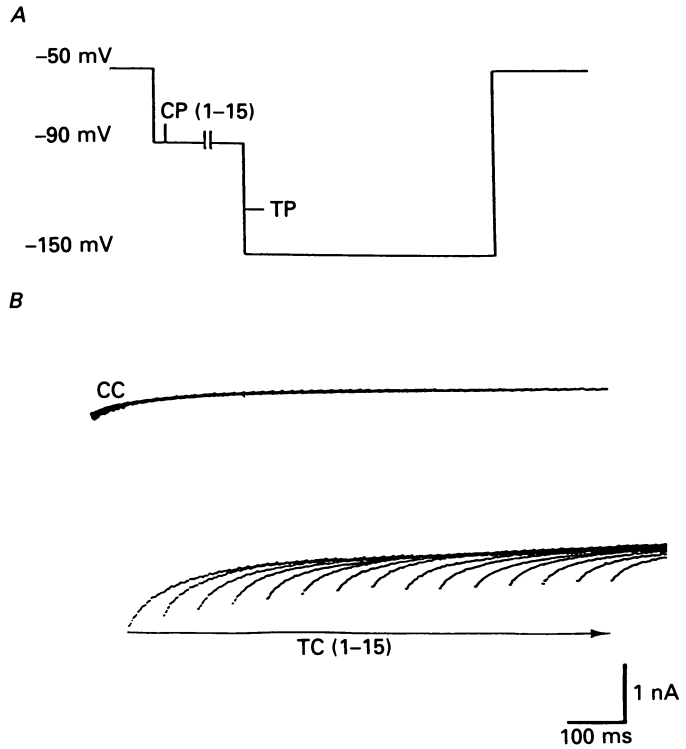

Fig. 5. Inactivation of i_{K1} at −90 mV. Panel *A* is the voltage-clamp protocol, and current traces are shown in panel *B*. In panel *A*, the inactivation of the i_{K1} channel at −90 mV from a holding potential of −50 mV was determined by the application of conditioning pulses (CP) of variable duration. At the end of each CP, a test pulse (TP) was applied to −150 mV, a potential at which a sizeable i_{K1} is elicited (TC; panel *B*). Progressively increasing CP duration (i.e. CP 1–15) from 60 to 900 ms, and therefore the amount of inactivation at −90 mV progressively decreased the peak value of TC. The time course of channel inactivation at −90 mV is represented by the time course of the decrease in TC1...15. TTX (30 μM) and cobalt (2 mM) present in Tyrode solution.

takes place at −90 mV, the peak current value when pulsing to −150 mV (TC) should depend on the extent of this inactivation. Thus, by progressively increasing CP duration (i.e. CP 1...15) from 60 to 900 ms, and therefore the amount of inactivation at −90 mV, the peak value of TC should progressively decrease. Such a decrease was indeed obtained in three preparations. In the experiment shown in panel *B*, a progressive reduction in TC 1...15 can be clearly seen. The time course of channel inactivation at −90 mV is represented by the time course of the decrease in TC 1...15, with a time constant of 270 ms. In this same experiment, at more negative potentials (−100 to −150 mV) the inactivation process could be fitted by two time

constants, one fast and one slow. For example, at −150 mV the time constants were 61 and 634 ms. Thus, the experiment shows that in the tricuspid valve cell, an inactivation of the i_{K1} occurs at less negative potentials than has been previously reported for other cardiac cells (cf. Biermans *et al.* 1987).

Delayed rectifier current, i_K

The delayed rectifier, which is activated during the action potential, has kinetic properties that suggest its involvement in repolarization as well as in the generation of the pacemaker potential (Nakayama, Kurachi, Noma & Irisawa, 1984; Shibata & Giles, 1985). We have also investigated the activation properties of the current in the isolated valve cells.

Application of depolarizing pulses of relatively long duration (3–5 s) from a holding potential of −40 or −50 mV produced in the four cells investigated (E and R) slow outward currents. In these experiments, the Tyrode solution contained cobalt (2 mM) and TTX (30 μM) to block, respectively, the inward calcium and sodium currents. Figure 6 shows data from an experiment in which i_K was elicited by the application of 4 s depolarizing pulses from a holding potential of −50 mV. In panel *A*, tail currents associated with the depolarizing clamp steps were analysed. Tail current magnitudes as fractions of the maximal tail amplitude ($I_{tail}/I_{tail,max}$) were expressed as a function of voltage. In panel *B*, the continuous curve in the plot is the Boltzmann distribution function, $1/(1+\exp[(V_h-V_m)/k])$, which best fitted the data points with a half-activation voltage (V_h) of 3·2 mV and a slope factor (k) of 7·8 mV.

Calcium current, i_{Ca}

Several studies have shown that a transient inward, TTX-insensitive current carried by calcium ions is present in cardiac pacemaker cells, and that the current kinetics and voltage range of activation suggest its involvement in the generation of pacemaker activity (Nakayama *et al.* 1984; Shibata & Giles, 1985). Recently, evidence has been presented (Hagiwara, Irisawa & Kameyama, 1988) for the contribution of two types of calcium currents ($i_{Ca,T}$ and $i_{Ca,L}$) to the pacemaker potential of the rabbit sinus node cell. The transient (T-type) calcium current has an activation threshold of around −55 mV and is sensitive to micromolar concentrations of nickel, while the long-lasting calcium current (L-type) is activated at −35 mV, blocked by cobalt and constitutes most of the current usually referred to as the slow inward current (see Hagiwara *et al.* 1988).

We investigated the L-type calcium current in the isolated valve cells. In this set of experiments, Tyrode solutions routinely contained TTX (30 μM) to block the transient inward sodium current. We studied the cobalt-sensitive calcium current by applying, from a holding potential of −50 mV, depolarizing clamp pulses (200 ms duration) in steps of 10 mV. Figure 7 shows selected traces of membrane current activated by the clamp steps in control conditions (panel *A*) and following superfusion with Tyrode solution containing cobalt (2 mM; panel *B*). Mean peak current (control conditions) in nine cells was 1·2 ± 0·5 nA. Panel *C* is an *I–V* plot from the same experiment as shown in panels *A* and *B*. The properties of the current shown – voltage of activation, reversal potential and sensitivity to cobalt – are

similar to those previously reported for the $i_{Ca,L}$ current in other cardiac pacemaker cells (Nakayama *et al.* 1984; Shibata & Giles, 1985).

It has been shown (Hagiwara *et al.* 1988) that superfusion of nickel (40 μM) slowed the spontaneous activity of sinus nodal cells as a consequence of the T-type calcium channel blockade, an effect that was further enhanced when the membrane was

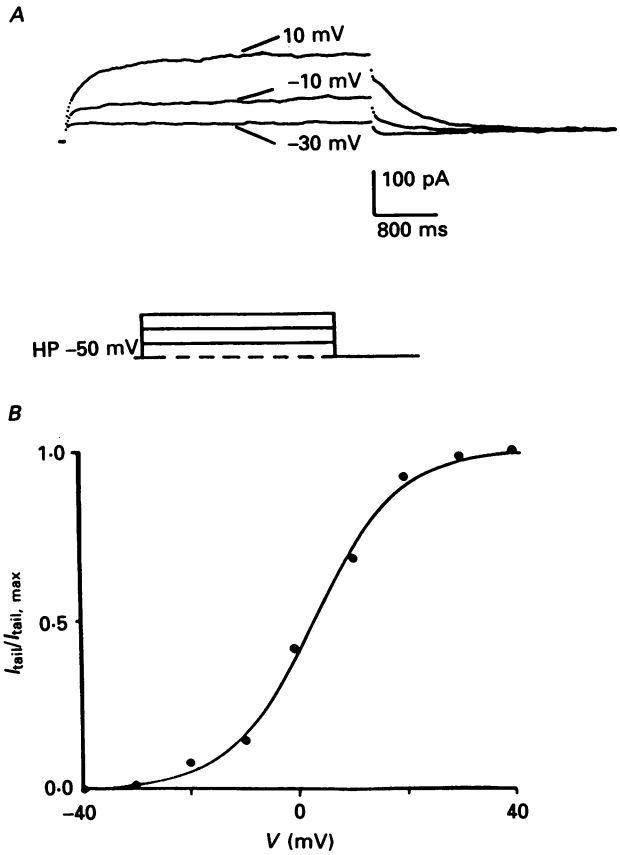

Fig. 6. Delayed rectifier current, i_K, in the valve cell. Currents were activated by 4 s depolarizing pulses from a holding potential of −50 mV (panel *A*, bottom). Panel *A* (top) shows activated currents, as well as their tails, by depolarizing to the annotated voltages. In panel *B*, tail current magnitudes were expressed as fractions of the maximal tail amplitude ($I_{tail}/I_{tail,max}$), and plotted as a function of voltage. The continuous curve in the plot is the Boltzmann distribution function, $1/(1+\exp[(V_h-V_m)/k])$, which best fitted the data points with a half-activation voltage (V_h) of 3·2 mV and a slope factor (k) of 7·8 mV. TTX (30 μM) and cobalt (2 mM) present in Tyrode solution.

hyperpolarized. Given the relatively negative maximum diastolic potential in valve cells, it is possible that the T-type channel, if present, should be involved in the pacemaker activity of the cells. Thus, similar to the sinus node, channel blockade should also result in a slowing of the rate of spontaneous activity. We investigated the effect of nickel in three spontaneously active cells, and in all three cases nickel (40 μM) consistently prolonged the spontaneous cycle length. One such experiment is illustrated in Figure 7*D*. In this panel, the top trace is the control, and as shown by

the lower trace superfusion with nickel indeed slowed the rate of the discharge; spontaneous cycle length was increased by about 56% of control. The same concentration of nickel prolonged the spontaneous cycle length by 38% in one cell and by 43% in another, of the other cells investigated. This effect of nickel was reversible (not shown). Notice that in our experiments, this significant slowing of rate

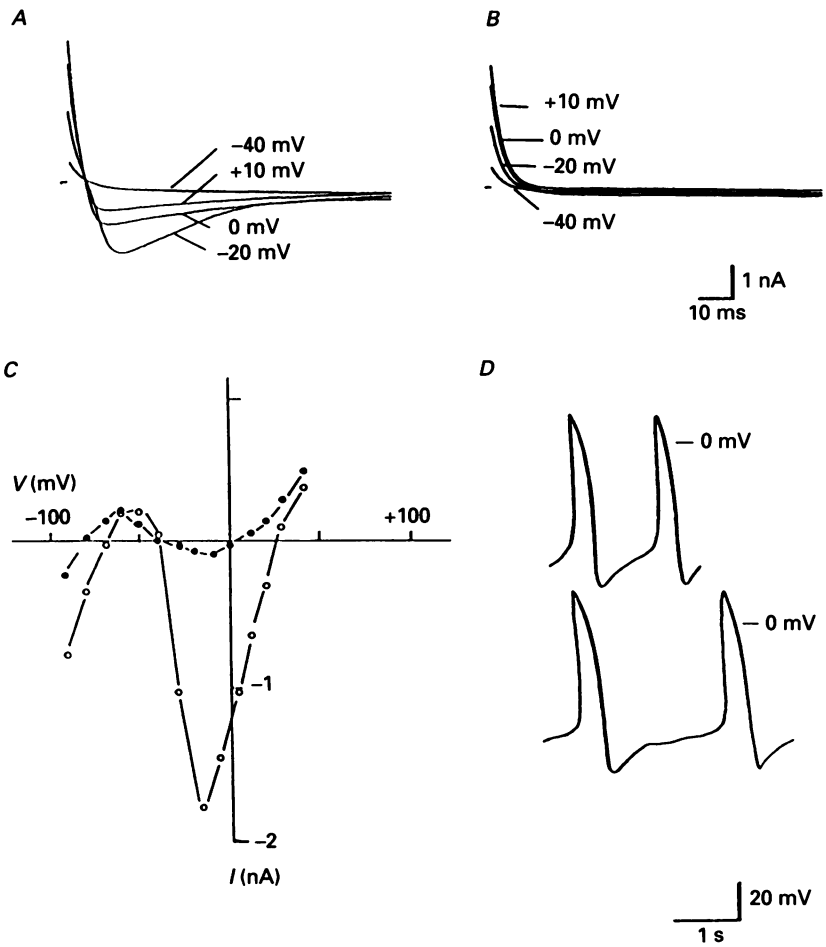

Fig. 7. Activation of the calcium current, $i_{Ca,L}$, in the valve cell. Selected traces of membrane current activated by the clamp steps to indicated voltages in control (panel *A*) and following superfusion with Tyrode solution containing cobalt (2 mM; panel *B*). Panel *C* is an *I–V* plot from the same experiment as shown in panels *A* and *B*. Open circles and closed circles represent current in control and in the presence of 2 mM-cobalt respectively. In another cell a nickel-induced slowing of the spontaneous discharge rate is shown in panel *D*. Top trace, control; bottom trace, during superfusion of nickel (40 μM). TTX (30 μM) present in Tyrode solution.

in the presence of nickel was achieved without hyperpolarizing the cell. In contrast, Hagiwara *et al.* (1988) observed a similar slowing (60·7% prolongation of spontaneous cycle length) in discharge rate of the sinus node cell only after the cell was hyperpolarized (cf. their Fig. 10).

TTX-sensitive inward current

Rozanski & Jalife (1986) examined the effect of sodium channel blockade on impulse generation in the valve tissue and reported that during TTX superfusion there was no significant effect on action potential amplitude, and that the spontaneous cycle length increased by only 15·3%. Given that the valve cell is

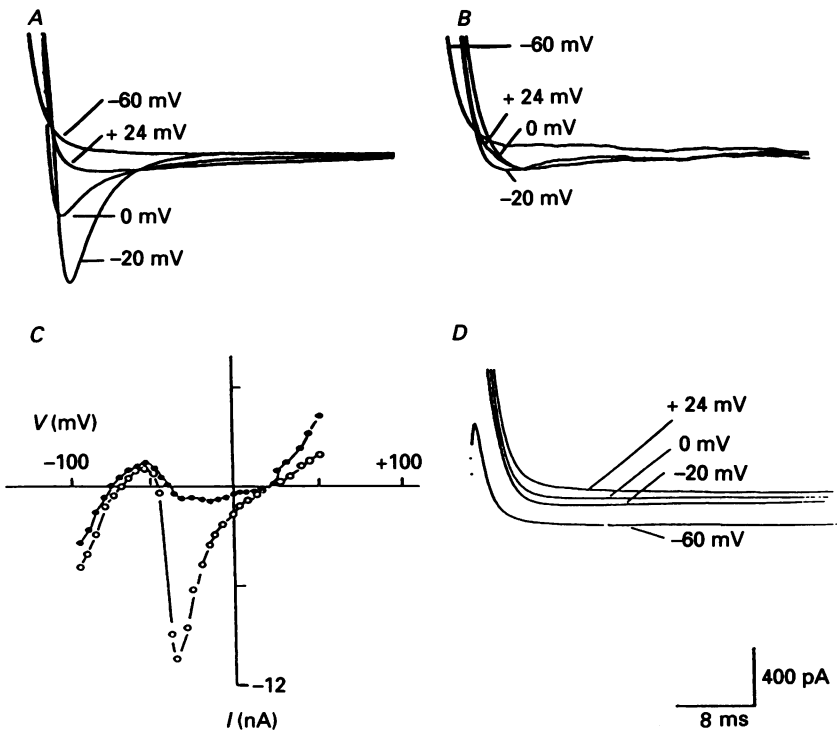

Fig. 8. TTX-sensitive transient inward current in the valve cells. Currents were elicited by depolarizing pulses of 40 ms duration (HP = −100 mV) to the voltages indicated in control (panel *A*) and in the presence of 30 μM-TTX (panel *B*). The *I–V* plots in panel *C* are for currents in control conditions (○), and during superfusion with TTX (●). Data points were obtained from the experiment shown in panels *A* and *B*. Panel *D* shows traces of currents from a cell in which the TTX-sensitive inward current could not be elicited. Cobalt (2 mM) present in Tyrode solution.

relatively well polarized (MDP = −82 mV) and therefore sodium channels, if present, would not all be in the inactivated state, they attributed the lack of sensitivity of valve action potential to TTX to a process of sodium channel accommodation, such as could occur during the initial phase of diastolic depolarization. However, even in the presence of such an accommodation process, the $\dot{V}_{max}$ values reported for the tricuspid valve in the present experiments, as well as previous studies (see Rozanski & Jalife, 1986), suggest that action potential upstrokes cannot be completely explained on the basis of the slow channel.

The properties of the transient inward sodium current (i_{Na}) are such that any

quantitative analysis of the current requires that stringent experimental protocols be used (see Johnson & Lieberman, 1971). In the experiments described below, we investigated the presence of a TTX-sensitive current in the valve cells in low-sodium (40 mM) and cobalt (2 mM)-containing Tyrode solution. However, since no further procedures ensuring adequate voltage control in the experiments were taken, quantitative data interpretation may be limited. In eight cells, depolarizing pulses of 40 ms duration (HP = −100 mV) were applied to various voltages, as shown in Fig. 8. In six of the cells (four R and two E cells), the pulses elicited transient inward currents of small and variable peak amplitude (< 12 nA), which were abolished in the presence of TTX (30 μM). In panels *A* and *B* of the figure are shown current traces with annotations indicating voltages to which the membrane potential was stepped, from a holding potential of −100 mV. Control currents are shown in panel *A*, while currents in the presence of TTX are shown in panel *B*. The *I–V* plots in panel *C* are for currents in control conditions (○), and during superfusion with TTX (●). Data points were obtained from the experiment shown in panels *A* and *B*.

However, in two cells (R and E), no such TTX-sensitive current could be elicited. Panel *D* of Fig. 8 shows traces of currents elicited in one of the cells. The same voltage-clamp protocol as applied to the cell in panel *A* was used in this cell. This was an R cell with a resting membrane potential of −70 mV.

DISCUSSION

The results of our study show that the myocytes in the tricuspid valve can easily be isolated, and that they have properties similar to those described in previous whole-tissue electrophysiological studies (Wit *et al.* 1973; Makarychev *et al.* 1976; Rozanski & Jalife, 1986). A few limitations of this study are, however, noteworthy: experiments were essentially carried out at room temperature (20–23 °C) which is expected to affect properties of the ionic currents studied. In addition, since it was not the purpose of this study, neither a detailed analysis of the kinetics of individual currents nor an estimation of current density was carried out. These limitations notwithstanding, our single-cell study confirms and expands previous studies from this laboratory in isolated tissue preparations and provides a more quantitative basis for determining and interpreting the electrophysiology of the tricuspid valve. Furthermore, this voltage-clamp study of the valve cell provides some insight into the ionic mechanism(s) of the spontaneous electrical activity in the cells of the primary pacemaker, or in similar pacemaking cells of the atrioventricular node.

Morphology and viability of atrioventricular valve cells

Previously regarded as purely passive structures that moved only following haemodynamic pressure changes in the chambers of the heart, the AV valves have subsequently been shown to be engaged in active processes, e.g. contractile, that may be involved in the closing of the valve (Sonnenblick, Napolitano, Daggett & Cooper, 1967; Priola, Fellow, Moorehouse & Sanchez, 1970). Light microscopic and ultrastructural studies have since described the musculature, vascularization and innervation of atrioventricular valves (Fenoglio, Pham, Wit, Bassett & Wagner, 1972; Ellison & Hibbs, 1973; Bassett, Fenoglio, Wit, Myerburg & Gerband, 1976).

Furthermore, the studies showed muscle fibres from the atrium extending onto the valve leaflets, with plexuses of nerve fibres originating from the atrium and the ventricle.

In our study, we have successfully isolated the tricuspid valve muscle cells. Morphological analysis by light microscopy immediately after dissociation (i.e. with the cells in the incubating KB medium) shows that the cells are predominantly spindle-shaped, and that by a process of intertwining, form muscle bundles that encircle the upper rim of the valve. Occasionally, some of these fibres branch off the bundle and terminate singly on the leaflets (J. M. B. Anumonwo, M. Delmar & J. Jalife, unpublished). As previously noted, we usually found that a few of the dissociated cells were rod-shaped. It is possible that these cells are a contamination from the atrium, which could result from the enzyme perfusion method used. Alternatively, they could simply be valve cells closest to the atrium. These cells have characteristic input resistance values more comparable to those of non-pacemaking cardiac cells. Not surprisingly, we rarely observed spontaneous rhythmic contractions in such cells. It must be noted, however, that not all pacemaking cells become rounded following isolation (DiFrancesco *et al.* 1986; Denyer & Brown, 1987). One observation of Rozanski & Jalife (1986) is perhaps noteworthy here, and may explain the two populations of cells (E and R) reported in the present study. These investigators observed 'islands' of spontaneously contracting cells on their isolated valve leaflet preparations. Could these be the spontaneously active (R) cells described in this present study? However, caution must be taken in interpreting data on cell morphology following cell isolation, since the employed dissociation procedure may affect the observed cell morphology (see Denyer & Brown, 1987).

The exact origin of the myocardial cells in the valve, although not determined in the present study, will eventually have to be determined since even though atrial fibres have been shown to extend onto the valve tissue, there are also fibres running from the AV node to the septal leaflets of the tricuspid valve – the dead-end fibres (Anderson, Janse, Van Cappelle, Billette, Becker & Durrer, 1974). It will be interesting to compare single-cell electrophysiological data from the two types of tissue. The results of our study, however, show that the tricuspid valve myocytes, whatever their origin, generate spontaneous electrical activity.

Although the majority of the isolated valve cells did undergo a change of shape on exposure to calcium, our electrophysiological data indicate that the cells are viable. For example resting membrane potential, maximum diastolic potential and input resistance values are in the range considered normal and are comparable to those reported for other cardiac pacemaking cells. Interestingly, we observed that the majority of these spontaneously active cells have morphological similarities with pacemaking cells isolated from mammalian nodal tissues (Nakayama *et al.* 1984). Thus following isolation, cells from mammalian nodal tissues kept in recovery medium (Ca^{2+}-free solution) are essentially elongated with clear striations, but when exposed to calcium ions most cells become rounded, lose their striations and develop spontaneous rhythmic contractions.

Ionic currents under voltage-clamp conditions

Our voltage-clamp experiments show that the valve cells have transmembrane ionic currents qualitatively similar to those described for other cardiac cells. Importantly, our results show that valve cells have the three main currents implicated in diastolic depolarization of pacemaker cells: i_K, i_{Ca} and i_F. Furthermore, the results of the experiment shown in Fig. 7*D* suggest that the T-type calcium channel is present in the valve cells. To conclusively show its presence will require additional experiments with an appropriate voltage-clamp protocol to elicit the T-type calcium current, that should show a sensitivity to nickel or tetramethrin (Hagiwara *et al.* 1988).

Two important considerations are noteworthy: the presence of the inward rectifier current as well as the transient, TTX-sensitive current in the valve cells. While the inward rectifier current has been described as being very small or completely absent in cells isolated from the sinus node (Noma, Nakayama, Kurachi & Irisawa, 1984), the current is present in valve cells, which may indeed explain their more negative diastolic potential values.

In our experiments, the presence of a transient TTX-sensitive inward current, with relatively small peak amplitude, is consistent with the lack of sensitivity of the valve action potential to TTX as previously shown by Rozanski & Jalife (1986). These workers reported that TTX (10 μg/ml) had no effect on valve action potential, and caused only a slight increase (15·3%) in the pacemaker cycle length. In fact, in two of the eight cells investigated by us, no such TTX-sensitive current could be elicited. The relatively small peak current amplitude is also consistent with the relatively low $\dot{V}_{max}$ value (6·3 $\pm$ 0·6 V/s) obtained in our study. However, as previously stated, quantitative interpretation of the data on the current may be limited because of the stringent requirements necessary for an adequate investigation. Thus, as shown in Fig. 8, current kinetics will be poorly resolved in the presence of a slow relaxation of the capacity currents (Fig. 8*B*), and peak current values may have been underestimated (Fig. 8*C*). Also, the probable effect of cobalt on the sodium current (see Frelin, Cognard, Vigne & Lazdunski, 1986) was not considered.

Ionic currents underlying diastolic depolarization in the tricuspid valve cell

The main voltage- and time-dependent current systems implicated in pacemaker activity of mammalian cardiac cells are i_K, i_{Ca} and the controversial 'pacemaker current' i_F (for a review see Brown, 1982). More recently (see Giles, Van Ginneken & Shibata, 1986; Irisawa & Hagiwara, 1988), it has also been suggested that the high input resistance of pacemaker cells could allow other small current sources such as a pump (Na^+–K^+), or an exchanger mechanism (Na^+–Ca^+) to produce significant changes in voltage during diastolic depolarization. As previously mentioned, the spontaneous electrical activity in the tricuspid valve, as well as other subsidiary pacemakers, indicates these tissues are potential sites for generating arrhythmias. Therefore, the mechanisms underlying such a pacemaker activity need to be thoroughly understood.

The diastolic depolarization of the valve cell has properties, e.g. its biphasic profile and the relatively negative value of the MDP (−82 mV), that suggest a complex

underlying mechanism. In one previous study, Rozanski (1987) characterized two separate phases (D_1 and D_2) on the basis of slope, within the diastolic potential of the tricuspid valve, and he showed a differential sensitivity of the phases to caesium ions. The results of our experiments in Fig. 2, which show a marked prolongation of cycle length following i_F blockade (Fig. 1), as well as those on the effect of nickel on spontaneous cycle length (Fig. 7*D*), indeed suggest a complex interplay of ionic currents in the process of diastolic depolarization. The possible roles played by the different ionic currents in diastolic depolarization of the tricuspid valve cells will now be discussed on the basis of previous studies (Rozanski & Jalife, 1986; Rozanski, 1987), as well as the voltage-clamp data present here. The roles played by the pump or the exchanger mechanisms will not be considered.

Potassium currents (i_K and i_{K1})

The involvement of i_K in diastolic depolarization of pacemaker cells is reasonably well established (Trautwein & Kassebaum, 1961; see Brown, 1982, for a review). Further data supporting this contention have also been obtained from single-channel analysis of the potassium current in nodal cells from the rabbit (Shibasaki, 1987). This current, which is activated during the upstroke of an action potential, is responsible for repolarizing the membrane, and is characterized by a deactivation process with slow kinetics (Nakayama *et al.* 1984; Shibasaki, 1987). This slow deactivation causes a gradual membrane depolarization, in the presence of a background inward current. Our data suggest that the i_K should play such a similar role in depolarizing valve cells during the pacemaker potential.

The properties of the inward rectifier current i_{K1} in the valve cells are such that the current might also be involved in the process of diastolic depolarization. For example, given the time constant of inactivation of i_{K1} (270 ms) within the diastolic potential (see Fig. 5), this current, if sizeable, should play a role similar to the i_K in nodal cells (i.e. a decreasing K^+ conductance) in bringing the membrane to threshold as in nodal cells. However, to determine the extent to which this is the case will require a more extensive investigation of the kinetics of the inactivation process at the range of voltages in the valve diastolic depolarization.

Hyperpolarization-activated current (i_F)

The role of i_F in the process of diastolic depolarization in the pacemaker activity of the sinus node has been controversial. Because the maximum diastolic potential of nodal cells rarely exceeds -65 mV, and given that the i_F starts activating at -40 to -50 mV, very little of the current would be activated in these cells. Consequently, it has been suggested that i_F may be responsible for diastolic depolarization in more hyperpolarized cells such as Purkinje cells (Noble, 1985).

In fact, it was reported that low Cs^+ concentrations reduce the discharge rate of the nodal tissues by only 20–30% (see Noble, 1985), which is consistent with the activation of only a small amount of the current in these tissues. However, the relatively well-polarized valve cell (MDP $= -82$ mV) implies that a sizeable amount of i_F will be activated during diastolic depolarization. This was indeed consistent with our experimental results, in which there was a marked prolongation of spontaneous cycle length following i_F blockade (Fig. 2). Rozanski (1987) also showed a similar effect of caesium on the spontaneous cycle length in valve tissue

preparations. However, his experiments, as well as ours, show that pacemaker activity continued in the presence of low concentrations of caesium, i.e. following i_F channel blockade. These results therefore suggest that, although more of i_F should be activated in the valve cell (as compared to the sinus node) and consequently more slowing of rate is expected following channel blockade, the current plays a role only in the *modulation* (and not in the *generation*) of pacemaker activity in the cells.

Calcium currents ($i_{Ca,T}$ and $i_{Ca,L}$)

In cardiac pacemaker cells, the calcium current which is activated during diastolic depolarization is suggested to be responsible for the final phase of depolarization to threshold, ultimately producing the upstroke (Noma, Kotake & Irisawa, 1980; Yanagihara & Irisawa, 1980; Noma, Morad & Irisawa, 1983; for a review see Brown, 1982). Initially, the idea that the current could play a role was controversial, since diastolic depolarization in the pacemaker cells occurs between −60 and −40 mV while the calcium current is activated between −30 and −40 mV. However, after the demonstration in cardiac pacemaker cells of the two types of calcium channel (T- and L-type) with activation voltages of around −55 and −30 mV respectively (Hagiwara *et al.* 1988), the issue then became the extent to which the current participates in the process. In fact, further support for this hypothesis has been recently presented in the action potential clamp experiments of Doerr, Denger & Trautwein (1989).

Our nickel experiments are indicative of the presence of the T-type channel in the valve cell. Given the voltage activation range of the T-channel (−55 to −60 mV), and because the take-off potential in the valve is −56 mV, some T-type current should be activated before the upstroke, with full activation of both types (T and L) occurring during upstroke. Since our results do indeed show an effect of nickel on the final phase of diastolic depolarization, it will therefore be important, in the future, to determine the effect of T-type channel blockade on the upstroke of the valve action potential.

In conclusion, the results of this study show that the cardiac myocytes in the tricuspid valve are normally spontaneously active, and can be used to study underlying mechanisms of pacemaker activity. The valve model should be particularly useful as it does not appear that its histology is as complicated as that of the sinus node. The process of diastolic depolarization in the cells involves an interplay of several ionic channels. Thus, this simpler model may provide a very useful means to study the complex interaction of ionic currents generating diastolic depolarization in pacemaker cells. Finally, it is also concluded that the electrophysiological properties of the tricuspid valve myocytes, as shown by previous whole-tissue as well as in the present single-cell investigations, clearly indicate that they are a potential source of ectopic impulse formation, which can be involved in precipitating arrhythmias of supraventricular origin.

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