The role of Na^+–Ca^{2+} exchange current in electrical restitution in ferret ventricular cells

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1. The mechanisms underlying electrical restitution (recovery of action potential duration after a preceding beat) were investigated in ferret ventricular cells. The time to 80% recovery (t_{80}) of action potential duration was ~204 ms.

2. At a holding potential of −80 mV, the Ca^{2+} current (I_{Ca}) reactivated and the delayed rectifier K^+ current (I_K) deactivated very rapidly (t_{80}: ~32 and ~93 ms, respectively). The kinetics of both currents are too fast to account for electrical restitution alone.

3. The putative inward Na^+–Ca^{2+} exchange current ($I_{Na\text{-}Ca}$) produced by the Na^+–Ca^{2+} exchanger in response to the intracellular Ca^{2+} transient reprimed (t_{80}: 189 ms) with the same time course as mechanical restitution (recovery of contraction) and with a similar time course to electrical restitution.

4. Substantial reduction of inward $I_{Na\text{-}Ca}$, by buffering intracellular Ca^{2+} with the acetyl methyl ester form of BAPTA, shortened the action potential and greatly altered the electrical restitution curve. Subsequent addition of nifedipine (to block I_{Ca}) or 4-aminopyridine (4-AP) (to block the transient outward current, I_{TO}) further altered the electrical restitution curve.

5. Any time-dependent current that contributes to the action potential is likely to affect the time course of electrical restitution. Although I_{Ca}, I_K and I_{TO} were previously thought to be the only currents involved in electrical restitution, we conclude that inward $I_{Na\text{-}Ca}$ also plays an important role.

If a test action potential is triggered immediately after a control action potential, the test action potential is shorter than the control. The test action potential recovers, as the test interval between the control and test responses is increased. A plot of test action potential duration against the test interval represents the time course of recovery of action potential duration and is known as an electrical restitution curve. The electrical restitution curve reflects the repriming of the different time-dependent currents involved in the action potential. Electrical restitution is important clinically because in ischaemic conditions ventricular arrhythmias are frequently initiated following a premature beat and experiments to investigate the electrical restitution curve may provide important clues to arrhythmogenesis and its control.

In early work it was suggested that electrical restitution resulted from the repriming of the Ca^{2+} current (I_{Ca}) or the delayed rectifier K^+ current (I_K). During an action potential, I_{Ca} is inactivated and I_K is activated. Gettes & Reuter (1974) suggested that incomplete reactivation of I_{Ca} at short test intervals and the consequent decrease in the amplitude of I_{Ca} during the test action potential could explain the shortening of the test action potential, whereas De Hemptinne (1971) and Hauswirth, Noble & Tsien (1972) suggested that incomplete deactivation of I_K and the consequent additional I_K during the test action potential could explain the shortening. Although it is possible that I_{Ca} and I_K play a role in electrical restitution, other time-dependent currents may also make an important contribution. The Ca^{2+} transient stimulates the generation of inward current by the Na^+–Ca^{2+} exchanger and this inward Na^+–Ca^{2+} exchange current ($I_{Na\text{-}Ca}$) is well known to be responsible for the 'low' plateau (approximately −40 mV) in rat ventricular cells and is also believed to make an important contribution to the higher plateau (+40 to −20 mV) in ventricular cells in other species (Mitchell, Powell, Terrar & Twist, 1984; Noble, Noble, Bett, Earm, Ho & So, 1991; Janvier & Boyett, 1996). At short test intervals, the Ca^{2+} transient and, consequently, the contraction are known to be depressed (Wier & Yue, 1986). The decrease in the Ca^{2+} transient is expected to result in a decrease in inward $I_{Na\text{-}Ca}$ and this may also contribute to the shortening of the test action potential.

To understand the mechanisms underlying electrical restitution we measured electrical restitution and the

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repriming of all the currents likely to be involved in electrical restitution (I_{Ca}, I_K and inward $I_{Na\text{-}Ca}$). Whereas in the early studies multicellular preparations were used with well-known problems of voltage control and extracellular ion changes, in the present study single cells were used. Repriming of currents is voltage dependent and, therefore, in the present study repriming was studied at −80 mV, close to the normal resting potential. Specific blockers have also been used to assess the contribution of inward $I_{Na\text{-}Ca}$ and I_{Ca} (and the transient outward current, I_{TO}) to the electrical restitution curve. A preliminary account of this work has been presented to the Physiological Society (Janvier, Harrison & Boyett, 1995).

METHODS

Experiments were performed on isolated rat or ferret ventricular cells. Rats (male or female, ~250 g body weight) were killed by cervical dislocation and ferrets were anaesthetized by intraperitoneal injection of 90–150 mg sodium pentobarbitone. The heart was rapidly excised and placed in isolation solution containing 750 μM $CaCl_2$. Isolation solution contained (mM): NaCl, 130; KCl, 5·4; $MgCl_2$, 1·4; NaH_2PO_4, 0·4; creatine, 10; taurine, 20; Hepes, 10; glucose, 10; pH 7·3 at room temperature (~25 °C) and equilibrated with 100% O_2. The aorta was cannulated and the heart retrogradely perfused at 20–23 ml min^{-1} with isolation solution containing 750 μM Ca^{2+} for sufficient time to clear the heart of blood (2–3 min). The perfusate was then switched to Ca^{2+}-free isolation solution (isolation solution plus 0·1 mM EGTA) for 4 min. Finally, the hearts were perfused for 6 min (rat) or 10 min (ferret) with enzyme solution – isolation solution containing 1 mg ml^{-1} collagenase (Worthington Type II; Lorne Laboratories), 0·1 mg ml^{-1} protease (Sigma) and 50 μM $CaCl_2$. This solution was recirculated through the heart. The ventricles were cut away from the atria, finely chopped, placed in a conical flask and digested with the enzyme solution supplemented with 10% (w/v) bovine serum albumin for a further 5 min. The tissue was shaken gently during this period. This process was repeated four times and the cells from each 5 min period were harvested by filtration and pelleted by centrifugation at 400 r.p.m. for 40 s. Cells were washed by resuspending them in isolation solution containing 750 μM Ca^{2+} and then recentrifuging them. The cells were stored in isolation solution containing 750 μM Ca^{2+} at 4 °C until required. The isolation procedure was performed at 37 °C.

Cells were pipetted into a small tissue bath (volume, 0·2 ml) attached to the stage of an inverted microscope (Nikon Diaphot). The cells were allowed to settle for several minutes onto the glass bottom of the chamber before being superfused at a rate of ~1·6 ml min^{-1} with Tyrode solution of the following composition (mM): NaCl, 136·9; KCl, 5·4; $CaCl_2$, 2; $MgCl_2$, 0·57; NaH_2PO_4, 0·37; Hepes, 5; glucose, 5·6; pH 7·4 at 37 °C. In some experiments NaCl was replaced with LiCl at the same concentration to block inward $I_{Na\text{-}Ca}$. In this case, the flow rate was increased to ~3 ml min^{-1} to reduce the solution exchange time in the chamber. In other experiments, the acetyl methyl ester form of BAPTA (BAPTA AM), nifedipine or 4-aminopyridine (4-AP) was added to the Tyrode solution to buffer intracellular Ca^{2+} or block I_{Ca} or I_{TO}, respectively. A 1 mM BAPTA AM stock solution was made by dissolving the drug in dimethyl sulphoxide (DMSO). Cells were perfused with Tyrode solution containing 5 μM BAPTA AM (0·5% DMSO) for 5–10 min. During this period, the size of the contraction dwindled as a result of the buffering of intracellular Ca^{2+}. When the contraction was almost completely abolished, the BAPTA AM was washed off. On wash-off of BAPTA AM the contraction did not recover (BAPTA remained trapped within the cells as a result of the cleavage of the AM group by intracellular esterases). Measurements were made only after more than 2 min of washing. As a control, cells were exposed to Tyrode solution containing 0·5% DMSO only for 10 min; this had no effect on the action potential, membrane current and accompanying contraction ($n = 4$ cells). Nifedipine was dissolved in methanol to give a 20 mM stock solution and a 300 mM stock solution of 4-AP was prepared by dissolving the drug in double-distilled water (pH adjusted to 7·6 at room temperature with HCl).

Miniature solenoid valves (Lee Products Ltd, Gerrards Cross, Buckinghamshire, UK) controlled which of four solutions flowed to the chamber. The fluid level in the chamber was controlled using the system described by Cannell & Lederer (1986). The temperature of the solution was maintained at 37 ± 0·5 °C by a heating coil wrapped around the solution inflow tube at the entrance of the chamber. Solution temperature was monitored by a thermistor mounted in the side of the chamber and controlled by a feedback circuit, which regulated the flow of current to the heating coil.

Action potentials were recorded with conventional microelectrodes (15–30 MΩ) filled with 1 M KCl and a Dagan 8800 amplifier (Dagan corporation, Minneapolis, USA) in the current clamp mode. Cells were stimulated using a 5 ms current pulse. $I_{Na\text{-}Ca}$ was recorded with conventional microelectrodes and the switch clamp technique (Dagan 8800 amplifier; switching frequency, ~4·5 kHz). Conventional microelectrodes were used for the recording of action potentials and $I_{Na\text{-}Ca}$, because this involves minimal intracellular dialysis and thus minimal disturbance of intracellular Na^+ and Ca^{2+} (important determinants of $I_{Na\text{-}Ca}$). Using conventional microelectrodes, stable recordings of up to and over 1 h were possible. The capacitance of the microelectrode was compensated just prior to impalement. The electrode potential upon switching between current injection and voltage recording was monitored on an oscilloscope throughout an experiment to ensure that the electrode potential settled between current injection pulses.

I_{Ca} and I_K were recorded using the whole-cell patch clamp technique to allow dialysis with the solutions below to optimize the recording of the relevant current. The resistance of the patch pipettes ranged from 1 to 3 MΩ. When i_{Ca} was recorded, the patch pipettes were filled with internal solution containing (mM): CsCl, 80; TEA-Cl, 20; $MgCl_2$, 4; Hepes, 5; Na_2ATP, 3; EGTA, 10; pH 7·1 adjusted with CsOH at 37 °C. The internal solution contained Cs^+ (rather than K^+) and TEA to block K^+ currents. EGTA was present in the pipette solution to buffer bulk intracellular Ca^{2+} and block cell contraction and Ca^{2+}-dependent currents (e.g. inward $I_{Na\text{-}Ca}$ in response to the Ca^{2+} transient). In these experiments, the Tyrode solution also contained 20 mM Cs^+. When I_K was recorded, the patch pipettes were filled with internal solution containing (mM): potassium aspartate, 120; KCl, 20; KH_2PO_4, 1; $MgCl_2$, 5·5; EGTA, 5; Hepes, 5; Na_2ATP, 3; Na_3GTP, 0·1; pH 7·2 at 37 °C. In these experiments, 1 μM nifedipine and 5 mM 4-AP were present in the bath solution to block I_{Ca} and I_{TO}, respectively. Membrane potential and current were recorded using an Axopatch-1C amplifier (Axon Instruments). At the start of the experiment, the pipette capacitance was compensated and in the whole-cell configuration cell capacitance and series resistance were compensated for. The current signal was filtered by a Bessel-type low-pass filter with a cut-off frequency of 1 kHz (−3 dB). Peak I_{Ca} was measured with reference to the current 195 ms after the start of a depolarizing pulse.

Timing units (Hi-Med, Reading, UK) or Cambridge Electronic Design voltage clamp software (Cambridge Electronic Design, Cambridge, UK) were used to control restitution protocols and synchronize other recording equipment. Cell length was recorded using an optical system based on a photodiode array (Boyett, Moore, Jewell, Montgomery, Kirby & Orchard, 1988), and action potential duration was measured electronically at −65 mV (Kentish & Boyett, 1983). Membrane potential and current were displayed on an oscilloscope (Tektronix 5000 series). Membrane potential and current, cell length, twitch shortening and action potential duration were displayed on a chart recorder (Gould 2600S, 2400 or RS 3600) and simultaneously recorded on videotape using a pulse code modulator (Neuro-Corder DR-890; Neuro Data Instruments Corp., New York, USA) and a video recorder. Membrane voltage and current and cell length were also digitized using an analog-to-digital converter (1401 plus; Cambridge Electronic Design, Cambridge, UK) and stored on an IBM-compatible computer running the Cambridge Electronic Design voltage clamp software.

Data are presented as means ± S.E.M., n indicates the number of cells.

RESULTS

Electrical and mechanical restitution

Figure 1 illustrates the protocol used to determine electrical restitution in a ferret ventricular cell. Action potentials and their accompanying contractions in Fig. 1*A* are shown on a slow time base on the left. Control beats were triggered at 1 Hz. In this example an extrasystole or test beat (T) was triggered at a test interval of 180 ms after the preceding control action potential. The control and test action potentials and contractions are shown on a fast time base on the right of Fig. 1*A*. The test action potential was shorter and the test contraction was smaller than the preceding controls. The results from such an experiment can be displayed as restitution curves: the duration of the test action potential and amplitude of the accompanying contraction (expressed as a percentage of the preceding controls in this case) are plotted against the test interval between the control and test beats (Fig. 1*B*). The top curve in Fig. 1*B* is known as an electrical restitution curve and the bottom curve is known as a mechanical restitution curve. At short test intervals the test action potential was short and the accompanying contraction was small. However, as the test interval was increased, the duration of the test action potential and the size of the contraction increased towards their control values. Full restitution of both action potential duration and contraction occurred after a test interval of ~1000 ms. Similar results have been observed in more than thirty cells.

Time course of reactivation of I_{Ca}

Figure 2*A* shows records of I_{Ca} during 200 ms voltage clamp pulses from −40 to 0 mV. Cs^+ and TEA were present to block K^+ currents and intracellular Ca^{2+} was buffered by EGTA to reduce or eliminate inward $I_{Na\text{-}Ca}$ activated by the Ca^{2+} transient (see Methods). Control pulses were applied at 1 Hz and test pulses were interpolated as before. Superimposed records of I_{Ca} triggered at different test intervals after a control pulse are shown in Fig. 2*A*. I_{Ca} triggered at a short test interval was smaller than the control I_{Ca} as a result of incomplete recovery from the inactivation that developed during the control pulse. As the test interval was increased, I_{Ca} during the test pulse increased. In Fig. 2*C*, peak I_{Ca} during the test pulse expressed as a percentage of the preceding control is plotted against the test interval. The curve with the open squares represents the time course of reactivation of I_{Ca} at −40 mV. At −40 mV, I_{Ca} reactivated with a time course similar to the electrical restitution curve in Fig. 1 and might, therefore, be thought of as controlling it. However, when the

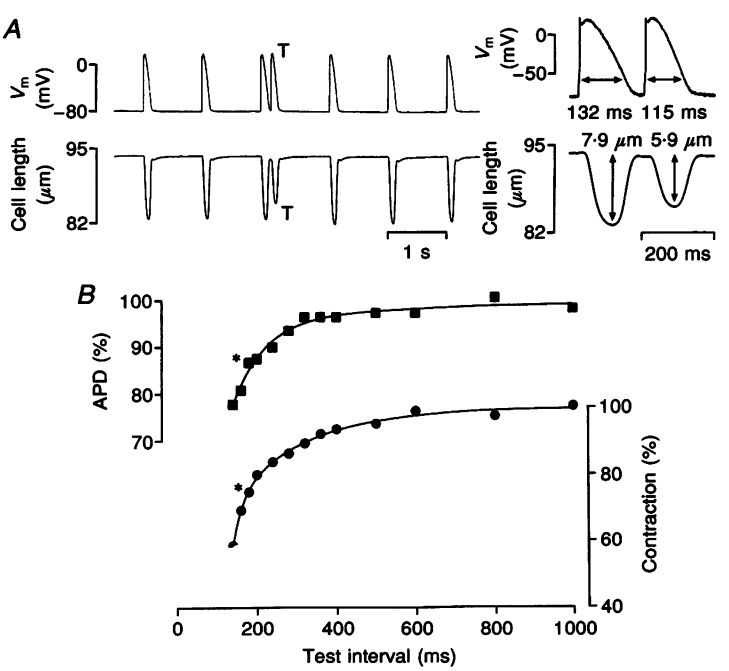

Figure 1. Electrical and mechanical restitution

A, experimental protocol. Slow time base records of action potentials (membrane potential, V_m; upper trace) and contractions (cell length; lower trace) are shown on the left. After the third action potential an extrasystole or test beat (T) was triggered at a test interval of 180 ms. The test interval is the time interval between the test stimulus and the preceding control stimulus. The test action potential and contraction and the preceding controls are shown on a fast time base on the right. Horizontal arrows show the measurements of action potential duration at −65 mV and vertical arrows the amplitude of contraction (cell shortening measured). *B*, electrical (upper) and mechanical (lower) restitution curves. The duration of the test action potential (■) and amplitude of the accompanying contraction (●) are expressed as a percentage of the preceding control and plotted against the test interval. Asterisks mark the results from *A*. Unless otherwise stated, in this and all subsequent figures, the data are fitted with smooth curves to guide the eye. Basic rate, 1 Hz. Ferret ventricular cell. Conventional microelectrode recordings.

holding potential was changed to −80 mV, close to the resting potential of a ferret ventricular cell, the reactivation of I_{Ca} was greatly altered. In this case, we applied a 10 ms prepulse from −80 to −40 mV to inactivate i_{Na} before stepping to 0 mV for 190 ms to activate I_{Ca} as shown in Fig. 2*B*. Figure 2*B* shows superimposed records of membrane current when the test interval between the control and test pulses was varied. Figure 2*C* also shows the reactivation of I_{Ca} at the holding potential of −80 mV (filled squares). Unlike the electrical restitution curve and reactivation of I_{Ca} at −40 mV, the reactivation of I_{Ca} at −80 mV was very rapid. I_{Ca} returned to its control value after a test interval of only 250 ms (Fig. 2*C*). Furthermore, at longer test intervals there is an overshoot in the curve when the test I_{Ca} was larger than the control (Fig. 2*C*); this can also be seen in Fig. 2*B*. If restitution of action potential duration was determined principally by I_{Ca}, the electrical restitution curve would be expected to display a similar overshoot, but this was not observed in ferret ventricular cells under normal conditions (Fig. 1*B*) (this argument depends on the assumption that restitution of I_{Ca} with action potentials is the same as that with voltage clamp pulses).

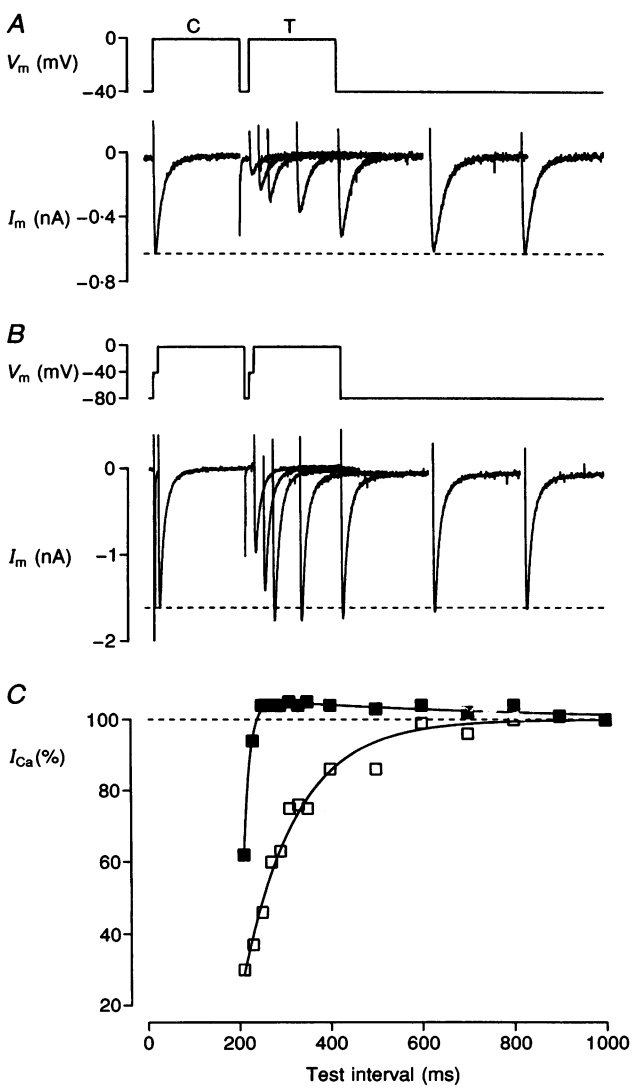

Figure 2. Reactivation of I_{Ca}

A and *B*, membrane current (I_m, principally I_{Ca}) (lower panel) during control and test voltage clamp pulses (upper panel). Membrane currents from eight runs with different test intervals are superimposed. The holding potential was −40 mV in *A* and −80 mV in *B*. In *A*, 200 ms voltage clamp pulses were applied from −40 to 0 mV. In *B*, a 10 ms prepulse from −80 to −40 mV was applied to inactivate i_{Na} before stepping to 0 mV for 190 ms. The dashed lines mark the peak I_{Ca} during the control pulse. *C*, reactivation of I_{Ca} at holding potentials of −40 (□) and −80 (■) mV. I_{Ca} is plotted as a percentage of the preceding control. The dashed line corresponds to I_{Ca} during the control pulse, i.e. 100%. All data from the same ferret ventricular cell. Basic rate, 1 Hz. Whole cell patch clamp technique.

Time course of deactivation of I_K

Figure 3 shows how we investigated the deactivation of I_K. I_K was recorded in the presence of nifedipine and 4-AP to block I_{Ca} and I_{TO}, respectively, and intracellular Ca^{2+} was buffered by EGTA to reduce or eliminate inward $I_{Na\text{-}Ca}$ produced in response to the Ca^{2+} transient (see Methods). Voltage clamp pulses (200 ms duration) from −80 to +40 mV were used to activate substantial I_K. Although 5 mM 4-AP was present to block I_{TO}, some I_{TO} was present at the start of each pulse (Fig. 3*A*). Control pulses were applied at 1 Hz and test pulses were interpolated. Figure 3*A* shows superimposed records of membrane current when the test interval between the control and test pulses was varied. During the control pulse, there was a gradual activation of I_K. After the control pulse, on return to −80 mV, I_K deactivated. When the test pulse was applied soon after the control pulse, deactivation of I_K was not complete and the current at the start of the test pulse (after the decay of I_{TO}) was more outward than at the equivalent time during the control pulse (Fig. 3*A*). During an action potential, this additional outward current is expected to result in a shortening of the action potential. In

the case of the test pulse applied soon after the control pulse, because I_K was already substantially activated at the start of the pulse, the amplitude of I_K at the end of the test pulse was greater than that at the end of the control pulse. As the test interval between the control and test pulses was increased, I_K deactivated by increasing amounts before the test pulse and, therefore, the current at the start of the test pulse (after I_{TO}) and the current at the end of the test pulse were both less outward (Fig. 3*A*). In Fig. 3*B* (filled circles) the mean amplitude of current from three cells at the start of a test pulse (after I_{TO}; measured with respect to the equivalent current during the control pulse) is plotted against the test interval. The data have been fitted with an exponential function with a time constant of 42 ms.

The analysis above relies on the assumption that the current at the start of the pulse (after I_{TO}) is a measure of I_K, whereas the amplitude of the I_K tail current is normally used as a measure of I_K. As shown in Fig. 3*A*, after the test pulse the membrane potential was clamped to −40 mV for 900 ms to measure the I_K tail current. An example of a tail current (after the earliest test pulse) is shown in Fig. 3*A*; other tail currents are not shown for clarity. As the test interval was increased, the amplitude of the tail current decreased and mean data from three cells are plotted against the test interval in Fig. 3*B* (open circles). The amplitude of the tail current decayed with a similar time constant to the current at the start of the pulse (the time constants are 44 and 42 ms, respectively) and was complete at a test interval of ~350 ms. Although the incomplete deactivation of I_K at short test intervals is appropriate to explain some of the shortening of the test action potential, the deactivation of I_K is quicker than electrical restitution (see Discussion) and other time-dependent currents are, therefore, also likely to play a role in electrical restitution.

The time course of the decay of a single tail current (like that in Fig. 3*A*) is also a reflection of the deactivation of I_K (at −40 mV). In three cells, it declined with a time constant of 119 ± 14 ms. This is greater than the time constant of deactivation of I_K at −80 mV (Fig. 3*B*).

Involvement of repriming of inward $I_{Na\text{-}Ca}$ in the electrical restitution curve in rat ventricular cells

It is possible that the Na^+–Ca^{2+} exchanger plays an important role in electrical restitution. Figure 1 shows that a test contraction is smaller than the preceding control. It is known that this is the result of a decrease in the underlying Ca^{2+} transient (Wier & Yue, 1986). The reduction in the Ca^{2+} transient is expected to decrease Ca^{2+} efflux on the Na^+–Ca^{2+} exchanger and, therefore, inward $I_{Na\text{-}Ca}$ during the test action potential. Such a loss of inward current will tend to shorten the test action potential (Janvier, Harrison & Boyett, 1997). It is widely accepted that the low plateau (at approximately −40 mV) of the rat ventricular action potential is generated by inward $I_{Na\text{-}Ca}$ activated by the Ca^{2+} transient (Noble *et al.* 1991; Janvier & Boyett, 1996). Initial

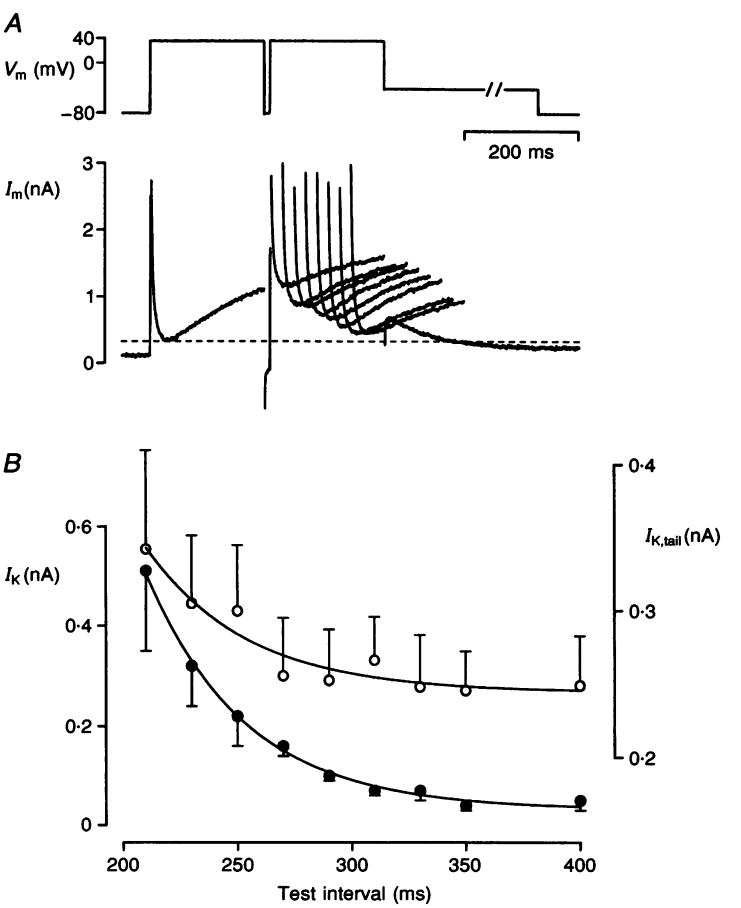

Figure 3. Deactivation of I_K

A, membrane current (lower traces) during control and test voltage clamp pulses (upper traces). Membrane currents from eight runs with different test intervals are superimposed. Voltage clamp pulses of 200 ms duration were applied from −80 to +40 mV. After the test pulse the membrane potential was clamped to −40 mV for 900 ms to measure the K^+ tail current ($I_{K,tail}$). Only one record of membrane current during the control pulse (left-hand part of trace) and only one tail current (right-hand part of trace) are shown for simplicity. Although 5 mM 4-AP was present to block I_{TO}, some I_{TO} remained at the start of each pulse. The dashed line shows the level of current after the inactivation of I_{TO} and before the activation of I_K during the control pulse. *B*, deactivation of I_K. The level of current after the inactivation of I_{TO} and before further activation of I_K during the test pulse (●) and the amplitude of the I_K tail current at −40 mV after the test pulse (○) are plotted against the test interval. Means ± S.E.M. from three ferret ventricular cells are shown. Single exponential functions with time constants of 44 (○) and 42 (●) ms are fitted. Basic rate, 1 Hz. Whole cell patch clamp technique.

experiments were, therefore, carried out on rat ventricular cells to establish whether inward $I_{Na\text{-}Ca}$ can be involved in electrical restitution.

A control rat ventricular action potential triggered at a rate of 1 Hz is shown in the left panel of Fig. 4*A*. Superimposed on this action potential is a test action potential (T), triggered at a test interval of 120 ms. Whereas the control action potential displayed a prominent low plateau at potentials negative to −40 mV, the test action potential did not. This is to be expected, as the test contraction at an interval of 120 ms (not shown) was smaller than the control (the small underlying Ca^{2+} transient is expected to activate less inward $I_{Na\text{-}Ca}$). Figure 4*B* and *C* (filled squares) shows the electrical restitution curve for the low plateau: the duration of the low plateau (measured at −60 mV) in milliseconds (Fig. 4*B*) or as a percentage of the preceding control (Fig. 4*C*) is plotted against the test interval. The mechanical restitution curve (plot of amplitude of the test contraction against test interval) is also shown in Fig. 4*C* (filled circles). As the test interval was increased, the amplitude of the test contraction and the duration of the low plateau increased in parallel. This result supports the hypothesis that repriming of inward $I_{Na\text{-}Ca}$ (determined by the repriming of the Ca^{2+} transient) determines restitution of the low plateau in rat ventricular cells. We have further tested this hypothesis by reducing the inward $I_{Na\text{-}Ca}$ produced in response to the Ca^{2+} transient by buffering intracellular Ca^{2+} (and thus reducing the Ca^{2+} transient) with 5 μM BAPTA AM. The right panel of Fig. 4*A* shows a pair of control and test action potentials (test interval, 120 ms, as in the left panel of Fig. 4*A*) after the application of BAPTA AM. Buffering intracellular Ca^{2+} is reported to suppress the low plateau by inhibiting inward $I_{Na\text{-}Ca}$ (Mitchell *et al.* 1984; Janvier & Boyett, 1996) and this is confirmed by Fig. 4*A*: the control action potential after the application of BAPTA AM (right panel) lacked the low plateau possessed by the control action potential under normal conditions prior to the application of BAPTA AM (left panel).

After the application of BAPTA AM, the electrical restitution curve for the low plateau was altered, as shown by Fig. 4*A* (right panel). Unlike under normal conditions, after the application of BAPTA AM the test action potential was longer than the control action potential. The electrical restitution curve after the application of BAPTA AM is shown in Fig. 4*B* and *C* (open squares). The electrical restitution curve shows that a test action potential triggered immediately after the control was the longest. As the test interval was increased, action potential duration at −60 mV (potential corresponding to the low plateau) declined towards its control value (Fig. 4*C*). Because the inward $I_{Na\text{-}Ca}$ produced in response to the Ca^{2+} transient was suppressed, the electrical restitution curve for action potential duration at −60 mV (corresponding to the low plateau) after the application of BAPTA AM should reflect

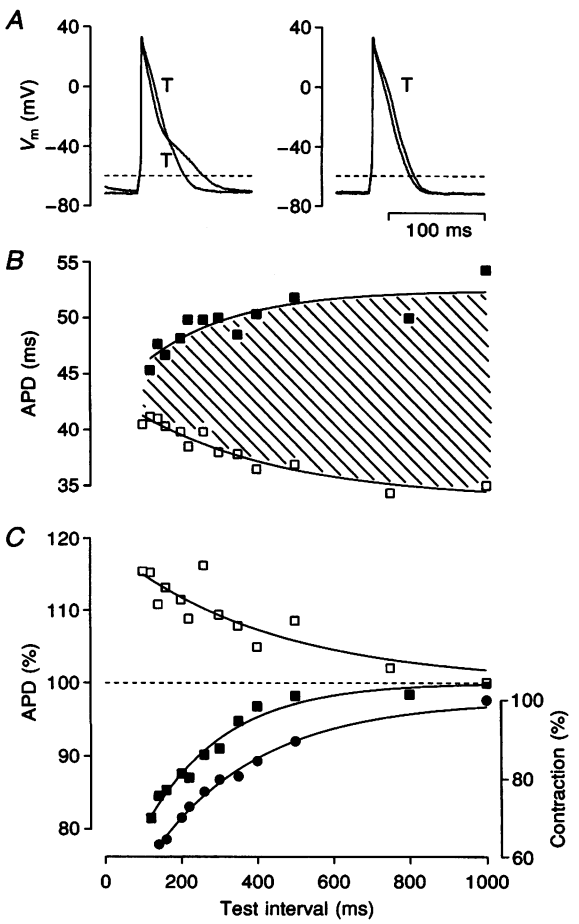

Figure 4. Electrical restitution in rat ventricular cells before and after application of 5 μM BAPTA AM

A, action potentials under normal conditions (left panel) and following the application of 5 μM BAPTA AM (right panel). In each case a test action potential (T) triggered after a test interval of 120 ms is superimposed on the preceding control. Action potential duration was measured at −65 mV, marked by the dashed line. *B* and *C*, electrical restitution. The duration of the test action potential (in ms in *B*; as a percentage of the preceding control in *C*) is plotted against the test interval under normal conditions (■) and after the application of 5 μM BAPTA AM (□). The mechanical restitution curve is also plotted in *C* (●) – the amplitude of the test contraction (as a percentage of the preceding control) is plotted against the test interval. In *C* the dashed line corresponds to 100%. Basic rate, 1 Hz. The curves are averaged from two rat ventricular cells. Conventional microelectrode recordings.

the restitution of other currents. These currents may be the same as those involved in the restitution of the high plateau (at potentials around 0 mV), because the high plateau of a test action potential was prolonged compared with the preceding control regardless of whether BAPTA AM had been applied or not (Fig. 4*A*).

Results similar to those in Fig. 4 were obtained from a total of five cells. These experiments suggest that inward $I_{Na\text{-}Ca}$ produced in response to the Ca^{2+} transient can be involved in electrical restitution.

Time course of repriming of inward $I_{Na\text{-}Ca}$

In a previous study of ferret ventricular cells, we showed that after the application of BAPTA AM a transient inward component of current was inhibited during depolarizing voltage clamp pulses (Janvier *et al.* 1997). It was argued that the BAPTA-sensitive current was most likely to be inward $I_{Na\text{-}Ca}$ produced in response to the Ca^{2+} transient. Experiments were carried out to determine whether the BAPTA-sensitive current (putative inward $I_{Na\text{-}Ca}$) undergoes a repriming process in ferret ventricular cells.

Figure 5 shows how we measured the repriming of the BAPTA-sensitive current. Pulses from −80 to 0 mV of 200 ms duration were applied at a rate of 1 Hz and test pulses were interpolated as before. This procedure was repeated before and after the application of 5 μM BAPTA AM. Membrane current recorded after the application of BAPTA AM was subtracted from current recorded under normal conditions before the application.

Figure 5*A* shows mean BAPTA-sensitive currents on the left and the accompanying contractions on the right during a control pulse and at test intervals of 210 and 250 ms. During the control pulse, the inward BAPTA-sensitive current had a time course similar to that of a Ca^{2+} transient. At the shortest test interval of 210 ms the BAPTA-sensitive current and accompanying contraction were greatly reduced compared with the controls. After a test interval of 250 ms, the BAPTA-sensitive current and contraction had increased. Figure 5*B* and *C* shows the repriming of the inward BAPTA-sensitive current and contraction, respectively, from four cells – the amplitude of the current or contraction are plotted against the test interval. As the test interval was increased, the BAPTA-sensitive current and accompanying contraction during the test pulse increased to their control values. Both restitution curves are fitted with double-exponential functions with time constants of 11 and 163 ms for the BAPTA-sensitive current and 10 and 162 ms for contraction. These results are consistent with the hypothesis that restitution of both the BAPTA-sensitive current and contraction depend on the restitution of the Ca^{2+} transient. It is concluded, therefore, that inward $I_{Na\text{-}Ca}$ (as measured by the BAPTA-sensitive current) does undergo a repriming process in ferret ventricular cells (although the repriming is not an intrinsic characteristic of $I_{Na\text{-}Ca}$ and instead is the result of the repriming of the underlying Ca^{2+} transient). The time course of the repriming of the putative inward $I_{Na\text{-}Ca}$ (Fig. 5*B*) is similar to that of the electrical restitution curve (Fig. 1), which suggests that inward $I_{Na\text{-}Ca}$ might play an important role in electrical restitution.

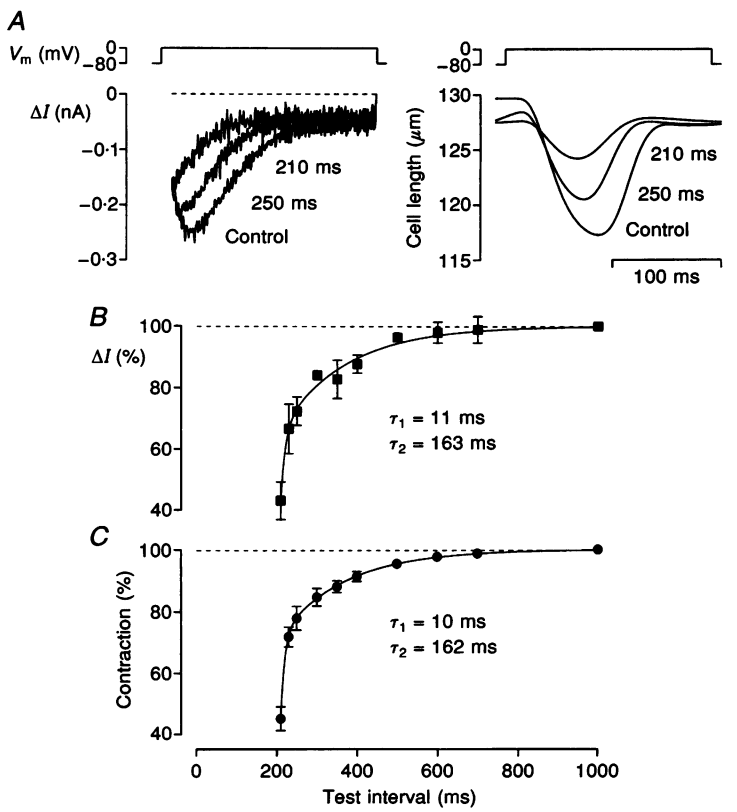

Figure 5. Correlation of the repriming of BAPTA-sensitive current with mechanical restitution

A, left panel, superimposed BAPTA-sensitive currents (lower panel) recorded during control pulses and during test pulses (upper panel) at test intervals of 210 and 250 ms. Membrane potential was clamped from −80 to 0 mV for 200 ms during all pulses. Current traces are an average of five. The dashed line corresponds to zero current. BAPTA-sensitive currents were obtained by subtracting current after the application of 5 μM BAPTA AM from current under normal conditions. Right panel, contractions recorded under normal conditions during the control and test pulses. *B*, repriming of the BAPTA-sensitive current. BAPTA-sensitive current during the test pulse (expressed as a percentage of the current during the preceding control pulse) is plotted against the test interval. *C*, mechanical restitution. Contraction during the test pulse (expressed as a percentage of the contraction during the preceding control pulse) is plotted against the test interval. In *B* and *C* the points show means ± S.E.M. from five cells and have been fitted by double-exponential functions whose time constants (τ) are shown. Basic rate, 1 Hz. Ferret ventricular cells. Switch clamp technique.

Possible involvement of repriming of inward $I_{Na\text{-}Ca}$ in the electrical restitution curve in ferret ventricular cells

To test whether inward $I_{Na\text{-}Ca}$ is involved in the electrical restitution curve in ferret ventricular cells, the effect of suppressing inward $I_{Na\text{-}Ca}$, by buffering intracellular Ca^{2+} with BAPTA AM, on the electrical restitution curve in ferret ventricular cells was investigated. A typical result is shown in Fig. 6. Action potentials are shown in Fig. 6*A* under normal conditions and following application of BAPTA AM. In each case a test action potential (T) triggered after a test interval of 200 ms, is superimposed on the preceding control (triggered at a rate of 1 Hz). Complete electrical restitution curves under normal conditions and after the application of BAPTA AM are shown in Fig. 6*B* and *C*. The duration of the test action potential is shown in milliseconds in Fig. 6*B* and as a percentage of the preceding control in Fig. 6*C*.

After the application of BAPTA AM the control action potential was shorter than the control under normal conditions due to the loss of the BAPTA-sensitive current (putative inward $I_{Na\text{-}Ca}$) – this confirms an earlier study (Janvier *et al.* 1997). This is shown in both Fig. 6*A* and *B* (in Fig. 6*B* the points at a test interval of 1000 ms, corresponding to 1 Hz, represent the duration of the control action potentials). After the application of BAPTA AM electrical restitution was modified. Figure 6*A* shows that under normal conditions before the application of BAPTA AM the test action potential was shorter than the control (left panel), whereas after the application of BAPTA AM the test action potential was longer than the control. Under normal conditions, a test action potential triggered after a short test interval was short in duration and the duration monotonically increased as the test interval was increased (Fig. 6*B* and *C*). After the application of BAPTA AM, action potential duration recovered to its control value quickly, increased beyond its control value and then slowly declined to its control value (Fig. 6*B* and *C*). This resulted in an overshoot in the electrical restitution curve. Similar results were obtained from a total of twenty cells. These results are analogous to those for the low plateau in rat ventricular cells (Fig. 4) and suggest that in ferret ventricular cells, as well as in rat ventricular cells, repriming of inward $I_{Na\text{-}Ca}$ plays a role in electrical restitution. For ferret ventricular cells, this conclusion was confirmed by the substitution of extracellular Na^+ by Li^+ to block inward $I_{Na\text{-}Ca}$ (not shown). In four cells substitution of extracellular Na^+ for Li^+ also resulted in an overshoot of the electrical restitution curve.

Possible involvement of reactivation of I_{Ca} in the electrical restitution curve

After the elimination of inward $I_{Na\text{-}Ca}$, the electrical restitution curve (Fig. 6*B* and *C*, open squares) resembles the time course of reactivation of I_{Ca} at −80 mV (Fig. 2*C*) and it is possible that the reactivation of I_{Ca} has a major effect on the time course of electrical restitution under these

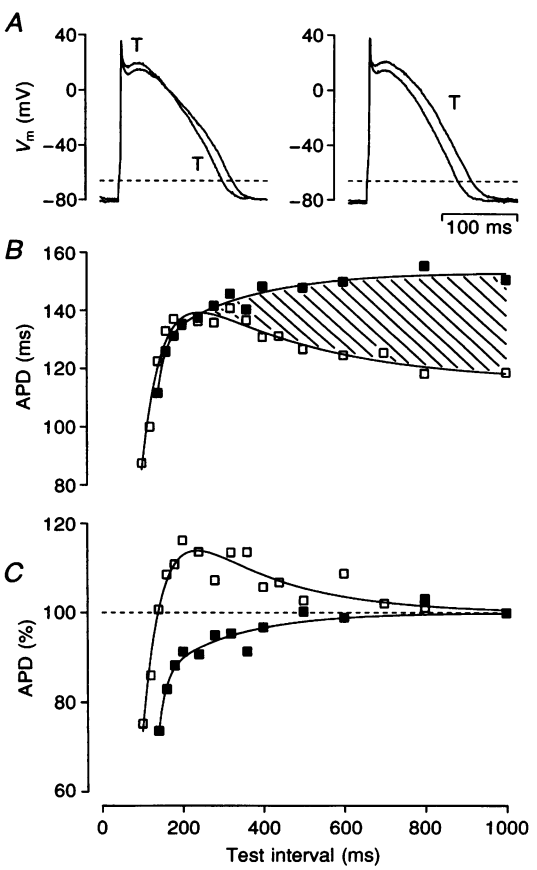

Figure 6. Effect of 5 μM BAPTA AM on electrical restitution

A, action potentials under normal conditions and following the application of 5 μM BAPTA AM. In each case a test action potential (T) triggered after a test interval of 200 ms is superimposed on the preceding control. Action potential duration was measured at −65 mV, marked by the dashed line. *B* and *C*, electrical restitution. The duration of the test action potential (in ms in *B*; as a percentage of the preceding control in *C*) is plotted against the test interval under normal conditions (■) and after the application of BAPTA AM (□). In *C* the dashed line corresponds to the duration of the control action potential, i.e. 100%. Basic rate, 1 Hz. Ferret ventricular cell. Conventional microelectrode recordings.

conditions. This hypothesis was tested by investigating the effect of block of I_{Ca} by nifedipine on the electrical restitution curve. Under normal conditions, nifedipine is expected to block both I_{Ca} and the inward $I_{Na\text{-}Ca}$ produced in response to the Ca^{2+} transient, because the block of I_{Ca} will lead to the loss of the Ca^{2+} transient. Therefore, BAPTA AM was first applied to the cell to buffer intracellular Ca^{2+} to inhibit the inward $I_{Na\text{-}Ca}$ produced in response to the Ca^{2+} transient and then nifedipine was added. The left panel of Fig. 7*A* shows superimposed control (1 Hz stimulation) and test (test interval, 250 ms) action potentials after the application of BAPTA AM. The test action potential was longer than the control. The electrical restitution curve in Fig. 7*B* and *C* (open squares) had an overshoot as before. Next we applied 20 μM nifedipine to block I_{Ca}. Control and test action potentials (test interval, 160 ms) after the application of nifedipine are shown on the right of Fig. 7*A*. As expected, block of I_{Ca} resulted in a marked reduction in the action potential plateau and duration. After block of I_{Ca} the test action potential was no longer prolonged compared with the control. This is confirmed by the electrical restitution curve in Fig. 7*B* and *C* (filled triangles), which shows that the earliest test action potential was slightly shorter than the control and the duration of the test action potential increased as the test interval was increased. Although this result is consistent with the possibility that I_{Ca} is involved in the electrical restitution curve, this interpretation is qualified (see Discussion).

Possible involvement of reactivation of I_{TO} in the electrical restitution curve

I_{Ca} is not the only current possibly involved in the overshoot of the electrical restitution curve after the application of BAPTA AM. Another candidate is I_{TO}. During an action potential, I_{TO} will inactivate and after the action potential it will reactivate. If a test action potential is triggered before reactivation is complete, I_{TO} will be reduced and the action potential will be prolonged. Figure 8 shows the effect of block of I_{TO} by 3 mM 4-AP on the electrical restitution curve. The effect of 4-AP was investigated after the application of BAPTA AM. Superimposed control (1 Hz stimulation) and test (test interval, 160 ms) action potentials before and after the application of 4-AP are shown in Fig. 8*A* and the corresponding electrical restitution curves are shown in Fig. 8*B* and *C*. After the application of BAPTA AM the test action potential was longer than the preceding control (Fig. 8*A*, left panel), but after the further application of 4-AP the test action potential was shorter (Fig. 8*A*, right panel). The electrical restitution curves in Fig. 8*B* and *C* show that 4-AP greatly reduced the overshoot, but did not abolish it, in this example.

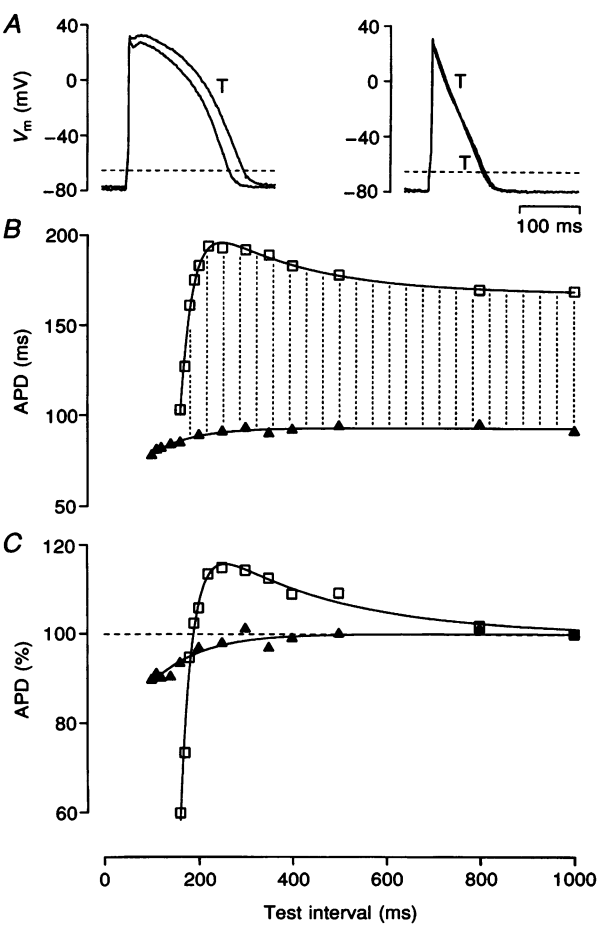

Figure 7. Effect of 20 μM nifedipine on electrical restitution

A, action potentials after the application of 5 μM BAPTA AM (left panel) and after the further application of 20 μM nifedipine (right panel). In each case a test action potential (T) triggered after a test interval of 250 (left) or 160 (right) ms, is superimposed on the preceding control. Action potential duration was measured at −65 mV, marked by the dashed line. *B* and *C*, electrical restitution. The duration of the test action potential (in ms in *B*; as a percentage of the preceding control in *C*) is plotted against the test interval after the application of BAPTA AM (□) and after the further application of nifedipine (▲). In *C* the dashed line corresponds to the duration of the control action potential, i.e. 100%. Basic rate, 1 Hz. Ferret ventricular cell. Conventional microelectrode recordings.

To summarize the effects of BAPTA alone, BAPTA plus nifedipine and BAPTA plus 4-AP on the electrical restitution curve, for a particular cell the test interval was determined at which the duration of the test action potential was maximum after the application of BAPTA AM (this ranged from 160 to 400 ms in different cells) and then the duration of the test action potential at this test interval (as a percentage of the preceding control) was measured in the particular cell under normal conditions, in the presence of BAPTA alone, in the presence of BAPTA plus nifedipine and in the presence of BAPTA plus 4-AP. Analysis showed that, whereas the test action potential was shorter than the control under normal conditions (93 ± 1% of control; $n = 20$), it was longer after the application of BAPTA AM (109 ± 1% of control; $n = 22$), and it was once again shorter after the further application of nifedipine (98 ± 1% of control; $n = 4$) or 4-AP (97 ± 2% of control; $n = 9$). This suggests that I_{TO}, as well as I_{Ca}, may play a role in the overshoot of the electrical restitution curve.

The effect of block of I_{TO} by 4-AP on the electrical restitution curve under normal conditions is shown in the inset in Fig. 8*B*. The effect is comparable to the effect of 4-AP after the application of BAPTA AM. Both under normal conditions and after the application of BAPTA AM, the test action potentials were similar in duration before and after the application of 4-AP at the shortest test intervals, but as the test interval was increased the difference in the duration of the test action potentials before and after the application of 4-AP increased, reaching a maximum at a test interval of ~400 ms. Effects of 4-AP similar to those in Fig. 8 were observed in a total of seven cells after the application of BAPTA AM and two cells under normal conditions.

DISCUSSION

In the present study we have measured repriming of the three most likely currents to be involved in the electrical restitution curve, I_{Ca}, I_K and $I_{Na\text{-}Ca}$, at −80 mV, i.e. close to the resting potential, the potential at which repriming of the currents will occur *in vivo*. The data obtained are summarized in Fig. 9. Figure 9*A* shows the electrical restitution curve (it is similar to that in Fig. 1*B*; in this case mean data from four to six cells are shown). Figure 9*B* shows repriming of inward $I_{Na\text{-}Ca}$ (same data as Fig. 5*B*). Figure 9*C* shows reactivation of I_{Ca} at −80 mV (it is similar to that in Fig. 2*C*; in this case mean data from five cells are shown). Figure 9*D* shows deactivation of I_K (same data as Fig. 5*B* – amplitude of current at start of pulse shown; in this case the data have been inverted to allow comparison with the other sets of data). In Fig. 9*E* all sets of data are normalized and superimposed to allow comparison of time courses (for clarity only means are plotted – S.E.M. as well as means are plotted in panels *A–D*). The time to 80% recovery (t_{80}) of each curve is 204 ms (electrical restitution), 189 ms (inward $I_{Na\text{-}Ca}$), 32 ms (I_{Ca}) and 93 ms (I_K).

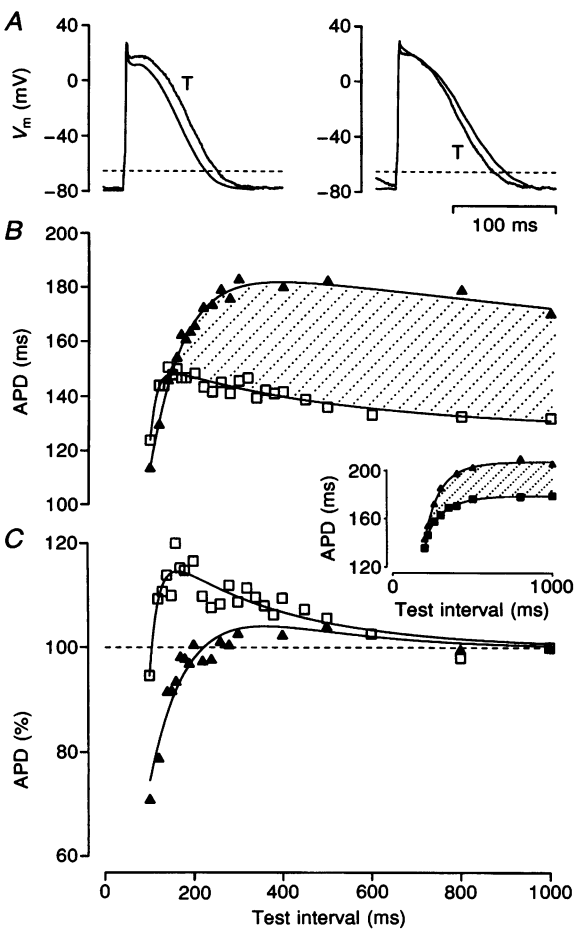

Figure 8. Effect of 3 mM 4-AP on electrical restitution

A, action potentials after the application of 5 μM BAPTA AM (left panel) and after the further application of 3 mM 4-AP (right panel). In each case a test action potential (T) triggered after a test interval of 160 ms is superimposed on the preceding control. *B* and *C*, electrical restitution. The duration of the test action potential (in ms in *B*; as a percentage of the preceding control in *C*) is plotted against the test interval after the application of BAPTA AM (□) and after the further application of 4-AP (▲). The inset in *B* shows the effect of 3 mM 4-AP on the electrical restitution curve under normal conditions. The duration of the test action potential (in ms) is plotted against the test interval under normal conditions (■) and after the application of 4-AP (▲). In *C* the dashed line corresponds to the duration of the control action potential, i.e. 100%. Basic rate, 1 Hz. Ferret ventricular cells. Conventional microelectrode recordings.

Reactivation of I_{Ca}

Previous studies have suggested that electrical restitution is the result of the reactivation of I_{Ca} or deactivation of I_K (De Hemptinne, 1971; Hauswirth *et al.* 1972; Gettes & Reuter, 1974). In the present study, at the usual holding potential used in the measurement of I_{Ca} (−40 mV), the time course of reactivation of I_{Ca} was comparable to that of the electrical restitution curve (compare Figs 2*C* and 1*B*). However, at a holding potential of −80 mV (close to the resting potential) this was not the case. At −80 mV, reactivation of I_{Ca} was rapid compared with the electrical restitution curve (t_{80}: I_{Ca}, 32 ms; electrical restitution curve, 204 ms) and, furthermore, over a wide range of test intervals I_{Ca} during the test pulse was larger than the preceding control (Fig. 9). Under normal conditions the electrical restitution curve did not have a comparable phase during which the test action potential was longer than the preceding control (Fig. 9). A similar overshoot of I_{Ca} has also been recorded in guinea-pig and dog left ventricular cells at holding potentials negative to −50 mV (Tseng, 1988). The overshoot of I_{Ca} has been attributed to Ca^{2+}-dependent facilitation of I_{Ca} due to activation of Ca^{2+}–calmodulin-dependent protein kinase and phosphorylation of a site on or near the Ca^{2+} channel (Yuan & Bers, 1994). Electrical restitution cannot, therefore,

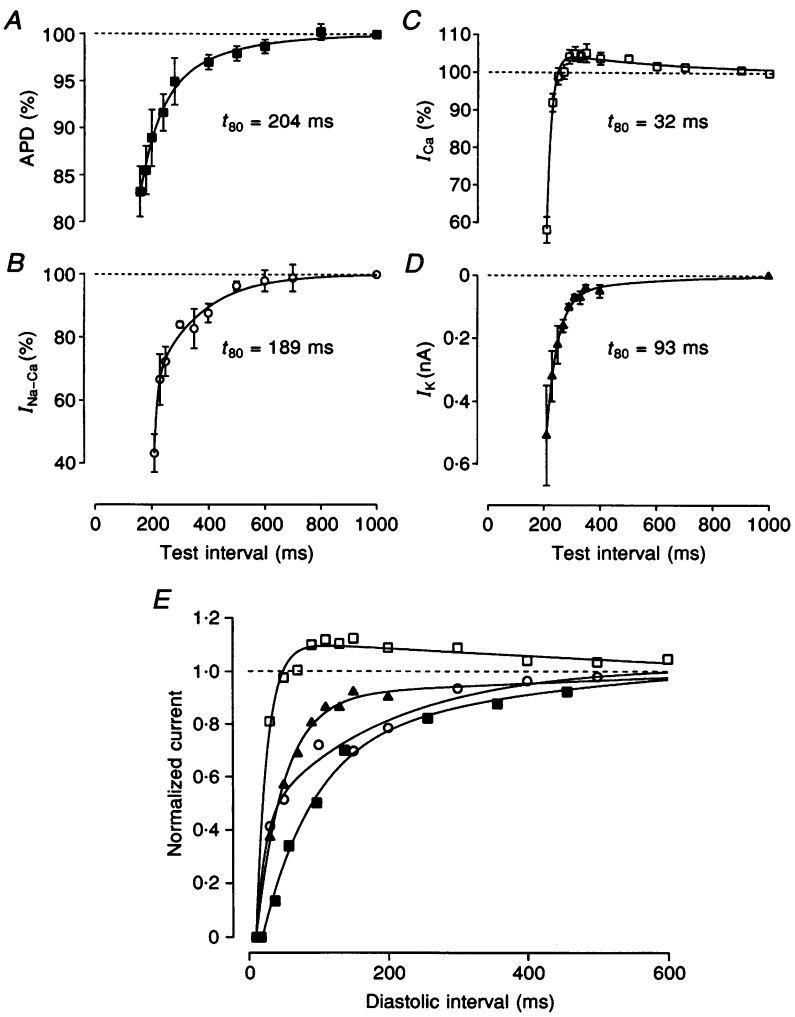

Figure 9. Comparison of the time course of electrical restitution and repriming of inward I_{Na-Ca}, I_{Ca} and I_K

A, electrical restitution curve under normal conditions (■). The duration of the test action potential (as a percentage of the preceding control) is plotted against the test interval. Means ± S.E.M. from five cells shown. Data from experiments like that in Fig. 1. *B*, plot of inward I_{Na-Ca} (○) during the test pulse against the test interval. Means ± S.E.M. from four cells shown. Same data as in Fig. 5*B*. *C*, plot of I_{Ca} (□) during the test pulse against the test interval. Means ± S.E.M. from three cells shown. From experiments like that in Fig. 2. *D*, plot of the level of current after the inactivation of I_{TO} and before further activation of I_K (▲) during the test pulse against the test interval. Note that the *y*-axis has been inverted to allow comparison of time courses. Means ± S.E.M. from three cells shown. Same data as in Fig. 3. The time to 80% recovery (t_{80}) of each curve is indicated. *E*, data from *A* to *D* normalized and superimposed. Means and curve fits only shown. The dashed lines show control values. Ferret ventricular cells.

be explained solely in terms of the reactivation of I_{Ca} (although the current will still play a role, as discussed later).

Deactivation of I_K

De Hemptinne (1971) and Hauswirth *et al.* (1972) suggested that deactivation of I_K could explain the electrical restitution curve at short test intervals (< 500 ms). However, Hauswirth *et al.* (1972) could not exclude the possibility that an inward current with a repriming time course similar to the time course of deactivation of I_K might also be involved. The t_{80} of I_K in our study was 93 ms (Fig. 9). Deactivation of I_K was, therefore, slower than reactivation of I_{Ca} (t_{80}, 32 ms), but faster than the electrical restitution curve (t_{80}, 204 ms) (Fig. 9).

Repriming of inward $I_{Na\text{-}Ca}$

In ferret ventricular cells, we have shown previously that BAPTA-sensitive current during a depolarizing pulse is likely to be inward $I_{Na\text{-}Ca}$ produced in response to the Ca^{2+} transient: the time course of the current is comparable to that of the Ca^{2+} transient; the amplitude of the current is increased by isoprenaline (which is expected to increase the Ca^{2+} transient); the current–voltage relationship of the current is appropriate for the Na^+–Ca^{2+} exchanger; and suppression of the normal mode of the Na^+–Ca^{2+} exchanger by replacement of extracellular Na^+ with Li^+ produces a similar change in current as BAPTA AM (Janvier *et al.* 1997). In the present study, the time course of repriming of the BAPTA-sensitive current after a voltage clamp pulse was almost identical to that of contraction (Fig. 5*B* and *C*). This behaviour is expected of inward $I_{Na\text{-}Ca}$ produced in response to the Ca^{2+} transient and further supports the earlier evidence above concerning the nature of the BAPTA-sensitive current. Figure 9 shows that the repriming of $I_{Na\text{-}Ca}$ is sufficiently slow (t_{80}, 189 ms) to play a role in electrical restitution (t_{80}, 204 ms). Figure 1 shows that in ferret ventricular cells restitution of contraction occurs along a similar time course to that of the electrical restitution curve. This also suggests that restitution of inward $I_{Na\text{-}Ca}$ is sufficiently slow to play a role in electrical restitution (contraction, like inward $I_{Na\text{-}Ca}$, depends on the Ca^{2+} transient).

The electrical restitution curve and the effects of block of inward $I_{Na\text{-}Ca}$, I_{Ca} and I_{TO}

An important role for inward $I_{Na\text{-}Ca}$ in electrical restitution is suggested by the effect of BAPTA AM on the electrical restitution curve. Reduction of inward $I_{Na\text{-}Ca}$ by BAPTA AM dramatically altered the electrical restitution curve in both rat and ferret ventricular cells (Figs 4 and 6). The plots of action potential duration in milliseconds against the test interval show the role of $I_{Na\text{-}Ca}$ (Figs 4*B* and 6*B*). In both species the duration of the test action potential was similar (Figs 4*B* and 6*B*) at the shortest test interval. This suggests that the inward $I_{Na\text{-}Ca}$ contributed little to action potential duration at the shortest test intervals. This is reasonable because the Ca^{2+} transient (and contraction – Fig. 1), which activates inward $I_{Na\text{-}Ca}$, is small at short test intervals. In both species the difference in action potential duration before and after the application of BAPTA AM increased as the test interval was increased; this difference, which is assumed to represent the increasing importance of inward $I_{Na\text{-}Ca}$ in the action potential, is hatched in Figs 4 and 6. In both species the increase in action potential duration ascribed to inward $I_{Na\text{-}Ca}$ occurred over a similar time course to the increase in contraction and in the ferret, at least, it occurred over a similar time course to the increase in putative inward $I_{Na\text{-}Ca}$, shown in Fig. 5*B*.

After application of nifedipine the overshoot in the electrical restitution curve (measured after application of BAPTA AM) was abolished and the curve became relatively flat (Fig. 7). This suggests that I_{Ca} generates the overshoot in the electrical restitution curve seen after the application of BAPTA AM. This is feasible, because at the normal resting potential there is a similar overshoot at comparable test intervals in the curve representing the time course of reactivation of I_{Ca} (Fig. 2). The plot of action potential duration in milliseconds against the test interval in Fig. 7*B* may show the role of I_{Ca} in the electrical restitution curve. The difference in action potential duration before and after the application of nifedipine (hatched in Fig. 7*B*) was small at the shortest test interval. This suggests that I_{Ca} makes a relatively small contribution to action potential duration at short test intervals. This is to be expected, because I_{Ca} will be small at these test intervals (Fig. 2). As the test interval was increased the difference in action potential duration increased markedly and then declined. This occurs in parallel with the expected changes in I_{Ca} (compare data at a holding potential of −80 mV in Fig. 2*C* with the hatched area in Fig. 7*B*). However, this conclusion must be regarded with caution. Application of nifedipine shortened and reduced the height of the action potential plateau (Fig. 7*A*). Such an effect on the action potential trajectory may alter the magnitude and time course of other currents (notably I_K and I_{TO}). This may influence the electrical restitution curve after the application of nifedipine and, therefore, the conclusion above.

After the application of BAPTA AM and nifedipine (to reduce or block inward $I_{Na\text{-}Ca}$ and I_{Ca}) the electrical restitution curve must reflect other currents. Two important currents are likely to be I_K and I_{TO}. Of these only I_K can explain the observed increase in action potential duration as the test interval was increased (I_{TO} will tend to shorten the action potential as explained below). Therefore, the electrical restitution curve after the application of BAPTA AM and nifedipine in Fig. 7*B* may reflect the contribution of deactivation of I_K to the electrical restitution curve. However, I_{TO} was not blocked in these experiments and, as explained below, the reactivation of I_{TO} will tend to oppose and minimize the changes in action potential duration as a result of the deactivation of I_K. In addition, the shortening of the action potential caused by the application of nifedipine will reduce the time- and voltage-

dependent activation of I_K during the control action potential and this will reduce the time-dependent changes in I_K after the action potential. Thus, although the electrical restitution curve after the application of BAPTA AM and nifedipine may reflect the deactivation of I_K, the role played by the deactivation of I_K in the electrical restitution curve under normal conditions is expected to be greater.

I_{TO} is partly responsible for phase 1 repolarization and the early notch in the ferret ventricular action potential (Campbell, Rasmusson, Qu & Strauss, 1993). The results from the present study suggest that I_{TO} also helps determine action potential duration (Fig. 8*A* and *B*). The reactivation kinetics of this current in ferret ventricular cells are strongly voltage dependent and are quicker at negative potentials (Campbell *et al.* 1993). In the study of Campbell *et al.* (1993), at a holding potential of −80 mV, I_{TO} recovered with a time constant of 30 ms; full recovery occurred after an interval of ~150 ms (comparable to a test interval in this study of 350 ms). At short test intervals a reduction of I_{TO} will tend to prolong the action potential, but as the test interval is increased I_{TO} will increase and tend to shorten the action potential. Reactivation of I_{TO}, therefore, can also explain the overshoot in the electrical restitution curve after blocking inward $I_{Na\text{-}Ca}$. This hypothesis is supported by the reduction of the overshoot when I_{TO} was blocked by 4-AP (Fig. 8). In this case, unlike blocking I_{Ca}, block of I_{TO} did not greatly alter action potential shape and, therefore, other currents are not expected to be greatly altered. We suggest that the overshoot in the electrical restitution curve after the application of BAPTA AM is the result of both I_{Ca} and I_{TO}. Removal of either current may be sufficient to abolish or greatly reduce the overshoot because the remaining current may not be sufficient to overcome the influence of I_K (which will tend monotonically to prolong the action potential as the test interval is increased). The plot of action potential duration in milliseconds against the test interval in Fig. 8*B* may show the role of I_{TO} in the electrical restitution curve. The difference in action potential duration before and after the application of 4-AP (hatched in Fig. 8*B*) was small at the shortest test interval (both before and after the application of BAPTA AM – Fig. 8*B*). This suggests that I_{TO} makes a relatively small contribution to action potential duration at short test intervals. This is to be expected, because I_{TO} will be small at these test intervals. As the test interval was increased the difference in action potential duration increased. The difference reached a maximum after a test interval of ~400 ms. Reactivation of I_{TO} is expected to be complete at this time (Campbell *et al.* 1993).

What currents determine electrical restitution? A working hypothesis

Any time-dependent current that contributes to the action potential is likely to affect the time course of electrical restitution. In the present study we have focused on the likely principal currents: I_{Ca}, inward $I_{Na\text{-}Ca}$, I_{TO} and I_K. We have argued that the hatched areas in Figs 4, 6, 7 and 8 represent the approximate contribution of I_{Ca} (Fig. 7), inward $I_{Na\text{-}Ca}$ (Figs 4 and 6) and I_{TO} (Fig. 8), and the electrical restitution curve after the application of BAPTA AM (to reduce inward $I_{Na\text{-}Ca}$) and nifedipine (to block I_{Ca}) (Fig. 7) may *roughly* reflect the contribution of I_K. These various components of the electrical restitution curve are combined in the schematic diagram shown in Fig. 10.

Immediately after the refractory period (Fig. 10*a*; shortest possible test interval) the action potential is short because of incomplete repriming of $I_{Na\text{-}Ca}$, incomplete reactivation of I_{Ca} and incomplete deactivation of I_K. As shown in Fig. 10 the roles of inward $I_{Na\text{-}Ca}$, I_{Ca} and I_K are substantial. Immediately after the refractory period (Fig. 10*a*) the action potential is short despite incomplete reactivation of I_{TO}; this will tend to prolong the action potential. As the test interval is increased from *a* to *b* the duration of the action potential increases as a result of greater reactivation of I_{Ca} and greater deactivation of I_K and despite greater reactivation of I_{TO}. However, as the test interval is increased from *b* to *c* further increase in action potential duration is the result of repriming of inward $I_{Na\text{-}Ca}$ and further reactivation of I_K. Over this range of test intervals (*b* to *c*) I_{Ca} declines and opposes the further prolongation of the action potential. Over the same range of test intervals, reactivation of I_{TO} is complete and thus I_{TO} is not a factor.

The present study was carried out on ferret ventricular cells, because we had previously shown that $I_{Na\text{-}Ca}$ helps determine action potential duration in these cells (Janvier *et al.* 1997). The results obtained may not be restricted to this species: in guinea-pig ventricular cells, $I_{Na\text{-}Ca}$ has also been shown to influence action potential duration (LeGuennec & Noble, 1994) and, therefore, $I_{Na\text{-}Ca}$ could play a role in restitution in this species. The role of $I_{Na\text{-}Ca}$ in the ventricular action potential in other species is not known. The restitution of $I_{Na\text{-}Ca}$ is presumably determined by the restitution of the Ca^{2+} transient and, because of this, intracellular Ca^{2+} dynamics is expected to influence electrical restitution.

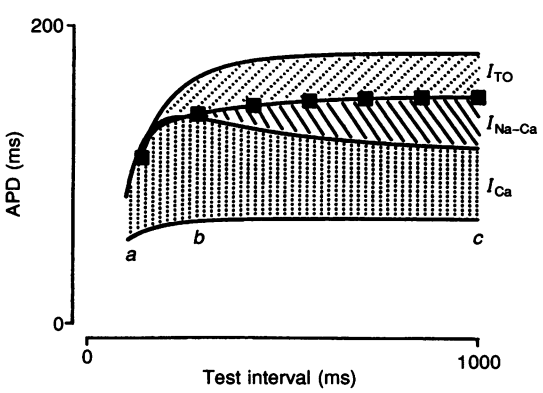

Figure 10
Schematic diagram of the various components of the electrical restitution curve.

Clinical importance of electrical restitution

Strong experimental evidence implicates increased dispersion of refractoriness as a major factor in a arrhythmogenesis (Janse & Wit, 1989). Under normal circumstances, the end of the refractory period, i.e. the time at which the cell becomes re-excitable, roughly corresponds to terminal repolarization of the action potential. Therefore increased spatial dispersion of action potential duration would favour arrhythmogenesis. In the clinical setting, arrhythmias are commonly initiated following an abrupt alteration in cycle length, e.g. following a premature beat (Janse & Wit, 1989). A premature beat would occupy a position on the early left hand part of the electrical restitution curve. As the impulse propagates across the ventricular myocardium, the time taken for propagation results in progressively longer coupling intervals (i.e. test intervals) as the wavefront progresses. Therefore, on the basis of electrical restitution, action potential duration would be expected to increase along the line of the activation path. In other words, as the action potential propagates it travels up its electrical restitution curve. The spatial dispersion of action potential duration will therefore be governed by the shape of the curve. The steeper the curve (or part of it) the greater the range of action potential duration for a given length of time in the spread of activation. However, in well-coupled cells, these action potential duration differences will be reduced as a result of electrotonic current; this is in keeping with the usually benign nature of ventricular ectopy in the absence of disease. Under pathological conditions, on the other hand, the time course of electrical restitution may play a significant if not critical role in the generation of arrhythmia. For example, any alteration in the time course of electrical restitution in an ischaemic zone of myocardium or the surrounding normal myocardium will alter the electrical gradients between the two regions and could be either pro-arrhythmic or anti-arrythmic. It has recently been shown that ischaemia results in a rapid flattening of the electrical restitution curve in the hearts of patients, which may facilitate arrhythmia formation by such a mechanism (Taggart, Sutton, Boyett, Lab & Swanton, 1996).

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