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MEMBRANE CURRENTS UNDERLYING DELAYED RECTIFICATION AND PACE-MAKER ACTIVITY IN FROG ATRIAL MUSCLE

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SUMMARY

1. A double sucrose gap method has been used to polarize and voltage clamp frog atrial muscle strips.

2. In response to steady depolarizing currents, normally quiescent strips often show pace-maker activity, and long lasting depolarization occurs when the current is terminated.

3. Voltage clamp experiments reveal the presence of two current components underlying delayed rectification.

4. The first of these components has a time constant which varies with potential and is approximately 500 msec at -90 mV. Its reversal potential usually lies between -70 and -40 mV and has always been found to be positive to the resting potential of normally quiescent fibres.

5. The time constant of the second component is extremely slow ($\tau \simeq 5$ sec at -90 mV). Its reversal potential is much more positive than that of the faster component.

6. The results confirm the presence of a component of inward current which is insensitive to tetrodotoxin (TTX), having an activation threshold about 20 mV positive to the sodium threshold. This current differs from the two components underlying delayed rectification both in its greater speed of activation and in showing inactivation. The inactivation of this TTX-insensitive current is also a fairly rapid process.

7. It is suggested that pace-making in sino-atrial muscle may depend upon the deactivation of the faster component of delayed rectification and that the TTX-insensitive inward current is also involved.

INTRODUCTION

Most of the quantitative analysis of the electrical properties of cardiac muscle in recent years has involved mammalian Purkinje fibres and comparatively little work on other types of heart tissue has been done. For

technical reasons it is difficult to attempt a quantitative study of the sinus region which forms the natural cardiac pace-maker. However, it is clear that the type of electrical response characteristic of atrial muscle bears a much closer resemblance to that of sinus muscle than does that of the Purkinje fibres. Moreover, under conditions in which atrial muscle can be induced to pace-make, the recorded action potentials are in many ways similar to the typical sinus response. We have used frog atrial muscle to begin an analysis of the slow membrane currents which may contribute to repolarization and pace-making in this region of the heart.

The first quantitative analysis of membrane properties of atrial muscle was that of Trautwein, Kuffler & Edwards (1956). Using fine external electrodes they measured changes in the time course and spatial spread of subthreshold electronic potentials applied to frog atrial trabeculae. Hence they were able to estimate the space constant and time constant in this tissue both in the resting state and after the application of acetylcholine.

Woodbury & Crill (1961) used micro-electrodes for mapping current spread in sheets of atrial muscle. From their results it was possible to make estimates of the electrical constants of the muscle membrane which were consistent with the theory of impulse spread by local electrical circuits. In principle, therefore, it should be possible to polarize large numbers of cells simultaneously since current flow between cells is not greatly restricted.

However, if a point source is used to polarize heart muscle, the resultant membrane polarization is extremely non-uniform. As Woodbury & Crill showed, the spatial decrement of voltage gives a curve which is not exponential but which can best be described by a Bessel function, so that the polarization is even more restricted to the area near the electrode than it is in single cable structures such as nerve or skeletal muscle fibres. This means that although point polarization can yield information about the resting properties of cardiac muscle, changes in membrane resistance may not be accurately estimated by this technique (see Noble, 1962).

For these reasons and also because the quantitative investigation of membrane currents requires uniform and controlled polarization, we have used a double sucrose gap technique to investigate the membrane currents in muscle fibres from frog atrium. The method is similar to that used by Rougier, Vassort, Garnier, Gargouïl & Coraboeuf (1969) and by Rougier, Vassort & Stämpfli (1968). These authors have shown that in response to a sudden depolarization there is an initial and very fast inward current, part but not all of which is blocked by TTX. This is followed by the onset of outward current exhibiting anomalous and delayed rectification.

We have investigated the characteristics of the currents underlying delayed rectification. In this first paper we describe the evidence we have obtained for the existence of two slowly rectifying current systems. Two

further papers will be concerned with the detailed individual analysis of these currents (Brown & Noble, 1969; Brown & Noble, in preparation).

METHODS

Preparation

The atria of the frog *Rana ridibunda* were isolated and opened. A thin strip of atrial muscle was then cut out. Atrial muscle is arranged in trabeculae (fine muscle bundles) and according to Barr, Dewey & Berger (1965) each trabeculum contains about 100 fusiform muscle fibres, the individual fibres being about 10 $\mu \times 200$ μ. We cut strips from the regions of the atria where the trabeculae appeared as parallel and unbranched as possible and each strip contained a small number of trabeculae, the strip dimensions being approximately 300 $\mu \times 4{\cdot}5$ mm. The final preparation was tied at each end with extremely fine nylon thread and placed in the perfusion bath.

Perfusion bath

Several perfusion baths have been used, all slight variations on the same basic pattern. These have been constructed for us in Perspex by Mr A. J. Spindler. To allow separate perfusion of different regions of the muscle strip, each bath is divided by four transverse partitions into five chambers (numbered 1 to 5 in Fig. 1). The bath consists of an upper and a lower section. These are identical except that across the partitions of the lower half runs a narrow groove (150 μ deep and 300 μ wide) for insertion of the muscle strip. To prevent communication between the chambers all surfaces and the groove were greased with silicone grease. Once the preparation was secured in the groove, the upper section of the bath was placed like a lid on top of the lower section and finally screwed down to hold it in place.

For most of the experiments described in this paper a bath was used in which the central chamber was 500 μ wide, but other baths with central chambers 400 or 200 μ wide have also been used. This dimension is important since it determines the length of muscle fibre involved in the voltage clamp circuit and this is discussed in some detail below (see *Uniformity of voltage clamp*). The internal widths of chambers 2 and 4 were both 800 μ and the Perspex partitions were 400 μ wide. Silver/silver chloride electrodes were fixed in chambers 1, 3 and 5 as shown in Fig. 1.

Chambers 2 and 4 were perfused with isotonic sucrose solution and chamber 3 with normal Ringer throughout the experiments. Chambers 1 and 5 were perfused either with normal Ringer or with high potassium Ringer (see below, *Gap potential*).

Solutions

Normal Ringer solution. NaCl 90 mM; $NaHCO_3$ 20 mM; KCl 2 mM; $MgCl_2$ 1 mM; $CaCl_2$ 1·1 mM; glucose 1 g/l., saturated with 5 % CO_2 in O_2.

High-K Ringer solution. KCl 92 mM; $KHCO_3$ 20 mM; $MgCl_2$ 1 mM; $CaCl_2$ 1·1 mM; glucose 1 g/l., saturated with 5 % CO_2 in O_2.

Tetrodotoxin (Sankyo Co., Tokyo). This was used in concentrations of 5×10^{-7} g/ml. in normal Ringer solution.

Electronic apparatus

Figure 1 is a diagram of the bath and the two alternative circuits used, one for passing square current pulses and the other for voltage clamping. Current was passed into chamber 5, and flowed to earth through one of the electrodes in chamber

3 via a 1 K resistor across which it was measured. Voltage was measured between chamber 5 and a second electrode in chamber 3. A voltage calibrator was inserted between chamber 3 and the oscilloscope. For most of the experiments described in this paper we used only one electrode in chamber 3 but the possibility of polarization of the electrode carrying the bulk of the current has encouraged us more recently to use separate voltage and current electrodes.

The stimulator gave square pulses of durations from 10 msec to 15 sec and amplitudes of up to 100 V. The oscilloscope used was a Tektronix 502A and the operational amplifier for the voltage clamp circuit was a Solartron DC amplifier Type 1023. The records were also displayed on a four-channel pen recorder (Devices Limited), whose

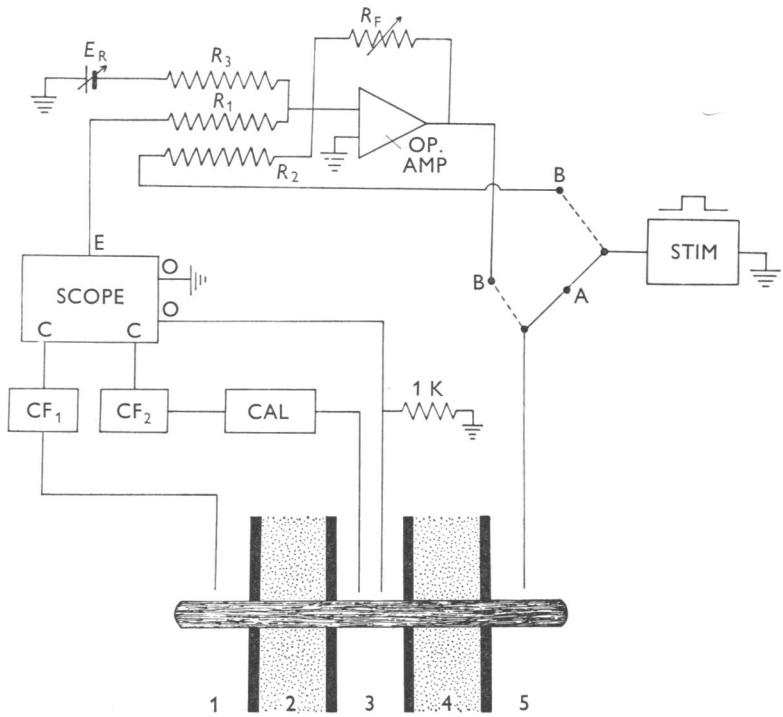

Fig. 1. Schematic diagram of bath and circuit used in polarizing and clamping atrial strips. The chambers of the perfusion bath are labelled 1 to 5. CAL, calibrator providing 10 and 1 mV steps; CF_1 and CF_2, ME 1400 cathode follower units; C, inputs to amplifier of Tektronix oscilloscope recording voltage; O, inputs to amplifier of Tektronix oscilloscope recording current; E, cathode follower output from Tektronix oscilloscope 502A (scope) giving voltage output proportional to the voltage input at C; OP. AMP, Solartron operational amplifier Type 1023; E_R, variable voltage determining holding potential; R_1, R_2 and R_3, input resistors determining current flow into OP. AMP; R_F, feed-back resistor determining the gain of the voltage clamp circuit; STIM, square voltage stimulator giving pulses of duration 10 msec to 15 sec and amplitude of up to 100 V; A, position of switches for constant current experiments; B, position of switches for voltage clamp experiments; 1 K, resistance monitoring current flow.

frequency response was flat up to about 50 Hz. The records shown in this paper are photographed pen recordings.

Gap potential

At the start of an experiment chambers 1, 3 and 5 were perfused with normal Ringer. Once the action potential no longer propagated through the sucrose chambers, i.e. the external resistance of the preparation in the sucrose gap had reached a sufficient magnitude to prevent extracellular current flow, the solutions perfusing chambers 1 and 5 were changed to high potassium (112 mM-K^+) Ringer. This change caused a negative going base-line shift (see Fig. 2). Provided that the external

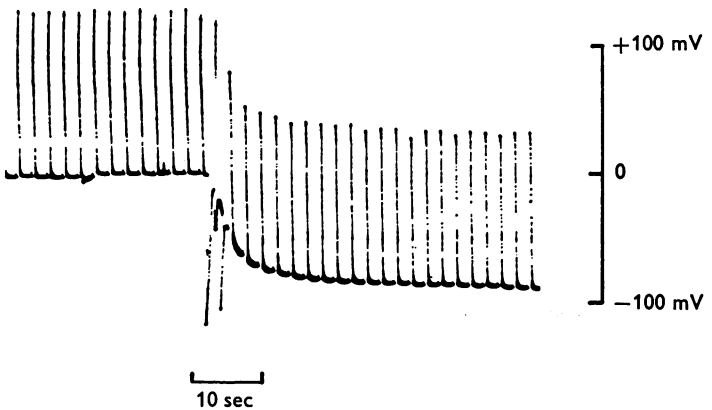

Fig. 2. Voltage record taken during change of fluid perfusing the outer chambers of the bath (chambers 1 and 5) from normal Ringer (2 mM-K^+) to high potassium Ringer (112 mM-K^+). The negative going base-line shift (the gap potential) approximates to the resting potential of the muscle fibre membranes. In this case the initial base line has been made to coincide with the zero level on the voltage calibration. The magnitude of this gap potential is about −80 mV.

resistance of the preparation in the sucrose gaps is great enough, this 'gap potential' is theoretically equal to the average resting potential of the fibres in the preparation. It can be compared to an injury potential where there is no short circuiting of uninjured regions of the membrane.

The values of gap potentials we recorded lay mainly between 70 and 90 mV and were associated with action potentials ranging between 90 and 130 mV.

Our gap potential measurements appear to agree well with values of resting potentials recorded intracellularly from atrial muscle by a number of workers. For example, Glitsch, Haas & Trautwein (1965) recorded from frog atrium resting potentials of $75 \pm 4{\cdot}6$ mV and action potentials of $89 \pm 5{\cdot}2$ mV. We have therefore used our gap potential measurements as a guide to the general state of the preparation and apparatus. For example, a preparation giving a large action potential but only a small gap potential would suggest some fault in the electrical recording or perfusion systems. When the two values are in good correspondence we consider the gap potential an estimate of the resting potential.

In addition to allowing us to estimate the resting potential of the preparation in this way the change to high potassium Ringer in chambers 1 and 5 gave a further advantage. Current should, under these conditions, enter the preparation more

easily since the conductance is usually substantially increased when the membrane is depolarized. This should, in turn, reduce the proportion of current flowing externally and so increase the effectiveness of the voltage clamp.

Uniformity of voltage clamp

Estimates of the space constant of atrial muscle vary. Trautwein *et al.* (1956) give values between 230 and 410 μ for frog atrial muscle strips. The widths of the clamp chambers used varied between 500 and 200 μ and since a small spread of silicone grease into this gap from either end was almost inevitable as the two halves of the bath were brought together, the region of muscle exposed to the voltage clamp currents would be less than the width of the chamber and probably varied between about 0·5 and 1·0 space constant.

The condition that all the intracellular current must leave the cells within the region of the gap requires that the strip within the gap be treated as an open-circuited short cable (cf. Weidmann, 1952; Adrian & Freygang, 1962). Assuming that this cable is linear, lengths between 0·5 and 1·0 space constant would be subject to voltage decrements of about 10 and 30 % respectively.

Although the strips were cut so that they showed as little branching as possible, some were certainly more geometrically favourable for clamping than others. This became evident when the squareness of the voltage trace was considered and only those preparations giving a sharply defined voltage response have been used for analysis. The larger gaps, although unsuitable for clamping the fast sodium current, were favourable for studying the smaller slow current changes in which we were interested. We noticed no difference between the delayed responses in gaps of different widths.

Since many of the results described in this paper were obtained using a 500 μ gap it is important to estimate the error due to voltage decrement which this gap might produce, for example, in establishing the value of current thresholds. The effect will be to reduce the threshold, but since the voltage recorded is the average of the total voltage change, this reduction will probably be not more than one half of the maximum 30 % already referred to. The likely maximum error therefore must be of the order of 15 %.

Nomenclature

Resting potential. Our recorded gap potential values are sufficiently similar to intracellularly recorded values of resting potentials for us to take them as an estimate of the true resting potential of the fibre membranes. Throughout this paper, however, we refer to 'resting potential' in inverted commas to emphasize that our measurement is not direct.

Holding potential. This is the potential to which the preparation is being clamped over a period of time, measured in mV above or below zero potential. The holding potential is set by dialling a steady current on R_3 (Fig. 1). Depolarizations and hyperpolarizations of up to 15 sec duration can be superimposed on the holding potential.

Deactivation. This term refers to the switching off of a current by a potential change which is in the opposite direction to that which switches it on, i.e. deactivation is the reverse of activation.

Inactivation. This refers to the switching off of a current at the same voltage as switches it on. Inactivation is thus not the opposite of activation but an additional process.

RESULTS

Pace-making in atrial fibres

Many of the trabeculae which were dissected from the atria, although naturally quiescent, could be induced to pace-make in response to depolarzing current pulses of relatively low magnitude and several seconds duration. For such activity there were invariably upper and lower limits of membrane potential above and below which pace-making would not occur

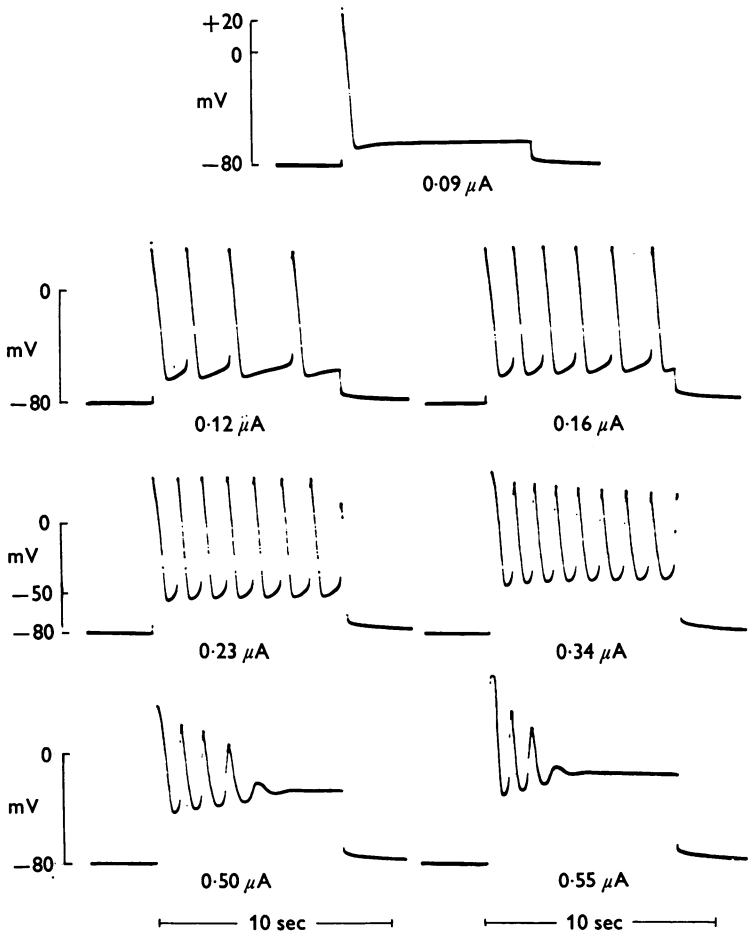

Fig. 3. Responses of an atrial strip to long (7·5 sec) pulses of depolarizing current of various magnitudes. (The current producing a given response is indicated beneath the corresponding record.) Maximum pace-making frequency was developed in this preparation by depolarization to about −50 mV.

and an optimum level of depolarization at which maximum frequency was developed (see Fig. 3). This optimum potential was frequently found to be around -50 mV.

One of the preparations showed spontaneous activity which persisted even after the addition of high potassium Ringer solution to the outer chambers of the bath. In this case the activity must have been generated

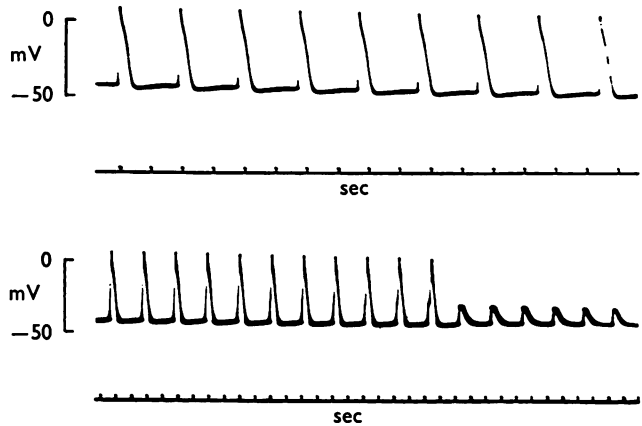

Fig. 4. Record of a spontaneously active preparation. The voltage scale has been placed so as to correspond exactly with the actual recording. (Action potential 50 mV; 'resting potential' $-42{\cdot}5$ mV. Overshoot 7·5 mV.) The lower trace shows the change which took place following the addition of TTX (5×10^{-7} g/ml.) to the normal Ringer perfusing the central chamber.

by the portion of the strip lying in the central chamber. In this preparation (which probably included a section of sinus muscle) the action potentials were small and the 'resting potential' was low (see Fig. 4. AP + 50 mV; 'RP' $-42{\cdot}4$ mV).

We investigated the effect of TTX (5×10^{-7} g/ml.) on this spontaneously beating preparation. Rougier, Vassort, Garnier, Gargouïl & Coraboeuf, (1969) have already shown that TTX in this concentration successfully eliminates the rapid inward sodium current. As can be seen from the record shown in Fig. 4, this concentration of TTX causes the action potential to be reduced in amplitude but not completely abolished. It would appear that the changes underlying pace-maker activity are unaffected by TTX in doses of this magnitude. Evidence in support of this will be described when the results of voltage clamp analysis are discussed.

Effect of prolonged depolarizing currents on atrial fibres

When atrial fibres are subjected to constant depolarizing currents of large magnitude and long duration, a considerable post-stimulus response

appears during which the voltage returns only extremely slowly to the resting level (see Fig. 5). The same effect, very much reduced in magnitude, is seen following the smaller long depolarizing currents effective in inducing pace-making.

This result might arise from the very slow activation of a membrane current which is inward going in the range of the resting potential. Voltage clamp experiments are needed to confirm this suggestion.

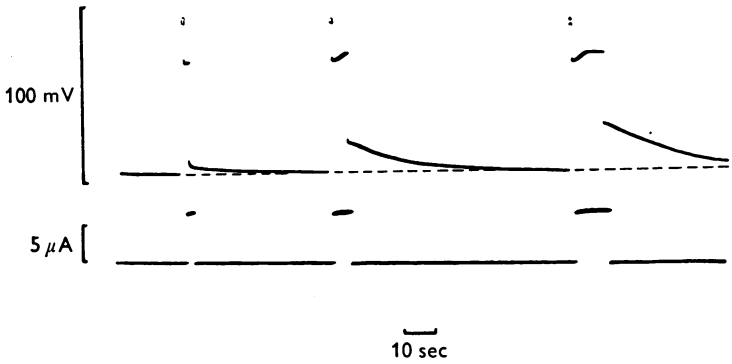

Fig. 5. Record of voltage changes during and after application of long current pulses to an atrial muscle preparation. Note the persistent depolarization after the end of the current pulse which increases in magnitude with the duration of the pulse.

Preliminary voltage clamp analysis

Voltage clamp records were obtained from fibres held in gaps 500, 400 and 200 μ wide. No differences were found in the behaviour of preparations held in gaps of different widths. From all the preparations, including those which showed little or no tendency to pace-make in response to depolarizing current pulses, consistent results of the type described below could be obtained.

Figure 6 shows the response from a preparation held in a 200 μ gap and depolarized from a holding potential of -45 to -10 mV for two different durations. The initial 'resting potential' of this preparation was -74 mV and the action potentials were 90 mV. It showed vigorous pace-making properties when constant current pulses were applied at the beginning of the experiment.

As can be seen in Fig. 6, when the fibre is repolarized to -45 mV following a clamp pulse of 3 sec a tail of outward current occurs. However, another depolarizing pulse of the same magnitude but of much longer duration (14·3 sec) is followed by a larger inward current tail on repolarization.

In the records shown in Fig. 7 the holding potential was -90 mV and

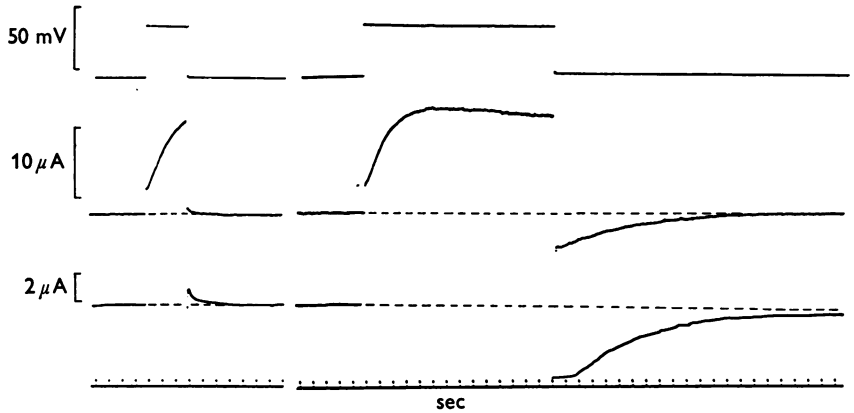

Fig. 6. Voltage clamp records of responses to two depolarizing pulses of different durations from a holding potential of −45 mV to one of −10 mV. Top trace, voltage record; middle trace, low gain current record; bottom trace, higher gain current record showing current tails only. Outward current upwards.

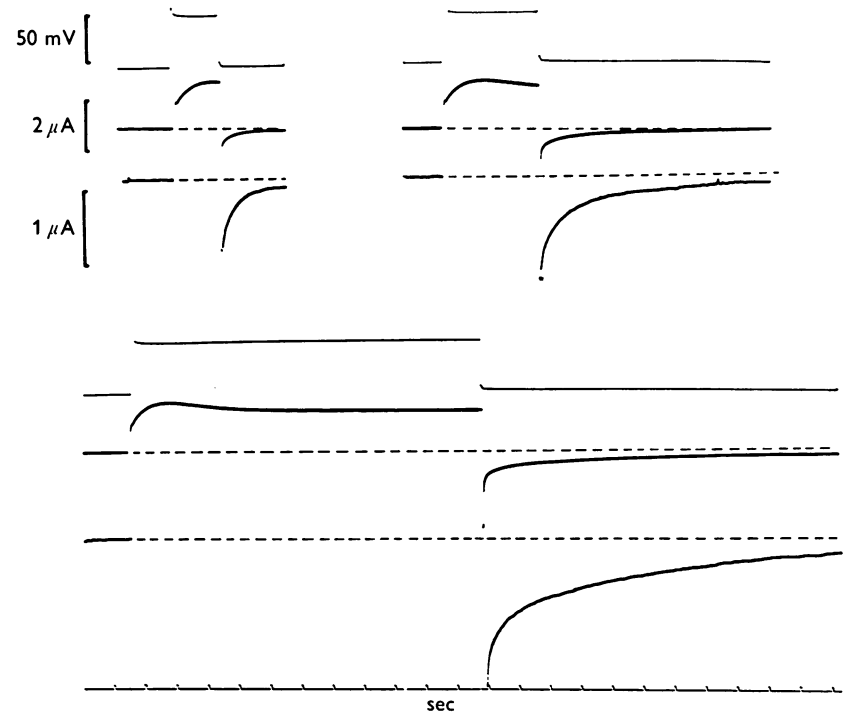

Fig. 7. Voltage clamp records of responses to three depolarizing pulses of different durations in which the potential is changed from −90 to −20 mV. Top traces, voltage records; middle traces, low gain current records; lower traces, high gain current records showing current tails only. Outward current upwards.

depolarizing pulses of +70 mV were used. The current tails on repolarization after pulses of durations of 1·6, 3·3 and 11·8 sec are shown. All three of these tails are of inward current and at this holding potential whatever duration of depolarizing pulse was used we never observed an outward current tail.

These results suggest the existence of two current systems which we will refer to as 1 and 2, differing in their reversal potentials and in their time course of activation. The holding potential at which the preparation was clamped in Fig. 6 (−45 mV) must be positive to the reversal potential of system 1 and negative to that of system 2, where the time course of activation of 1 is much faster than that of 2. Thus the shorter depolarizing pulse gives an outward current tail on repolarization and the longer pulse gives an inward current tail. The holding potential of the records shown in Fig. 7 (−90 mV) must be negative to the reversal potential of all slow systems, since only inward current tails can be recorded whatever duration of depolarizing clamp pulse is selected.

The decline in membrane current which is an obvious feature of the current records during the longer clamp pulses shown in both Figs. 6 and 7 could well be attributed to the activation of system 2, provided that the reversal potential (E_{rev}) of this system is positive to the potentials to which the preparations were clamped (−10 mV in Fig. 6 and −20 mV in Fig. 7). If this is so it follows that −10 and −20 mV are closer to $(E_{rev})_2$ than are the holding potentials (−45 and −90 mV) for the magnitude of the fall in the clamp currents is considerably less than the magnitude of the inward current generated when the preparation is returned to the holding potential.

More quantitative evidence for distinguishing between the two current systems underlying delayed rectification can be obtained by analysing current tails of the kind shown in Fig. 7 using semi-log plots of current against time. Figure 8*A* and *B* show the results of this analysis on the first and third of the current tails shown in Fig. 7. The fact that in Fig. 8*A* a straight line graph is obtained means that the relationship between i and t must be that of a simple exponential, i.e. $i_1 = i_{1_0} e^{-t/\tau_1}$, or $\ln i_1 = -t/\tau_1 + \ln i_{1_0}$, where i represents current, t time, 1 denotes current system 1, and τ_1 is the time constant of current component 1 at this holding potential. The value of τ_1 will therefore be given by the slope of this line. Our result, obtained by plotting log i against t, gives us a value for τ_1 of 450 msec at −90 mV.

In Figure 8*B* (lower curve) it is clear that the simple exponential relationship between ln i and t does not hold in this case. It might, however, be postulated that this curve obeys a double exponential relation. All the inward current due to the presence of component 1 will be deactivated within a brief period and the remaining current must be attributed to

component 2 alone. Assuming that this current also declines exponentially, it must be the case that, following total deactivation of 1, the curve will approximate to a linear relation such that

$$\ln i_2 = t/\tau_2 + \ln i_{2_0}.$$

Clearly this line upon extrapolation to $t = 0$ should supply a series of values between $t = 0$ and $t = x$ (x being the time at which all component 1 is deactivated), which, when subtracted from i_{total}, will give values of i_1 at time t.

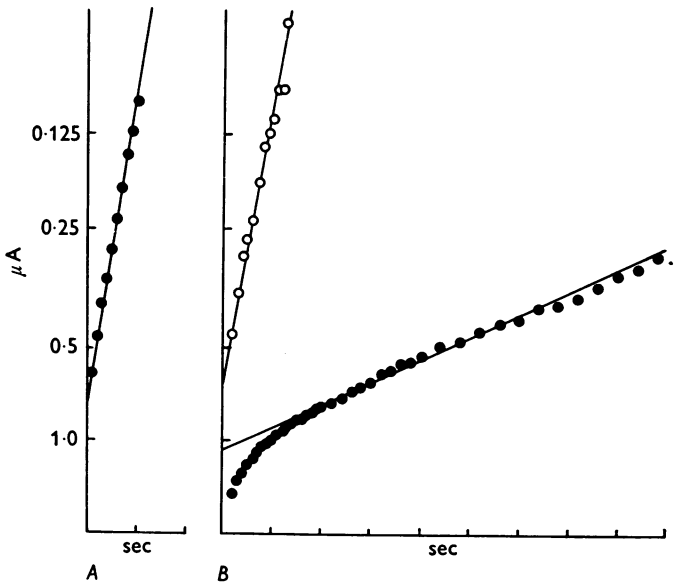

Fig. 8. Semi-log plots of current against time for two of the high gain current tails shown in Fig. 7. *A*, semi-log plot of current against time for the current tail following the first depolarizing pulse (1·6 sec duration) shown in Fig. 7. *B*, ●, semi-log plot of total current against time for the current tail following the third depolarizing pulse (11·8 sec duration) shown in Fig. 7; ○, semi-log plot of i_1 against time. Values of i_1 obtained as explained in the text.

A second estimate of the relationship between log i and t for component 1 can thus be obtained (see Fig. 8*B*, upper graph). This line is virtually identical with that shown in Fig. 8*A*. Here $\tau_1 = 500$ msec, while $\tau_2 = 5{\cdot}87$ sec. A similar analysis using the second of the three results shown in Fig. 7, although not illustrated here, gives a value of 575 msec for τ_1, sufficiently close to the other values obtained to be within the bounds of experimental error. These results are important in several respects. First, they show that both slowly rectifying systems obey first-order kinetics resulting in simple exponential current changes which suggests that they

might be analysed in terms of the Hodgkin–Huxley model for the behaviour of voltage-dependent membrane channels (Hodgkin & Huxley, 1952). Here $1/\tau = \alpha+\beta$, α and β being the rate constants for the opening and closing of the membrane channels at a given potential. Secondly, the results differentiate fairly conclusively between the possible mechanisms underlying system 2. Apart from the possibility already mentioned that this is a voltage-dependent current system with a very positive activation range, the result obtained when depolarizing current was applied to the preparations (see Fig. 5) could have arisen from the accumulation of K^+ ions in restricted extracellular spaces (cf. McAllister & Noble, 1966). It is also possible that both effects may occur and some of our later results are consistent with this possibility (see *Note added in proof*).

Thirdly, it is now apparent that the systems are not governed by inactivation variables. The fall in amplitude during the clamp pulses which appears in the current records shown in Fig. 6 and again in Fig. 7 must be due to the onset of inward current derived from system 2. It could not arise from inactivation of system 1, for then although deactivation of 1 when the clamp returns the membrane to the holding potential would still occur the resulting inward current would be much smaller than it is in Fig. 8*B*.

In order to attempt a quantitative analysis of system 1, the fast current components representing activation and inactivation of the sodium system should be removed. This is necessary since the reversal potential of system 1 generally lies between about -70 and -40 mV, at which potentials the sodium transients tend to be very large. We have therefore investigated the effects of tetrodotoxin on the voltage clamp currents. Figure 9 shows current records obtained using 40 mV depolarizing pulses from a holding potential of -30 mV. At this potential the fast sodium current is largely inactivated. Virtually no change was observed on application of 5×10^{-7} g/ml. TTX. Both records show there is activation of component 1 during the 1·4 sec pulse (which was too short to activate component 2). On repolarizing to -30 mV, component 1 deactivates and, since this potential is positive to the reversal potential of system 1, the result is a tail of outward current. It is clear from this result that TTX in doses sufficient to block the fast sodium current has no effect on component 1. This result is in agreement with Rougier, Vassort & Stämpfli (1968), who showed that delayed rectification is present in atrial muscle perfused with TTX solutions.

Figure 10 shows current records obtained from a preparation voltage-clamped in the presence of TTX. A holding potential of -90 mV was used throughout. In normal Ringer depolarizing pulses from this same holding potential gave current responses which included fast inward transient of up to 1 μA in amplitude.

The transients were rapidly abolished on application of TTX ($5 \times 10^{-$ g/ml.) and the records in Fig. 10 show the currents which remained and were obtained in response to short (600 msec) clamp pulses of various magnitudes of depolarization. These short pulses are followed on repolarization to -90 mV by inward current tails which we attribute to the deactivation of component 1.

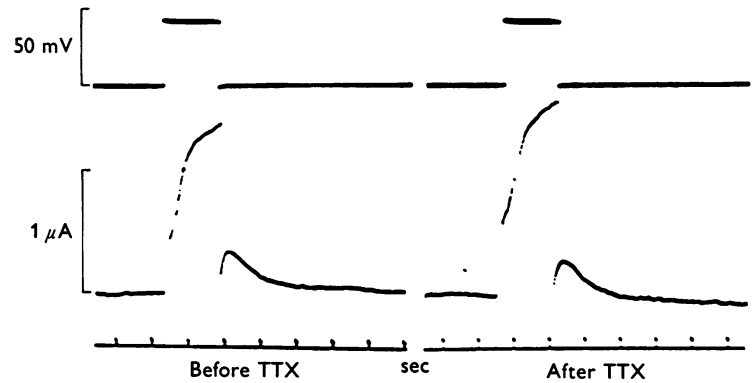

Fig. 9. Voltage clamp records before and after the application of TTX (5×10^{-7} g/ml.) in normal Ringer. Above, voltage record. Two identical clamp pulses are shown which changed the potential from -30 mV (the holding potential) to $+10$ mV. Pulse duration 1·4 sec. Below, current records. The two records shown were selected at random from large numbers of responses to identical pulses. The fact that the total current flow before TTX appears from the two records shown to be slightly smaller than the current flow after TTX is probably not significant; the *slow* changes in current flow were unaffected.

In response to small depolarizations (*a* to *d*) in Fig. 10, the current change during the depolarizing clamp pulses is also in the inward direction. Potentials up to about -50 mV must therefore be negative to $(E_{rev})_1$ in this preparation. During stronger depolarizations (*e* to *h*), however, the pattern of current flow changes. The slow current becomes outward during the pulse and it is preceded by a rapidly inactivated inward current.

Figure 11 shows the current–voltage relations obtained from the records illustrated in Fig. 10. The diagram at the top of the Figure shows how these measurements were made. The continuous horizontal line indicates the current zero obtained before the voltage clamp was applied and all the current measurements were made with respect to this zero. The continuous line in the graph (filled circles) shows the current–voltage relation obtained by measuring the currents at the end of each pulse. It can be seen that this current–voltage relation shows inward going rectification.

The open circles show the current–voltage relation immediately after

the capacity current has subsided. Beyond about −50 mV this relation curves steeply in the inward direction. Most of this inward change may be attributed to an inward current which is insensitive to TTX and whose threshold is about 20 mV positive to the threshold for the fast sodium current. Such a current has been described by Rougier, Vassort, Garnier, Gargouïl & Coraboeuf (1969), who attribute it, at least in part, to calcium ions. However, this component inactivates relatively rapidly so that

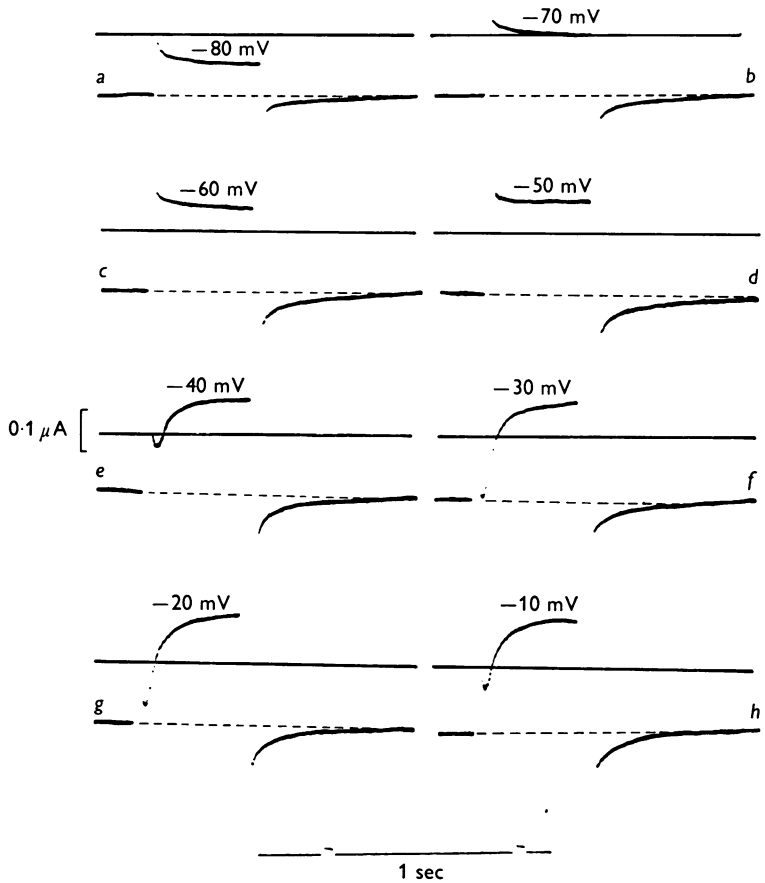

Fig. 10. Current records obtained in response to voltage clamp pulses in the presence of TTX (5×10^{-7} g/ml.). Holding potential −90 mV; pulse duration 600 msec. Zero current indicated by horizontal continuous line on each record. The depolarizing clamp pulses were increased in 10 mV increments; above each record is written the voltage level (mV) to which the pulse depolarized the preparation. Records *a* to *d*, current change during the clamp pulse is inward (downwards); *e* to *h*, current change during the pulse is now outward and is preceded by a faster inward current which is rapidly inactivated.

ideally the changes in component 1 could still be estimated in its presence. In practice only the records *f* and *g* were analysable in this experiment. This was done in the following way. The slower current changes were extrapolated back to the beginning of the pulse, assuming these changes to be roughly exponential. (This was not done in the case of records *e* and *h* since the shape of the slow current change during the pulse did not allow a simple extrapolation to be estimated.) The values obtained are represented by triangles in Fig. 11. The resulting curve is given by the

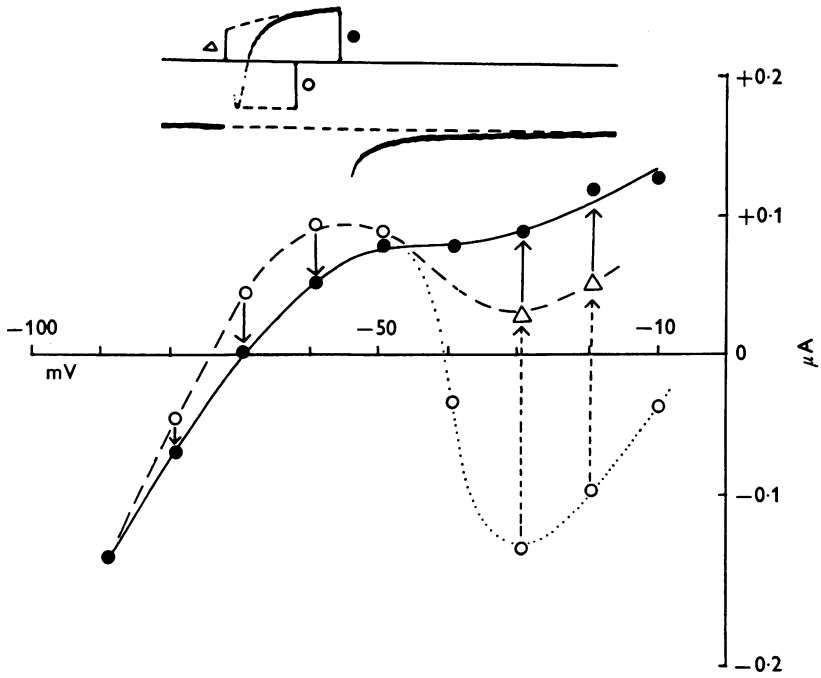

Fig. 11. Current–voltage relations obtained from records shown in Fig. 10. Above, one of the records from Fig. 10 showing how the current measurements plotted below were made. The continuous horizontal line represents zero current level, i.e. the position of the current trace in the absence of the voltage clamp. ●, magnitude of current at end of pulse; ○, magnitude of current at beginning of pulse, △, estimates of current at beginning of pulse obtained by extrapolation of the slow current change. Below, dashed line represents the current–voltage relation before activation of the slow outward current but not including the TTX-insensitive inward current; dotted line represents the current–voltage relation including the TTX-insensitive current; continuous line represents the current–voltage relation at the end of the pulse (600 msec); dashed arrows show the direction of current change due to inactivation of the TTX-insensitive inward current; continuous arrows show direction of current change due to activation of slow current. Holding potential −90 mV. Sodium threshold before application of TTX approximately −65 mV.

interrupted line which thus indicates the initial value of the current before activation of either component 1 or of the TTX-insensitive inward current.

In order to illustrate the temporal changes in the current–voltage relations which occur during the depolarizing pulses, the direction of the current change is indicated by arrows. Changes in the slow component 1 are indicated by the continuous arrows and it can be seen that this change reverses direction from inward to outward as the potential becomes positive to about − 50 mV. This result is consistent with previous ones in showing that the reversal potential of component 1 is negative to −30 mV but usually positive to the 'resting potential'. The interrupted arrows show the direction of the current changes caused by the rapid inactivation of the TTX-insensitive inward current.

The presence of this TTX-insensitive current which is sufficiently large to produce a region of net inward current in the current–voltage relations implies that, under suitable circumstances, it should be possible to produce regenerative responses in the presence of TTX. This was found to be the case in this preparation, although much larger stimuli were required in the presence of TTX than in its absence. Rougier, Vassort, Garnier, Gargouïl & Coraboeuf (1969) have also shown that regenerative depolarizations may be initiated in the presence of TTX.

DISCUSSION

The results described in this paper have established that at least two current-carrying systems underlie delayed rectification in the frog atrium. The first system (1) obeys relatively fast kinetics (time constant approximately 500 msec at −90 mV) and has a reversal potential between −70 and −40 mV. In our normally quiescent preparations reversal of this current takes place at potentials which are invariably positive to the 'resting potential' of about −80 mV.

The changes responsible for system 2 take place only very slowly (time constant approximately 5 sec at −90 mV). The reversal potential for this current usually lies in the range −30 to 0 mV. At potentials negative to $(E_{rev})_2$ this system must therefore carry an inward, depolarizing current. The presence of such a current, which deactivates only very slowly on repolarization, may therefore account for the 'after-depolarization' which we have observed in preparations subjected to constant depolarizing currents of several seconds duration (Fig. 5). During a normal atrial action potential, which lasts for only a few hundred msec and is usually triangular in shape, system 2 would not be significantly activated.

Delayed rectification in mammalian Purkinje fibres has also been shown to depend on more than one current-carrying system (Noble & Tsien, 1968, 1969) and it is of interest to compare our results with those on

Purkinje fibres. Component 1 resembles the current labelled i_{x_1} by Noble & Tsien. This current also reverses at a potential somewhat positive to the resting potential and is activated fairly quickly. Component 2 resembles Noble & Tsien's i_{x_2} in having a much more positive reversal potential and in activating extremely slowly. Of especial interest, however, is the absence in frog atrium of a pure potassium current underlying pace-making comparable to the s system in Purkinje fibres (Noble & Tsien, 1968). This explains why, in atrial fibres with large resting potentials, there is no tendency towards spontaneous activity. At large membrane potentials, the current flowing in system 1 will be inward and the deactivation of this current will lead to further hyperpolarization of the membrane and not to the depolarization which is required for pace-maker activity. However, if the membrane is artificially depolarized with applied current to a point positive to $(E_{rev})_1$, pace-maker activity should become possible. Our results show that in many atrial fibres this is indeed the case and steady depolarization is itself sufficient to induce pace-maker activity (see Fig. 3). It is also significant that spontaneous activity in atrial and sinus muscle occurs in fibres with low membrane potentials (see Fig. 4 and Hutter & Trautwein, 1956; Toda, 1968). We may reasonably suppose, therefore, that the deactivation of system 1 is involved in pace-maker depolarization in spontaneously active sino-atrial fibres, and that the main difference between spontaneously active (sinus) muscle and normally quiescent (atrial) muscle depends largely upon the existence of small variations in the total steady-state current determining the resting potential.

Another factor involved in pace-making will be the TTX-insensitive inward current shown in Figs. 10 and 11. This is activated over the appropriate voltage range (from about -50 to -30 mV). Although it seems reasonable to suppose that this current is involved in those cases where spontaneous activity may be recorded in the presence of TTX (see Fig. 4), the pace-maker depolarization itself would have to be initiated by the deactivation of an outward current, presumably component 1. In the case of the preparation which gave the results shown in Figs. 10 and 11, it can be seen that this deactivation would not alone be sufficient to produce a net inward current at any time and consequently spontaneous activity would not be expected.

The fact that both slow current systems in the frog atrium follow simple exponential time courses in response to voltage clamp steps suggests that the kinetics governing these changes are first order. Moreover, neither system is affected by concentrations of TTX sufficient to block the sodium conductance. Provided that it is possible to subtract or eliminate the rapidly inactivated TTX-insensitive inward current (see Figs. 10 and 11, and Rougier, Vassort, Garnier, Gargouïl & Coraboeuf, 1969) it should be

possible to make a complete analysis of the kinetics and other properties of both system 1 and system 2 without significant interference from other membrane currents. We shall describe such analyses in subsequent papers.

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REFERENCES

ADRIAN, R. H. & FREYGANG, W. H. (1962). The potassium and chloride conductance of frog muscle membrane. *J. Physiol.* **163**, 61–103.

BARR, L., DEWEY, M. M. & BERGER, W. (1965). Propagation of action potentials and the structure of the nexus in cardiac muscle. *J. gen. Physiol.* **48**, 797–823.

BROWN, H. F. & NOBLE, S. J. (1969). A quantitative analysis of the slow component of delayed rectification in frog atrium. *J. Physiol.* **204**, 737–747.

GLITSCH, H. G., HAAS, H. G. & TRAUTWEIN, W. (1965). The effect of adrenaline on the K and Na fluxes in the frog's atrium. *Arch. exp. Path. Pharmak.* **250**, 59–71.

HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**, 500–544.

HUTTER, O. F. & TRAUTWEIN, W. (1956). Vagal and sympathetic effects on the pacemaker fibers in the sinus venosus of the heart. *J. gen. Physiol.* **39**, 715–733.

MCALLISTER, R. E. & NOBLE, D. (1966). The time and voltage dependence of the slow outward current in cardiac Purkinje fibres. *J. Physiol.* **186**, 632–662.

NOBLE, D. (1962). The voltage dependence of the cardiac membrane conductance. *Biophys. J.* **2**, 381–393.

NOBLE, D. & TSIEN, R. W. (1968). The kinetics and rectifier properties of the slow potassium current in cardiac Purkinje fibres. *J. Physiol.* **195**, 185–214.

NOBLE, D. & TSIEN, R. W. (1969). Outward membrane currents activated in the plateau range of potentials in cardiac Purkinje fibres. *J. Physiol.* **200**, 205–231.

ROUGIER, O., VASSORT, G. & STÄMPFLI, R. (1968). Voltage clamp experiments on frog atrial heart muscle fibres with sucrose gap technique. *Pflügers Arch. ges. Physiol.* **301**, 91–108.

ROUGIER, O., VASSORT, G., GARNIER, D., GARGOUÏL, Y-M. & CORABOEUF, E. (1969). Existence and role of a slow inward current during the frog atrial action potential. *Pflügers Arch. ges. Physiol.* **308**, 91–110.

TRAUTWEIN, W., KUFFLER, S. W. & EDWARDS, C. (1956). Changes in membrane characteristics of heart muscle during inhibition. *J. gen. Physiol.* **40**, 135–145.

TODA, N. (1968). Influence of sodium ions on the membrane potential of the sino-atrial node in response to sympathetic nerve stimulation. *J. Physiol.* **196**, 677–691.

WEIDMANN, S. (1952). The electrical constants of Purkinje fibres. *J. Physiol.* **118**, 348–360.

WOODBURY, J. W. & CRILL, W. E. (1961). On the problems of impulse conduction in the atrium. In *Nervous Inhibition*, ed. FLOREY, E. Oxford: Pergamon Press.

Note added in proof

Further experiments performed since this paper was submitted have shown that depolarization is frequently followed by an inward current which decays even more slowly than component 2 and which does not obey first order kinetics. This current might result from extracellular potassium accumulation.