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A QUANTITATIVE ANALYSIS OF THE SLOW COMPONENT OF DELAYED RECTIFICATION IN FROG ATRIUM

BY HILARY F. BROWN AND SUSAN J. NOBLE
From the University Laboratory of Physiology, Oxford

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SUMMARY

1. The slow component of delayed rectification in the atrial muscle membrane of *Rana ridibunda* has been analysed quantitatively using a voltage-clamp technique.

2. It is shown that the current is proportional to a variable which obeys first-order kinetics. At negative potentials the time constant of this process is very long (τ = 3 sec at −55 mV) but it becomes faster at positive potentials (τ = 1·85 sec at +25 mV).

3. In the steady state the degree of activation is a sigmoid function of potential. The activation threshold varies substantially between preparations but is usually negative to −30 mV.

4. The instantaneous current–voltage relation is nearly linear and its reversal potential most frequently lies between zero and −30 mV.

5. The properties of this current system resemble those of the current system i_{x_2} found in mammalian Purkinje fibres.

INTRODUCTION

In a previous paper we have distinguished between two current systems underlying delayed rectification in atrial muscle of the frog *Rana ridibunda* (Brown & Noble, 1969). In this paper we shall describe the analysis of the more slowly activated of these two systems, that which we originally referred to as system 2. This system is simpler to analyse than is system 1, because in some atrial preparations the range of potential over which it becomes activated is sufficiently positive for little interference from other membrane currents to appear on the relevant voltage clamp records.

As the results in this paper will show, the characteristics of system 2 are in many ways similar to those of one of the current systems found by Noble & Tsien (1969) in mammalian Purkinje fibres and labelled by them i_{x_2}. For the sake of conformity, therefore, we shall refer in future to system 2 as i_{x_2}.

METHODS

Atrial trabeculae were dissected from the heart of *Rana ridibunda* and investigated using a double sucrose gap technique. Details of the preparation and method have been given earlier (Brown & Noble, 1969).

Nomenclature. For the definition of some of the terms used, e.g. holding potential, deactivation, 'resting potential' see Brown & Noble (1969).

RESULTS

The quantitative analysis of the current system i_{x_2} which is described in this paper was carried out on an atrial preparation which showed a 'resting potential' of -94 mV and an action potential of 130 mV. In addition several partial analyses have been performed on similar preparations, while estimates of the reversal potential and activation range of i_{x_2} have been observed in numerous experiments not specifically designed to study this system in detail.

To establish the range of membrane potentials over which the current became activated in this preparation, the following simple experiment was performed and is illustrated in Fig. 1*a*. The voltage clamp was set to give a holding potential of -45 mV. The membrane potential was then progressively increased in a positive direction by 8·5 mV steps. As can be seen the first two step changes produced no detectable slow current change at the amplification used. After this each subsequent voltage increment produced a time-dependent outward current flow, which initially increased with increasing potential and then began to diminish until, at a membrane potential of $+35$ mV, very little current remained to be activated.

Since the duration of each step was insufficient to allow a steady state to be reached, it is not possible to deduce the total magnitude of the slow current which may be activated at each potential. Nor was the current amplification great enough to give a precise value for the activation threshold of i_{x_2} although it is sufficient to show that the current becomes a steep function of voltage beyond about -30 mV.

The voltage dependence of the slow current is demonstrated somewhat differently in Fig. 1*b*. Here the initial holding potential of -45 mV was changed immediately to one of $+35$ mV and then decreased again in steps which were again 8·5 mV, apart from the last step change in this series which was 17 mV. This time each step was accompanied by slow deactivation of some of the current activated during the initial voltage change. Again the magnitude and rate of this decrease depends upon the membrane potential, but the steady state is not reached until the original holding potential is re-established.

It will be noticed that in Fig. 1*b* the initial positive going clamp pulse

of 80 mV results first in a sharp jump in the current record and then in a slow outward current which gradually reaches its maximum value after several seconds. This slow change is attributable to the i_{x_2} component which we analyse in this paper. The initial instantaneous current flow will also be discussed below.

These experiments demonstrate the voltage dependence of the current system i_{x_2}. They also enabled a holding potential to be selected from which further voltage clamp experiments could be usefully performed. The

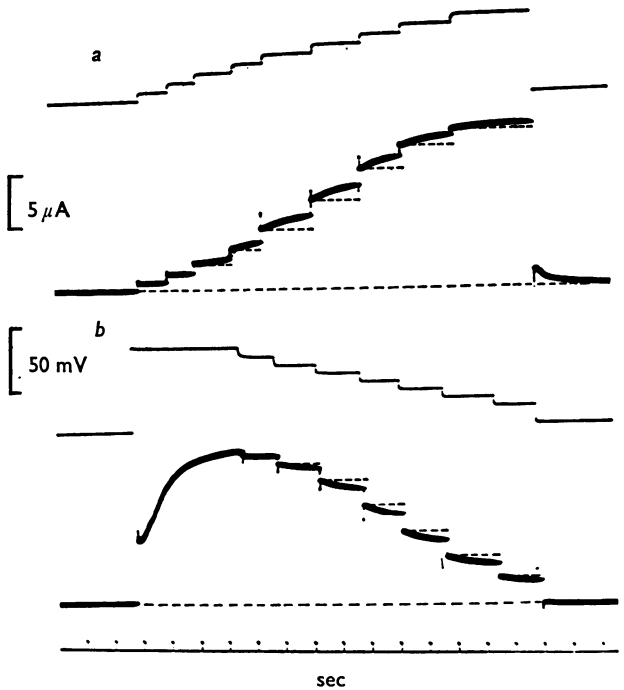

Fig. 1. Voltage clamp records showing the range of potential over which i_{x_2} is activated (*a*) and deactivated (*b*). Upper records, voltage; lower records, current. Potential range approx. −45 to +35 mV.

holding potential first selected on this basis was +25 mV. From Fig. 1 it will be clear that at this potential most of the i_{x_2} current is activated. We then performed numerous voltage clamp hyperpolarizations of the type illustrated in Fig. 2. A number of clamp depolarizations were also carried out, and responses to clamp pulses given from a different holding potential were later investigated.

The records shown in Fig. 2 allow two important measurements of slow current change to be made. Let the holding potential of +25 mV be referred to as E_{HP}, and the potential to which the membrane is clamped

as E_m. When the membrane potential is changed from E_{HP} to E_m a sudden fall in current occurs followed by a slow current decrease. The steady-state magnitude of this slow current decrease will be called i_A. i_A is due to the deactivation of some of i_{x_2} and its magnitude will depend upon the value of E_m. When the clamp returns the membrane potential to E_{HP}, another slow change in current flow follows the instantaneous response, this time representing activation of i_{x_2}. This change will be labelled i_B.

The variation of i_B with E_m will be dealt with first and its significance discussed. In Fig. 2 the two largest hyperpolarizing pulses give identical

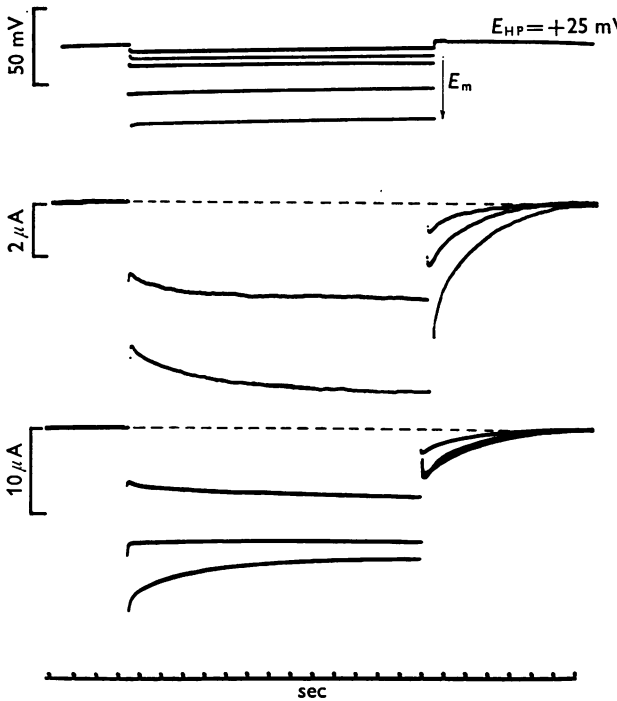

Fig. 2. Superimposed records of hyperpolarizing voltage clamp pulses given from a holding potential of +25 mV. Top record: voltage trace. The potentials (E_m) to which the five pulses hyperpolarized the preparation were +15, +5, −5, −35 and −74 mV. Middle record: high gain record of the currents recorded in response to the first, second and third hyperpolarizing pulses. At this gain, the current during the third of these pulses (E_m = −5 mV) is too large to be recorded but the current tail following this pulse does appear. Bottom record: lower gain current records during and after the third, fourth and fifth clamp pulses. Thus the top record in this group is the same as the bottom record in the previous group (E_m = −5 mV). The reversal potential for i_{x_2} is shown to be near E_m for the fourth pulse (= −35 mV). The current tails in response to the two largest pulses (E_m = −35 and −74 mV) can be seen to be nearly equal in value (see text).

values of i_B when the potential is returned to E_{HP} even though these values of E_m are very different, i.e. -35 and -74 mV. It is thus apparent that for i_B there is a maximum value of negative current. Similarly a maximum value of positive current was found by applying depolarizing pulses and measuring the initial magnitude of the positive current tail which occurs when the membrane potential returns to E_{HP}. The value of E_m at which i_B reached the maximum positive value was about $+50$ mV.

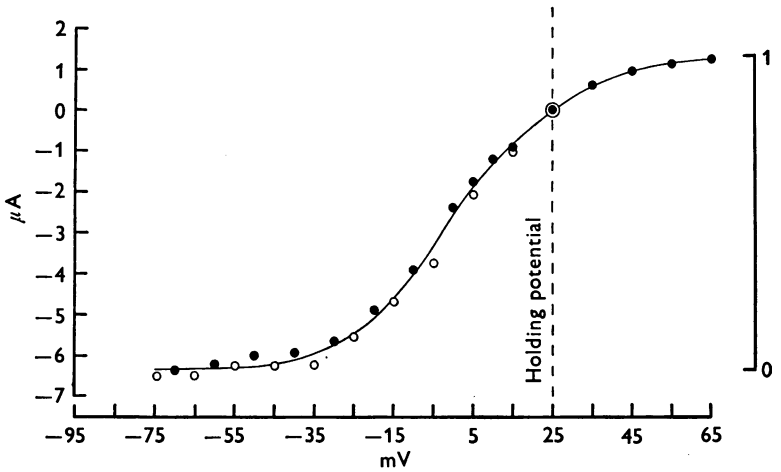

Fig. 3. Activation curve for the i_{x_2} current obtained as described in the text from measurements of voltage-clamp currents in response to pulses given from a holding potential of $+25$ mV. ●, measurements from one series of pulses; ○, measurements from a second series performed later in the experiment.

Since i_B is always measured at the same potential any direct influence of the membrane potential on the ionic current will be the same in all cases. The variation in i_B must therefore be due only to changes in the degree of activation of the current system, i.e. to the fraction of voltage-dependent membrane 'channels' which are available for current flow in moving from a given value of E_m to E_{HP}. We shall call this degree of activation x_2. A plot of i_B against E_m therefore gives the variation of x_2 with potential. This is shown in Fig. 3. It is evident that the curve is a sigmoid function of potential. Negative to -35 mV, the curve is flat and at these potentials no i_{x_2} is activated. Positive to about $+50$ mV the i_{x_2} system is fully activated. Following the usual convention we define x_2 at these potentials as equal to zero and 1 respectively. The experimental curve may now be expressed in terms of x_2 (see right-hand ordinate in Fig. 3). We shall refer to this normalized curve as the 'activation curve of the i_{x_2} system'.

We may now consider the information to be obtained from the other

slow current measurement, i_A, made during each clamp pulse. Figure 4 shows the current record, also reproduced in Fig. 2, of a voltage clamp hyperpolarization from $E_{HP} = +25$ mV to $E_m = +5$ mV. Beneath is shown our experimental activation curve, x_2. The arrows show the changes in x_2 which must occur during and following the hyperpolarization. Since x_2 must return to the same steady-state value, Δx_2 during the hyperpolarization must be equal to Δx_2 following the termination of the pulse.

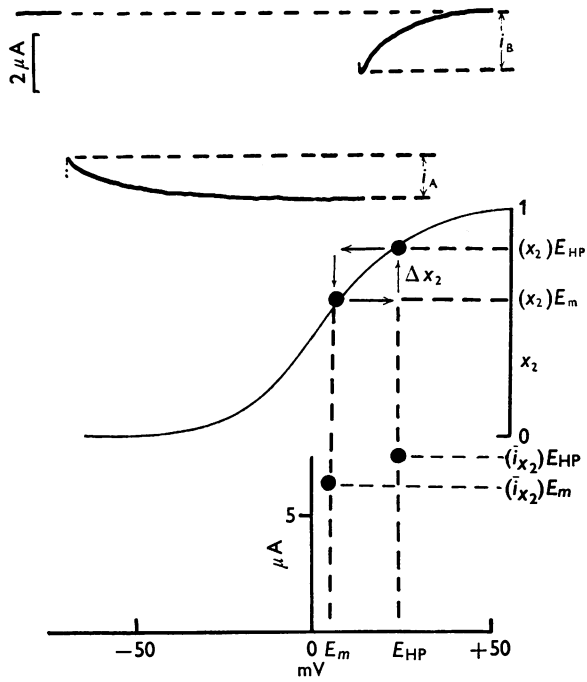

Fig. 4. Diagram illustrating method of analysis of current records. Top: current record in response to a hyperpolarizing pulse of 20 mV ($E_{HP} = +25$ mV; $E_m = +5$ mV). i_A and i_B are measured as shown (see text). Middle: activation curve for i_{x_2} as plotted in Fig. 3. The two points are values of x_2 at E_{HP} and E_m. Arrows show the change of $x_2(\Delta x_2)$ during and following this hyperpolarization. Bottom: points show values of $\bar{i}_{x_2}$ at E_{HP} and E_m calculated from Δx_2, i_A and i_B as described in the text.

Hence if x_2 were the only factor determining the magnitude of i_{x_2}, i_A would be equal to i_B. However, experiment shows that $i_A < i_B$. and thus the membrane potential must have an additional influence on i_{x_2}. Let $\bar{i}_{x_2}$ represent the current which would flow if the value of x_2 were equal to unity. This would be the case if all the voltage-dependent membrane 'gates' were open. Now

$$i_{x_2} = \bar{i}_{x_2} x_2.$$

Also $$i_B = \bar{i}_{(E_{HP})}\Delta x_2,$$

and $$i_A = \bar{i}_{(E_m)}\Delta x_2.$$

In this case Δx_2 has a value of 0·26, i_B of 2 μA, and i_A of 1·54 μA. Hence $\bar{i}_{(E_{HP})} = 7{\cdot}7\ \mu$A, and $\bar{i}_{(E_m)} = 6\ \mu$A. These points are filled in below the activation curve in Fig. 4. The actual curve of $\bar{i}_{x_2}$ obtained by calculating more points using different values of E_m and the procedure described above is plotted in Fig. 5. We will refer to this curve as the fully activated current–voltage relation of the system. This relation describes that part of the

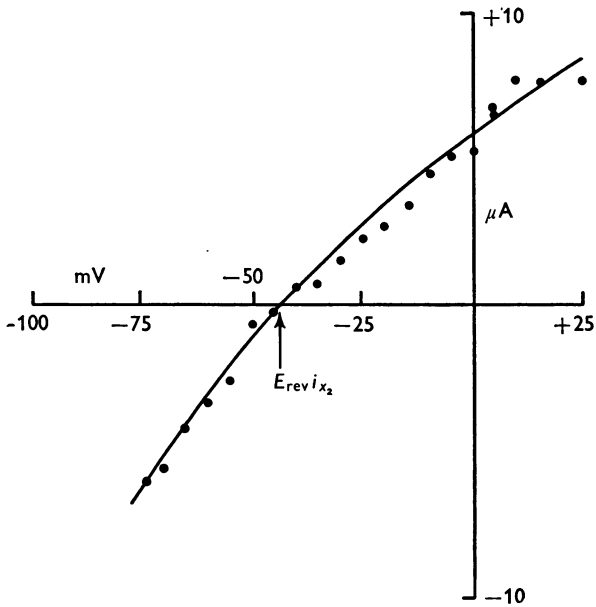

Fig. 5. Fully activated current voltage relation for the i_{x_2} current derived as described in the text and as illustrated in Fig. 4. From this graph the reversal potential of i_{x_2} is seen to be about −40 mV.

voltage dependence of i_{x_2} which results from the fact that the ionic current flows are functions of the electrochemical gradient. In general,

$$i = f(E_m - E_{rev}),$$

where f is a function which may vary linearly or non-linearly with voltage and must be zero when $E_m = E_{rev}$. From Fig. 5, therefore, the reversal potential for i_{x_2} is shown to be about −35 to −40 mV in this preparation.

In this case it is also evident that f is a nearly linear function and in particular does not show the inward-going rectification exhibited by other cardiac currents (Noble & Tsien, 1968, 1969).

In contrast to the time course of current change due to changes in x_2,

$\bar{i}_{x_2}$ is a virtually instantaneous function of voltage. Hence some of the current changes seen on experimental records could be attributable to changes in $\bar{i}_{x_2}$. However, since sudden current changes are also observed when x_2 is not activated (see Fig. 1*b*), other currents not analysed in this paper must also contribute to these sudden changes.

So far we have dealt only with the behaviour of i_{x_2} under steady-state conditions. We will now analyse its time dependence. When analysing the rate of change of current with time we found that it obeyed an exponential relationship such that

$$i_{x_2} = (i_{x_2})_0 e^{-t/\tau x_2},$$

where τ_{x_2} varies with the membrane potential and $(i_{x_2})_0$ is the initial value of the current at zero time.

Figure 6 shows semi-log plots of current against time obtained from experimental results of the type illustrated in Fig. 2. Here the current involved is that representing the activation of i_{x_2} when the potential is switched from various values of E_m back to E_{HP}. The value of E_{HP} (+25 mV) is the potential responsible for determining the magnitude of τ_{x_2} in this case.

As can be seen, these plots give linear, parallel relations between $\log i_B$ and t with a value for $\tau_{x_2,\,E_{HP}}$ of 1·85 sec. The fact that the experimental variation of current with time has been shown to be exponential suggests that the kinetics controlling the 'gating mechanism' should be first order. Thus

$$dx_2/dt = \alpha_{x_2}(1-x_2) - \beta_{x_2} x_2,$$

where α_{x_2} and β_{x_2} are voltage-dependent rate coefficients, and x_2 is the fraction of 'gates' open at time t.

When the steady state is reached,

$$\alpha_{x_2}(1-x_{2\infty}) - \beta_{x_2} x_{2\infty} = 0$$

and

$$(x_2) = \alpha_{x_2}/(\alpha_{x_2}+\beta_{x_2}).$$

The value of x_2 can be obtained for a given potential from the experimental activation curve shown in Fig. 3.

Now it is known that

$$1/\tau_{x_2} = \alpha_{x_2}+\beta_{x_2},$$

and thus

$$x_2\, 1/\tau_{x_2} = \alpha_{x_2}.$$

Values for β_{x_2} were obtained from the measurements of $1/\tau_{x_2}$ by subtraction.

We have plotted the curve of $1/\tau_{x_2}$ for this current system and derived α_{x_2} and β_{x_2} from it as indicated above. In Fig. 7, which shows our experimental curves, two holding potentials from which many estimates of τ_{x_2} were obtained are shown as filled symbols. These points we consider to be

the most reliable since they represent the average of large numbers of results. The open symbols are obtained from a measure of τ_{x_2} for the rate of change of i_A with time at potentials of various magnitudes. These points must be less reliable, depending as they do on one or two measure-

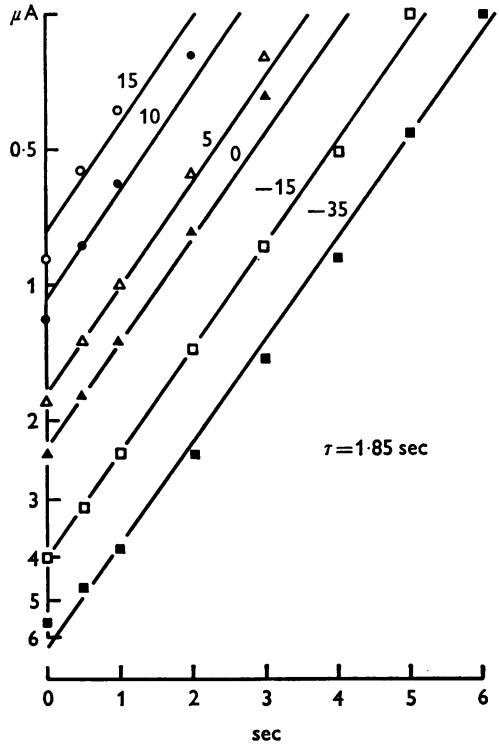

Fig. 6. Semi-log plots of current against time for activation of the i_{x_2} current. Current tails occurring after six hyperpolarizing clamp pulses of the type shown in Fig. 2 have been plotted against time. The number above each plot indicates the value in millivolts of the potential (E_m) to which the membrane had been clamped during the pulse. In each case the membrane potential was returned after the pulse to the holding potential of +25 mV. Note that, since all the curves are parallel, the time constant at E_{HP} is independent of the magnitude of the voltage clamp pulse.

ments only, but to improve our accuracy we have selected only those current changes giving an initial high gain amplitude of greater than 4 mm on the current record of the pen recorder (4 mm = 0·5 μA). This determination of the manner of variation of the voltage-dependent rate coefficients with membrane potential completes our analysis of the i_{x_2} current system.

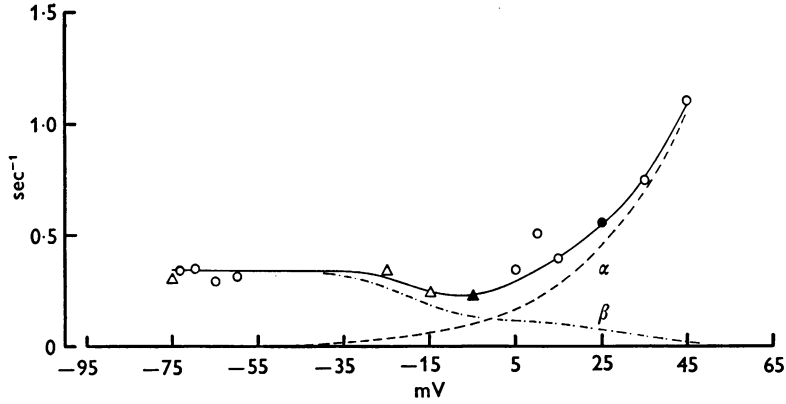

Fig. 7. Rate of change of i_{x_2} measured as the reciprocal of the time constant at each potential. Circles are values obtained using pulses from + 25 mV, triangles from − 5 mV. Measurements made at the holding potentials are indicated by filled symbols. The interrupted lines show calculated relations for α_{x_2} and β_{x_2} (see text).

DISCUSSION

The results of this analysis show that the atrial current system i_{x_2} obeys Hodgkin–Huxley kinetics. A physical interpretation of this result is that the i_{x_2} current flows through membrane pores or channels governed by a voltage-dependent 'gating mechanism'. The nature of this 'gating mechanism' remains unknown but it must carry charge if it is to move in a potential field. Assuming that this movement occurs across the whole of the potential field available, then we may calculate the minimum valency of the 'gates' (see Hodgkin & Huxley, 1952). The ratio of concentration of charge associated with the open and closed states will then obey the steady-state equation

$$\ln[(x_2)_\infty/(1-x_2)_\infty] = z\Delta E_m \mathrm{F}/\mathrm{RT},$$

where $\Delta E_m = E_m - E_{m(0\cdot5)}$. $E_{m(0\cdot5)}$ is the value at which $(x_2) = 0\cdot5$.

The value of z found to fit the results shown in Fig. 3 is 1·8. Since the valency of a particle cannot be fractional, it would seem reasonable to assume that the minimal charge associated with the 'membrane gates' is 2, thus allowing for experimental error.

From our analysis of the kinetics of x_2 it is evident that the rate $(\alpha_{x_2} + \beta_{x_2})$ is not a symmetrical function of voltage, since the β rate curve becomes flat at negative potentials. In the Purkinje fibre it is the α curve which behaves in this way. As yet it is not known why the rate functions vary as they do (see Tsien & Noble, 1969).

The reversal potential of i_{x_2} is somewhat variable, generally lying

between 0 and -30 mV. This suggests that it is not pure with respect to its ionic composition. We have not investigated the possibility that it might be a pure chloride current, but the extremely high intracellular chloride concentration needed for this to be the case would appear to rule this out.

The existence of i_{x_2} in atrial muscle raises the question whether it has a functional role. As is well known, the atrial action potential lasts for a few hundred milliseconds only and is triangular in shape. The duration of the depolarization is thus too brief to activate i_{x_2} and under these circumstances it might well appear to be redundant.

However, in pace-making fibres where the maximum negative potential seldom exceeds -50 mV, a steady background current attributable to a small activation of i_{x_2} would often be present, and when large enough might influence the frequency of pace-maker activity. This effect would only be significant in those preparations in which the activation of i_{x_2} occurs at more negative potentials than it does in the preparation analysed in this paper. In some of our quiescent atrial preparations a considerable fraction of i_{x_2} is activated at potentials negative to the reversal potential, the activation range being even more variable than the potential at which the system reverses. We have found no correlation between the actual reversal potential and the total activation range in the preparations we have studied.

The extent of involvement of i_{x_2} in pace-maker activity is a problem requiring more voltage clamp experiments to solve.

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