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THE MECHANISM OF OSCILLATORY ACTIVITY AT LOW MEMBRANE POTENTIALS IN CARDIAC PURKINJE FIBRES

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SUMMARY

1. The mechanism of oscillations at low membrane potentials in Purkinje fibres has been investigated using voltage clamp experiments.

2. The oscillations are generated by time-dependent variations in an outward current component, i_{x_1}, that is activated over the voltage range -40 to 10 mV. During normal activity, this current is responsible for initiating full repolarization to the resting potential (Noble & Tsien, 1969*b*) so that the oscillations represent a failure of the normal repolarization process, probably as a consequence of a small change in background (leakage) current.

3. These oscillations are distinct from the normal pacemaker activity of Purkinje fibres which is generated by a separate time-dependent current, i_{K_2} (Noble & Tsien, 1968). i_{K_2} shows no time-dependence when the membrane potential variations are entirely positive to -65 mV and cannot, therefore, be involved in the oscillatory activity apart from contributing a background outward current.

4. The amplitude and frequency of the oscillations are very sensitive to applied currents less than 1 μA/cm^2. Larger currents abolish the oscillatory activity.

5. The mechanism of the oscillations is discussed in relation to the possible mechanisms underlying the natural pacemaker activity of the sino-atrial (SA) node.

INTRODUCTION

It is now well established that, in conditions which give rise to low (i.e. less than -70 mV) membrane potentials, Purkinje fibres show a tendency to oscillate in a manner which appears different from the usual pace-maker activity at high membrane potentials. These oscillations have

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been recorded at low external K concentrations (Carmeliet, 1961; Müller, 1963*b*), in anoxia (Trautwein, 1964), in Cl-free solutions (Hutter & Noble, 1961), in solutions of very low ionic strength (Trautwein, Dudel & Peper, 1965), following treatment with ouabain (Müller, 1963*a*), dinitrophenol (DNP) and aconitine (Trautwein, 1964). Moreover, they are also sometimes observed in excised Purkinje fibres which have failed to fully recover from the initial disturbance of the excision.

Noble & Tsien (1968) have shown that the time-dependent K current which is responsible for generating normal pacemaker activity in Purkinje fibres is fully activated in the steady state at potentials positive to about -65 mV so that oscillatory activity which occurs entirely positive to this potential cannot be generated by the normal pace-maker K current. However, other time-dependent outward currents, which are probably carried largely but not exclusively by K ions, are activated at more positive potentials (Noble & Tsien, 1969*a*, *b*). The purpose of the present paper is to indicate how these currents are involved in oscillations at low membrane potentials.

METHODS

The methods were identical with those described previously (Noble & Tsien, 1968). The preparations used were short excised sheep Purkinje fibres which had failed to recover their normal high resting potential.

RESULTS

The mechanism of the low voltage oscillations was investigated by determining the membrane currents under voltage clamp conditions at potentials within the range of the oscillations. Figure 1 shows the result of such an experiment on a preparation that was spontaneously oscillating between -46 and -13 mV. At a certain point the potential was suddenly clamped at the maximum negative potential reached during the oscillation. The current immediately following application of the clamp was outward, but it then slowly changed towards a net inward value. This inward current would, of course, drive the potential in the depolarizing direction if the potential were not controlled by the clamp circuit.

In order to determine the behaviour of the membrane current in the voltage range of the oscillation, the membrane was depolarized in 10 mV steps. Depolarization to -36 mV produced an initial inward current followed by a slow increase in current towards a net outward value. Depolarization to -25 mV produced further activation of outward current. In the absence of the voltage clamp this outward current would have hyperpolarized the membrane and so would be responsible for the repolarizing phase of the oscillations. This repolarization would itself then deactivate the outward current. This is demonstrated by applying a 10 mV

hyperpolarization. The hyperpolarization was accompanied by a decay of the outward current which, in the absence of the voltage clamp, would allow the oscillatory cycle to recur.

This sequence of current changes provides a satisfactory qualitative description of the mechanism of the oscillation. A more quantitative description would require a detailed analysis of the kinetics and other properties of the current changes. In a previous paper (Noble & Tsien,

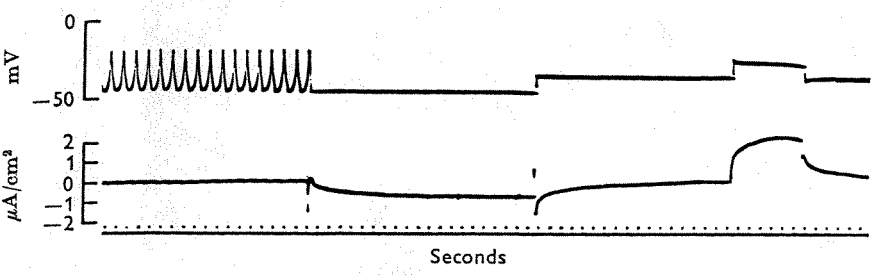

Fig. 1. Membrane currents recorded during step potential changes in voltage range of oscillation. The preparation was oscillating spontaneously and was initially clamped at the maximum negative potential (−46 mV) reached during oscillation. The membrane was then depolarized to −36 and −25 mV, followed by repolarization to −36 mV. Owing to the current-limiting properties of the current microelectrode the potential is not quite constant following depolarization to −25 mV. Note slow current changes activated by depolarizations and deactivated by hyperpolarizations.

1969*a*) it was shown that the slow current changes in this range of potentials are accounted for by two components of outward current, i_{x_1} and i_{x_2}. i_{x_1} is activated relatively quickly (time constant about 1 sec) and is responsible for initiating the repolarization process in preparations with normal resting potentials (Noble & Tsien, 1969*c*). Its time course is similar to that of the current changes shown in Fig. 1. Although it was not possible to obtain a full analysis of the kinetics of the current in this particular preparation, it seems reasonable to assume that they are similar to those determined in other preparations and that the slow current underlying the oscillations is i_{x_1}. Further justification for this assumption will be provided below (*Membrane currents at potentials negative to the oscillation range*).

We will therefore use the method of analysis used by Noble & Tsien (1969*b*) to give a semiquantitative description of oscillatory activity. In this analysis the time-dependent changes in membrane current are represented in terms of the movement of an instantaneous current–voltage relation and the trajectory of the voltage and current during activity. Since the net current is proportional to dV/dt, the result is a phase plane diagram. Figure 2 (bottom) illustrates the current–voltage

relations which may be assumed to apply at various times during th oscillation shown in Fig. 1. The filled circles and interrupted line show th experimentally determined steady-state current–voltage relation and th instantaneous current–voltage relations are based on the clamp record and on information concerning the shape of these relations in othe

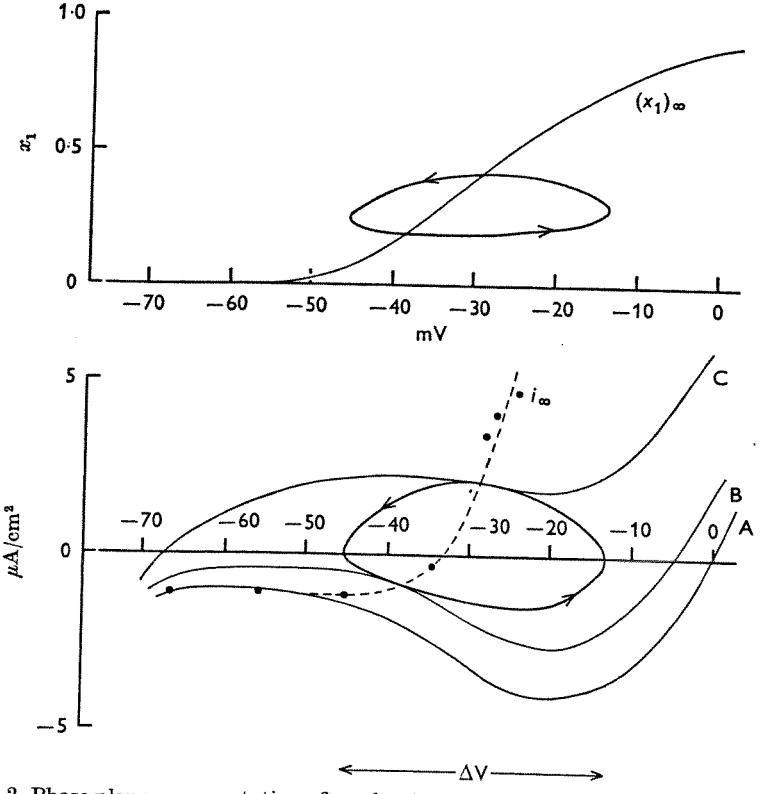

Fig. 2. Phase plane representation of mechanism of oscillation. Top: sigmoid curve shows experimental relation between $(x_1)_\infty$ and membrane potential (Noble & Tsien, 1969*a*). The closed trajectory shows possible variation in x_1 during oscillation.

Bottom: points show steady-state membrane current measured experimentally on preparation illustrated in Figs. 1 and 3. Curves labelled A, B and C show probable instantaneous current–voltage relations when x_1 is zero (A) and when x_1 has minimum (B) and maximum (C) values reached during oscillation. The closed trajectory illustrates way in which current and voltage may vary during oscillation. Further description in text.

preparations. A single cycle of the oscillation may be represented by a closed trajectory (Jones, 1961; FitzHugh, 1961). A point on this trajectory represents the potential and net ionic current at a particular time during the cycle. It also determines the position of the instantaneous current–voltage relation at that time. An upward movement of the current–voltage

relation is generated by activation of x_1, which approaches its steady-state value, $(x_1)_\infty$, according to a first order differential equation (Noble & Tsien, 1969*a*). The variation in x_1 may also be described by a trajectory in the x_1, V plane (Fig. 2 top). In the range of potentials over which the oscillations occur, the current–voltage relations are fairly flat (i.e. the slope conductance is always small), so that relatively small current changes will give rise to large variations in the points at which the current–voltage relation intersects the voltage axis. The variation in the stable point voltage (V_{SP}) during the oscillation may therefore be much larger than the variation in membrane potential itself, which is limited by the rate at which the membrane capacitance can be charged by the small net ionic currents. Conversely, since very small current changes are required, only small changes in the activation variable, x_1, will occur. Hence, the oscillations may occur at a frequency which is high compared to that which would be expected if time were needed for large changes in x_1 to occur. Thus, the time constant of x_1 is of the order of 1 sec, whereas each half cycle of the oscillation is often smaller than this.

Conditions for quiescence and instability

It can be seen from Fig. 2 that, during each cycle, x_1 must equal $(x_1)_\infty$ at two points corresponding to the maximum and minimum values of x_1. Since one of the conditions for quiescence is that $x_1 = (x_1)_\infty$, it may be asked what condition ensures that the oscillation should be maintained. A quiescent potential requires not only that $x_1 = (x_1)_\infty$ but also that the net ionic current should be zero. This condition is obviously not satisfied at the maximum and minimum values of x_1 in Fig. 2 since ionic current flowing at these times ensures that the potential should continue to change beyond the point at which $x_1 = (x_1)_\infty$. However, the condition for zero current will be satisfied at a point *within* the closed trajectory of x_1, V or of i, V, and this point is given by the intersection of the steady-state current–voltage relation with the voltage axis. This is shown in Fig. 2 as the interrupted line and the point at which it intersects the voltage axis is called the *centre* (Jones, 1961). In order for the centre to form a possible quiescent potential it must be stable. A sufficient condition for stability is that the slope of the instantaneous current–voltage relation at this point should be positive. In this case, the amplitude of the oscillation will depend on the initial conditions and the oscillation will be damped. However, some preparations which are initially quiescent and which do not show a marked negative slope conductance in the oscillation range may, nevertheless, oscillate for a prolonged period or even indefinitely following the application of a short pulse. In general, it can be shown that the condition for instability is

$$-(\partial i/\partial V)_t \geqslant \tau_1^{-1} C_m.$$

where $(\partial i/\partial V)_t$ is the instantaneous slope conductance. From this equation it can be seen that the condition for instability is that the slope conductance should be negative and that its magnitude should be greater than $\tau_1^{-1} C_m$. Since C_m is about 10 μF/cm^2 and τ_1^{-1} is about 1 sec^{-1} the minimum negative slope conductance required for instability is about 0·01 mmho/cm^2, or about 0·1 μA/cm^2/10 mV. In a typical preparation with a membrane area equal to about 0·005 cm^2, the current in response to a 10 mV step would be about 5×10^{-9} A. It is difficult to obtain reliable measurements of currents as small as this under voltage clamp conditions in most experiments so that the negative slope conductance in an oscillating preparation may not be very apparent experimentally.

Large oscillations of the kind illustrated in the present paper occurred in preparations with fairly marked negative slope conductances in the region of the intersection of the steady state current–voltage relation with the voltage axis. In this case, the equilibrium point, $i_\infty = 0$, is very unstable. The amplitude of the oscillation will not depend on the initial conditions but on the extent of the negative current region. This region may be modified by applied current, which may therefore modulate the oscillation amplitude (see Fig. 4).

Membrane currents at potentials negative to the oscillation range

The identification of the slow current changes underlying low voltage oscillatory activity with i_{x_1} depends partly on the fact that this activity occurs in the voltage range over which x_1 is activated in normal preparations. It may be questioned, however, whether preparations showing low voltage oscillatory activity are sufficiently normal for this assumption to be valid and, in particular, whether the oscillatory activity may perhaps represent another possible mode of activity of the normal pacemaker mechanism in fully repolarized preparations (Noble & Tsien, 1968). These questions may be resolved by recording the voltage clamp currents in an oscillating preparation over a wider range of membrane potentials.

Figure 3 shows the membrane currents following hyperpolarization of the membrane with a staircase voltage from the middle of the oscillation range to beyond E_K. In Fig. 3*A*, there is a slow decay of outward current on repolarization from -27 to -37 mV and to a lesser extent on repolarization to -47 mV. Very little time dependent change occurs at -57 and -67 mV (which is consistent with results in normal preparations—Noble & Tsien, 1969*a*, Fig. 1) and there is a small degree of negative slope in the current–voltage relation in this range. Hyperpolarization to -77 mV then produces a large slow current change. Stronger hyperpolarizations produce even larger slow current changes and so the clamp currents in the range negative to -77 mV are shown at lower amplification (Fig. 3*B*). Slow changes of current are evident at -85 and at -93 mV but very little slow change occurs at -100 mV, which may in fact be slightly beyond the potassium equilibrium potential in this preparation.

This result clearly shows that, as in more normal preparations, there are two distinct voltage ranges in which slow current changes may be observed. The slow current change negative to -70 mV is that previously identified as the current (i_{K_2}) responsible for the normal pace-maker activity of Purkinje fibres. There can be little doubt, therefore, that the low voltage oscillations are generated by changes in i_{x_1} and not by changes in i_{K_2}. The mechanism underlying oscillatory activity at low membrane potentials is therefore quite separate from the normal pacemaker mechanism. The results shown in Fig. 3 also suggest that preparations showing low voltage oscillations might be made to show more normal pace-maker activity if

)erpolarized into the voltage range in which changes in i_{K_2} occur (see . 4).

Influence of small changes in applied current on oscillatory activity

'he conclusion that fairly small variations in current are responsible generating low voltage oscillations makes it likely that the oscillations uld be very sensitive to applied currents. This was found to be the case.

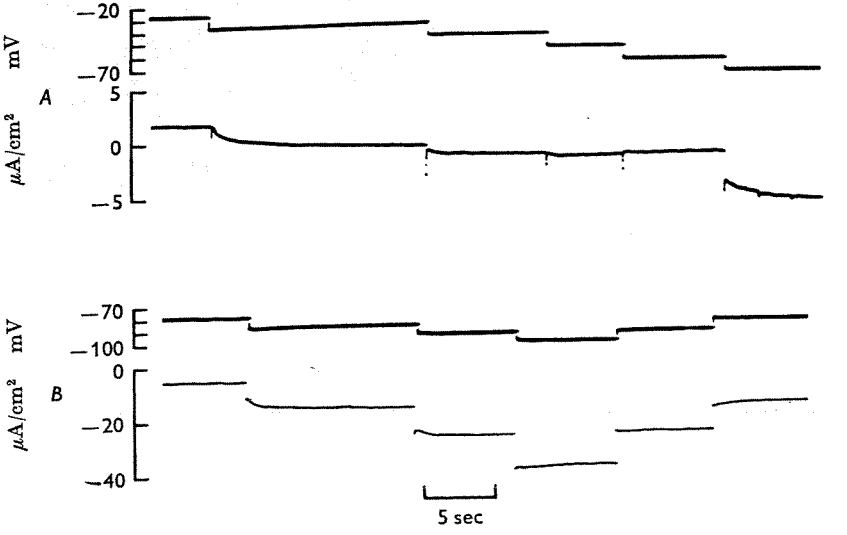

Fig. 3. Membrane current in response to 'staircase' hyperpolarization of the membrane from −27 to −100 mV. Step changes to −37 and −47 mV produce substantial slow current changes. At −57 and −67 mV there are virtually no slow current changes. On hyperpolarizing to −77 mV another large current change occurs. The responses to larger hyperpolarizations (−85, −93 and −100 mV) are shown on smaller amplification. Same preparation as in Fig. 1.

Figure 4 shows the effect of small variations in background current)duced by applying current from an external circuit. In the absence of plied current this preparation was quiescent at a depolarized potential 24 mV) in the plateau range. Oscillation begins when a small hyper- larizing current is applied. An increase in current produces larger and nsiderably slower oscillations which must require larger variations in A further increase in current gives rise to a quiescent potential at 45 mV. At this potential there must be a stable intersection of the rrent–voltage relation with the axis formed by the applied current level. ıe oscillations may therefore be modulated by a very small range of rrent (less than 10^{-8} A in this experiment). Outside this range of current, ə preparation may be quiescent. However, a further increase in hyper-

polarizing current was able in this case to induce pacemaker activity in the range of potentials at which the normal pace-maker current is activated (Fig. 4 bottom). This result is particularly striking since it shows that the same preparation may be made to show oscillatory activity in both voltage ranges in which slow current changes occur in voltage clamp conditions (Fig. 3).

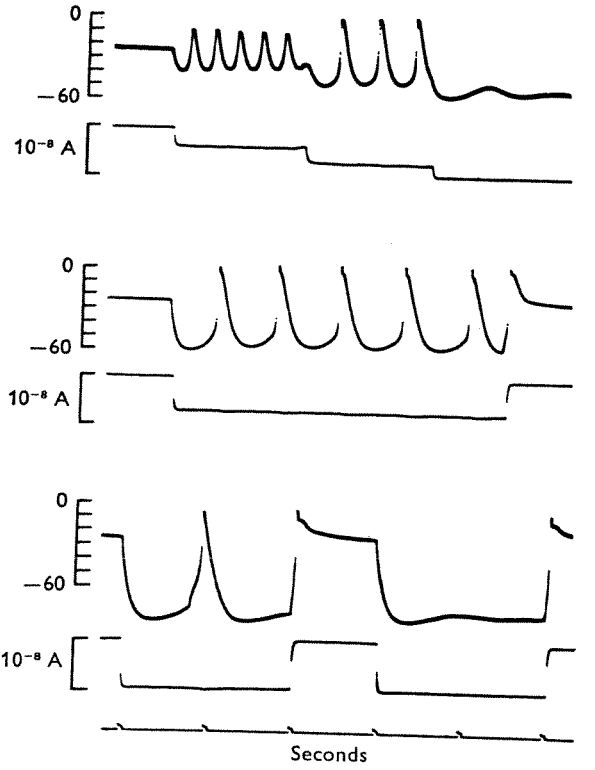

Fig. 4. Modulation of oscillatory activity by applied currents. Top: responses to $3{\cdot}3 \times 10^{-9}$ A, $6{\cdot}6 \times 10^{-9}$ A and 1×10^{-8} A. Middle: response to $7{\cdot}2 \times 10^{-9}$ A. Bottom: hyperpolarization to normal pace-maker range. The same current pulse was applied twice. A spontaneous response occurred during the first pulse. A damped oscillation occurred in response to the second pulse.

DISCUSSION

The conclusion of this paper is that the oscillatory activity in Purkinje fibres at low membrane potentials is generated by changes in the same outward current, i_{x_1}, that is responsible for initiating full repolarization in more normal preparations. Apart from the net background current change necessary to depolarize the membrane, the current changes recorded under

voltage clamp conditions in oscillating preparations are similar to those recorded in normal preparations. The implication of this observation is that it should be relatively easy to induce low voltage oscillatory activity in normal preparations by procedures which simply vary the amount of background current. The most direct way of doing this is to apply a steady depolarizing current. Trautwein & Kassebaum (1961) have shown that this produces low voltage oscillatory activity in preparations showing normal pace-maker activity in the absence of applied current. Other procedures which may induce low voltage oscillations were referred to earlier (see introduction). Many of these procedures are readily reversible, which further supports the view that low voltage oscillations represent a mode of activity of relatively normal membranes. Preparations which are recovering from damage due to excision or as a result of micro-electrode penetration sometimes show a train of oscillations before repolarizing back to the normal resting potential.

Although the current mainly responsible for the temporal characteristics of the oscillation is i_{x_1}, the properties of other currents are also important in determining the amplitude and frequency. In particular, a background inward current is required to allow the slow variations in outward current to produce a net membrane ionic current which varies periodically from inward to outward and vice versa. It seems likely that this inward current is carried largely by sodium ions and that it represents the residual sodium current which should occur when the inactivation variable (h) has reached its steady-state values (Weidmann, 1955; Noble, 1962). In keeping with this view, Carmeliet (1961) has shown that the oscillations in low K solutions disappear in the absence of Na ions. However, Trautwein *et al.* (1965, Figs. 7 and 8) have described low voltage oscillations in Na-free solutions of very low ionic strength. It is not yet clear what may carry the inward current in this case but it seems likely that the time-dependent outward current, i_{x_1}, is also responsible for the temporal characteristics of these oscillations since this current, in contrast to the normal pace-maker current, is not reduced in Na-free solutions (D. Noble & R. W. Tsien, unpublished). Moreover, the magnitude of the inward current required for instability is sometimes extremely small (see *Conditions for quiescence and instability*) so that currents several orders of magnitude smaller than the Na current underlying the normal action potential may be adequate to provide the negative slope conductance.

Low voltage oscillations do not appear to have any functional significance in Purkinje fibres (unless, perhaps, they occur in cardiac arrhythmias) and the major interest in the present results lies in the fact that these oscillations more closely resemble those of the SA pace-maker region than does the normal pace-maker activity of Purkinje fibres. Thus, the

record shown in the middle trace in Fig. 4 is very similar to the potential changes occurring in the SA node (Hutter & Trautwein, 1956; Toda, 1968). This raises the possibility that natural pace-maker activity in the SA node may be generated by similar current mechanisms as the low voltage oscillations in Purkinje fibres. On this view, normal pace-maker activity in fully repolarized Purkinje fibres is generated by changes in the pure K current, i_{K_2}, whereas pace-maker activity in the SA node may be generated by changes in a less specific K current similar to i_{x_1}. Only when the Purkinje fibre membrane is depolarized beyond the potential at which i_{K_2} is fully activated may it show spontaneous activity which closely resembles that of the SA node.

This interpretation suggests that the slow current changes in different regions of the heart may be qualitatively very similar and that regions which do not normally show spontaneous pace-maker activity may be induced to do so by introducing quantitative variations in the background conditions. Müller (1965) has shown that quiescent auricular and ventricular muscle preparations may be made to show pace-maker activity at low Ca and K concentrations. Although the explanation of his results is not yet clear, they lend further support to the view that the underlying similarities between different cardiac membranes may be at least as striking as the more obvious functional differences.

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