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THE EFFECT OF SUBTHRESHOLD POTENTIALS ON THE MEMBRANE CURRENT IN CARDIAC PURKINJE FIBRES

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SUMMARY

1. In sodium-containing solutions, up to 60% of the slow outward current in Purkinje fibres can be activated by depolarizations which are too small to activate the fast sodium conductance responsible for the depolarization phase of the action potential. This result reinforces the view that the slow outward current results from slow activation of the potassium conductance rather than from slow inactivation of the sodium conductance.

2. These subthreshold changes in membrane conductance are probably responsible for the dependence of the action potential and pace-maker potential durations on small changes in membrane potential in the subthreshold region.

INTRODUCTION

In a previous paper (McAllister & Noble, 1966) the voltage clamp currents observed in sheep Purkinje fibres were analysed in terms of three postulated components of the membrane conductance: (*a*) a fast sodium conductance (Deck & Trautwein, 1964) responsible for the depolarization phase of the action potential (Draper & Weidmann, 1951); (*b*) a component, g_{K_1}, of the potassium conductance showing inward-going rectification only (Hutter & Noble, 1960; Carmeliet, 1961; Hall, Hutter & Noble, 1963) and which is, therefore, very low during the plateau of the action potential; (*c*) a potassium conductance, g_{K_2}, showing inward-going rectification in response to fast changes in membrane potential but also slowly rectifying in the opposite (outward-going) direction. The latter conductance, which is responsible for initiating repolarization, is evident as a slow increase in current in an outward direction when the membrane is depolarized and as an outward current which decays even more slowly when the membrane is repolarized. Using voltage clamp pulses of similar duration to the normal action potential (about 400 msec) it appeared that the threshold potential for this current is roughly equal to the threshold potential for activating the fast sodium conductance and, therefore, to the threshold for initiating

an action potential (between -60 and -65 mV). Subthreshold depolarizations of this duration produced virtually no detectable time dependent conductance change, although potentials in the immediate vicinity of threshold were not systematically explored. However, since the time constants of the slow current are very long in the region of the resting potential (about -85 mV), it is possible that subthreshold depolarizations may activate some outward current too slowly to be observed unless the depolarization is long-lasting, particularly since the time course of onset may be sigmoid. We have therefore tested this possibility by using small depolarizations up to 10 sec in duration.

METHODS

The methods used were identical with those used by McAllister & Noble (1966). All solutions contained 140 mM-Na^+, 4 mM-K^+, 144·6 mM-Cl^-, 0·5 mM-Mg^{2+}, 1·8 mM-Ca^{2+}, 1·65 mM-HPO_4^{2-}, 0·7 mM-$H_2PO_4^-$ and 1 g/l. glucose. They were oxygenated and warmed before flowing over the preparations. The temperature was held constant at a value within the range 30–35° C.

RESULTS

Provided that long enough depolarizations were applied to the membrane, a substantial slow outward current could be observed at potentials negative to the sodium current threshold. Figure 1 shows currents in response to two levels of depolarization. One (16 mV) is a few mV below the threshold for activating the sodium conductance and, apart from the transient spikes of capacity current, the only time-dependent change in current is the slowly developed outward current. At the second level of depolarization (38 mV), the outward current switches on more quickly but is preceded by an inward sodium current. The maximum outward current recorded at this potential is larger than at the smaller depolarization but it is clear from the comparison that a considerable fraction (in this experiment, about 40 %) of the slow outward current may be activated by a potential which is too negative to activate the sodium conductance.

The magnitude of the slow outward current which may be activated by just subthreshold depolarizations is surprisingly large and this implies that current should be observed in response to depolarizations considerably smaller than threshold. This was found to be the case. In fact, it is possible to activate a measurable slow current with a depolarization as small as 1–2 mV. The results of a more complete experiment than that illustrated in Fig. 1 are plotted in Fig. 2 which shows the time courses of onset of the slow outward current at different levels of depolarization measured as the peak current on repolarization, as described by McAllister & Noble (1966). The major advantage of this method is that the currents are always

measured at the same membrane potential and, are, therefore, proportional to the instantaneous value of the slow conductance at this potential (although the degree of activation of this conductance is always determined

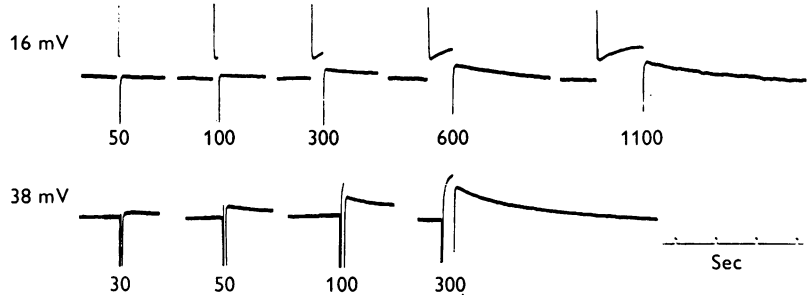

Fig. 1. Currents recorded during depolarizations of various durations and two magnitudes (16 and 38 mV). At 16 mV, apart from the fast transient capacitive currents at the beginning and end of the clamp pulses, the only time-dependent change is in an outward (upward) direction. At 38 mV, the initial capacitive transient is completely masked by an intense surge of inward sodium current which is then followed by outward current. The figures below each record indicate the clamp duration in msec. Holding potential −80 mV approx. Note: the fast transient components of the current records have been retouched.

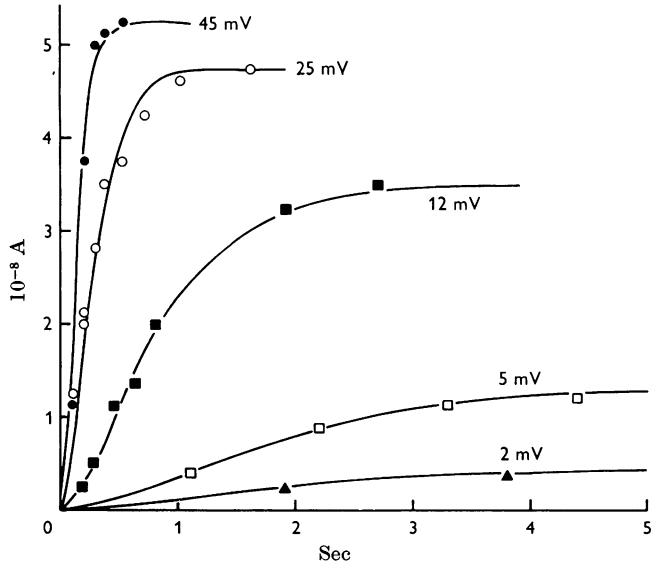

Fig. 2. Time courses of onset of slow outward current at subthreshold (2, 5 and 12 mV) and suprathreshold (25 and 45 mV) levels of depolarization. The currents were measured after repolarization to the holding potential (approx. −80 mV) from the depolarization level indicated and are shown as various symbols. The continuous curves are solutions of the equations discussed in the text. Temperature 31° C.

by some different potential). This means that non-linearities in the instantaneous current–voltage relations do not interfere with the analysis. However, the method has the disadvantage that a large number of voltage clamp pulses have to be applied to the membrane (frequently between 10 and 30 at each membrane potential) and this severely limits the amount of information which can be obtained from one preparation.

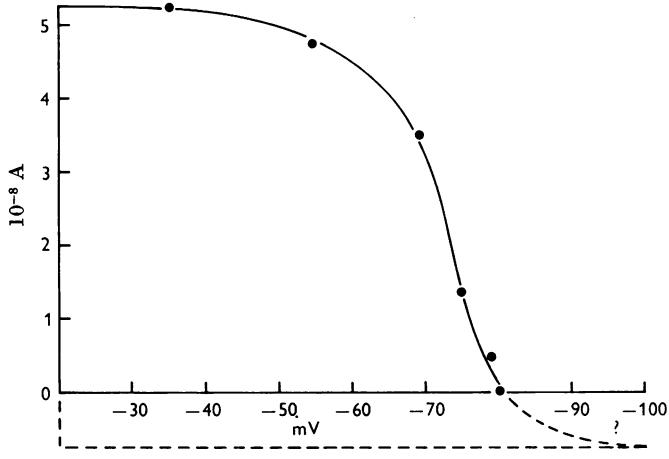

Fig. 3. Magnitude of steady state slow conductance activated by various levels of depolarization (filled circles). As explained in the text, it is probable that the steady-state conductance is not zero at the resting potential and the interrupted curve and abscissa in this diagram show a possible extension of the relation in the hyperpolarizing direction.

The depolarizations by 26 and 45 mV in Fig. 2 were strong enough to activate the sodium conductance in addition to activating most of the slow outward current. The other depolarizations (12, 5 and 2 mV) were too weak to activate any appreciable sodium conductance. It can be seen that, although the outward current switches on very slowly at these potentials, up to two thirds of the maximum current can be activated by subthreshold potentials. In fact, this is probably an under estimate since it assumes that no slow outward current is already activated at the holding potential (about − 80 mV). That this assumption is almost certainly wrong can be seen by plotting the steady state conductance as a function of the membrane potential at which it is activated. This has been done in Fig. 3. It is evident that the relation is quite steep in the region of the holding potential, which implies that some slow conductance is already present at this potential in the steady state. Unfortunately, our technique for measuring the slow conductance (see above) does not allow an accurate estimate of this resting steady state conductance. The difference between

the holding potential and the potassium equilibrium potential is quite small. If a more negative holding potential were used, it would become more difficult to measure the slow conductance since the driving force would then be reduced.

Although we cannot obtain an accurate estimate of the resting slow conductance in the steady state, it is probably less than 25 % of the maximum value of g_{K_2}. Thus, sodium removal, which greatly raises the threshold of the slow outward current (McAllister & Noble, 1966), has very little effect on the resting membrane conductance (Hall *et al.* 1963). A change in conductance greater than 10–20 % of the maximum value of g_{K_2} should be detected fairly easily. The absence of an appreciable change in conductance therefore suggests that the steady state value is small.

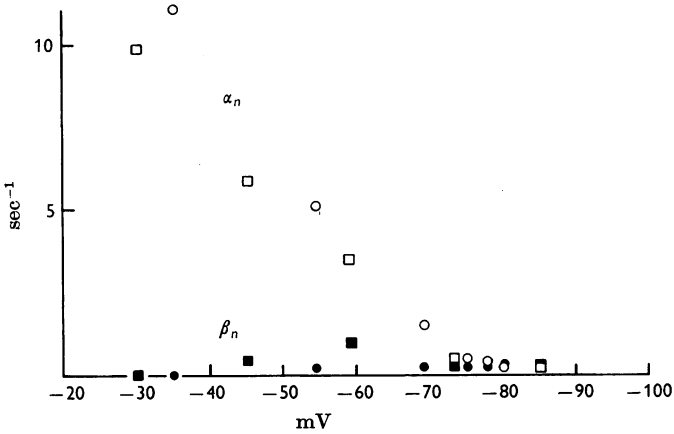

Fig. 4. Relations between α_n (open symbols) and β_n (filled symbols) and membrane potential. The calculated results of two experiments (circles and squares) are shown. In each case results were obtained for various assumed values of the steady state conductance at the holding potential and those obtained for an assumed value of 20 % are shown. These differ relatively little from the results obtained for other assumed values between 0 and 25 %. Discussion in text.

Although these results are not extensive enough to test fully any particular kinetic model, it is of some interest to analyse them in terms of the Hodgkin–Huxley n equations (for definitions of symbols, etc., see Hodgkin & Huxley, 1952). This has been done by assuming that

$$g_{K_2} \propto n^2$$

as also assumed by McAllister & Noble (1966) to fit the sigmoid rise in conductance which is frequently observed. n obeys the first-order equation:

$$dn/dt = \alpha_n (1-n) - \beta_n n.$$

The continuous curves in Fig. 2 have been drawn from these equations assuming that τ_n ($= 1/(\alpha_n+\beta_n)$) is 90 msec at 45 mV depolarization;

190 msec (25 mV); 600 msec (12 mV); 1·4 sec (5 mV) and 1·75 sec (2 mV). The steady-state value of g_{K_2} gives n_∞^2, which is equal to $[\alpha_n/(\alpha_n+\beta_n)]^2$, provided that the value of g_{K_2} at the resting potential is known. As indicated above, this is not known with any certainty and we have therefore calculated the values of the rate coefficients assuming various values for the steady state g_{K_2} at the holding potential between 0 and 25% of the maximum steady state value. Fortunately, the general characteristics of the α_n (E) and β_n (E) relations are not influenced much by the assumed value of g_{K_2} at the holding potential.

Several sets of experimental results have been analysed in this way and, although similar curves for α_n are obtained, the β_n curve is variable. This is shown in Fig. 4 in which the results of two experiments are plotted. It is seen that α_n is a monotonic function of E, and increases steeply with increasing membrane depolarization. β_n, however, is always very small and is not always a monotonic function of E. The relatively small dependence of β_n on E is also observed in squid nerve (Hodgkin & Huxley, 1952).

It is not yet clear how much significance should be attached to the fact that β_n is not always a monotonic function of the membrane potential. It may be the case that a different analysis of the kinetics would give simpler results for the β rate coefficient (or its equivalent in an alternative kinetic model) but more extensive experimental results would be required to test this point.

DISCUSSION

The results described in this paper show that a large fraction of the slow outward current in Purkinje fibres may be activated by membrane depolarizations which are insufficiently large to activate the fast sodium conductance. This conclusion reinforces the view that the slow current is not attributable to inactivation of the sodium conductance. However, two reservations might be made in connexion with this view. First, it may be that a small component of the slow current change is attributable to sodium inactivation which is obscured by a much larger change in potassium conductance. Vassalle (1966) has suggested that the initial part of the slow current changes may be a result of residual sodium inactivation and this possibility cannot be entirely excluded. Secondly, the slow current changes which occur in response to subthreshold depolarizations might be attributed to inactivation of a *resting* sodium conductance. This possibility can however, be excluded on various grounds. The slow current changes are too large to be attributed to changes in a relatively small sodium conductance. Also this theory would make it difficult to explain why the slow current reverses at a potential near the potassium equilibrium potential (Deck & Trautwein, 1964; Vassalle, 1966) and why the slope conductance changes (see Vassalle, 1966; and discussion in McAllister & Noble, 1966) are consistent with the view that changes in K conductance are involved.

Our results also help to explain the effects of small changes in membrane potential on the action potential and pace-maker potential in Purkinje fibres. Weidmann (1956; see also Noble, 1965, fig. 3) has demonstrated a dependence of the action potential duration on the initial value of the membrane potential (an effect which is additional to the well-known effect of membrane potential changes resulting from changes in extracellular potassium concentration) and this may be attributed to the fact that even very small depolarizations can activate sufficient slow K conductance to shorten the duration of the action potential. The post-cathodal depression and post-anodal enhancement described by Weidmann (1951) may also be attributed, at least partly, to changes in the slow K conductance. In pace-maker regions, for example, a small depolarization applied for a period of time can considerably lengthen the duration of the pace-maker potential (Weidmann, 1951, Fig. 4) which would be expected if g_{K_2} were temporarily increased. Likewise, a transient hyperpolarization should reduce g_{K_2} so that excitation will occur more quickly (Weidmann, 1951, fig. 3). These effects cannot be accounted for by a model (e.g. Noble, 1962) in which g_{K_2} is activated by strong depolarizations only.

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