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Adrenergic control of cardiac pacemaker currents

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Pacemaker activity in atrial muscle and in Purkinje fibres is generated by a time-dependent decay of potassium current that allows the membrane to be depolarized to the threshold for action potential initiation. The kinetics of the pacemaker potassium currents in these two parts of the heart are sufficiently different to indicate that they correspond to different membrane structures. This conclusion is strengthened by the discovery that the mechanisms of acceleration produced by adrenaline are also quite different. In Purkinje fibres, the activation threshold for the potassium current is shifted in a depolarizing direction with no change in maximum amplitude. This voltage shift is adequate by itself to explain the acceleration. In atrial fibres the pacemaker potassium current is increased in amplitude with no shift in threshold. By itself, this action of adrenaline would slow pacemaker activity and the acceleration in this case is dependent on a large increase in the current attributable to calcium ions. The rôles of cyclic 3′,5′-AMP and of intracellular calcium ions in mediating the pacemaker actions of adrenaline will also be discussed.

Introduction

The ionic mechanisms underlying the inhibitory action of acetylcholine on cardiac muscle have been known for some time (see review by Hutter 1957). The action is to increase the potassium permeability of the membrane in a highly specific way and so produce an inhibitory hyperpolarization similar to that observed at some central nervous inhibitory synapses. By contrast, the ionic mechanism of the excitatory actions of adrenaline and other catecholamines have remained mysterious until recently.

The reason for this situation is that the actions of adrenaline are unusual from a synaptic point of view since they have been found to involve changes in the behaviour of the voltage and time-dependent conductances underlying the nonlinear properties of the membrane. Their elucidation has therefore awaited the application of voltage-clamp techniques to cardiac cells.

In this paper we shall review recent work on the actions of adrenaline on the currents responsible for pacemaker activity. We shall show that the mechanisms by which acceleration occurs are very different in different parts of the heart. The two main preparations that have been studied and which show pacemaker activity are mammalian Purkinje fibres and frog atrial trabeculae. The latter show pacemaker activity in response to small depolarizing currents and this response strongly resembles spontaneous pacemaker activity in sinus tissue. Both preparations show similar potential changes on exposure to adrenaline, i.e. an acceleration of pacemaker depolarization and an increase in the maximum diastolic potential. The acceleration in frog atrium, however, is not always so pronounced as it is in the Purkinje fibre and the reasons for this lie in the fact that the ionic current changes responsible are quite different.

Pacemaker mechanism in Purkinje fibres

Noble & Tsien (1968) analysed the kinetics of a potassium current, I_{K_2}, whose decay following each action potential is responsible for the pacemaker depolarization in Purkinje fibres (Deck & Trautwein 1964; Vassalle 1966). This current is activated by a gating mechanism, s, whose threshold lies at -90 mV and which becomes fully activated in the steady state at -60 mV. This is also the range of potentials over which the pacemaker depolarization occurs, which means that the activation of the current I_{K_2} is strongly voltage dependent in this range.

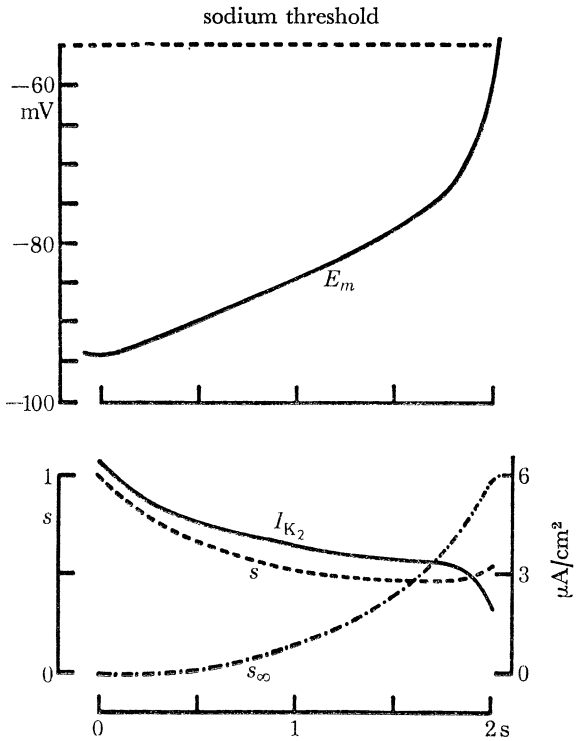

FIGURE 1. Calculated changes in s and I_{K_2} during pacemaker depolarization in Purkinje fibres (Noble & Tsien 1968). Note that rate of decay in s is reduced and halted as the membrane depolarizes to potentials at which the steady state degree of activation, s_∞, becomes significant. The position of the steady state activation curve therefore limits the speed of pacemaker depolarization.

During each action potential s becomes fully activated since the time constant of activation during strong depolarizations is very short compared to the duration of the action potential (McAllister & Noble 1966). The pacemaker depolarization therefore starts at a time when all the I_{K_2} channels are open. The time constant of decay of s at potentials in the pacemaker range is 1 or 2 s. As this decay occurs, the magnitude of the potassium current decreases so allowing an inward background current to depolarize the membrane. During a single pacemaker potential the number of I_{K_2} channels activated decays by about 50 %.

One of the factors that limits the speed at which the decay occurs, and hence the frequency of beating, is the position of the steady state activation curve. At the beginning of the depolarization, when the membrane potential lies near the threshold for activation of I_{K_2}, the degree of activation falls fairly rapidly as s moves from full activation towards zero activation. As the depolarization occurs, however, the steady state degree of activation increases and so the value

towards which s moves increases. The rate of decay of s therefore decreases. Indeed, as shown in figure 1, the rate of decay becomes zero when $s = s_\infty$. Beyond this point the channels begin to open up again and it is only the highly nonlinear behaviour of the channels themselves (which show inward-rectification and so carry much less current as the membrane becomes depolarized), and the activation of inward sodium current as the threshold is approached that allows the depolarization to continue beyond this point (see McAllister, Noble & Tsien 1975).

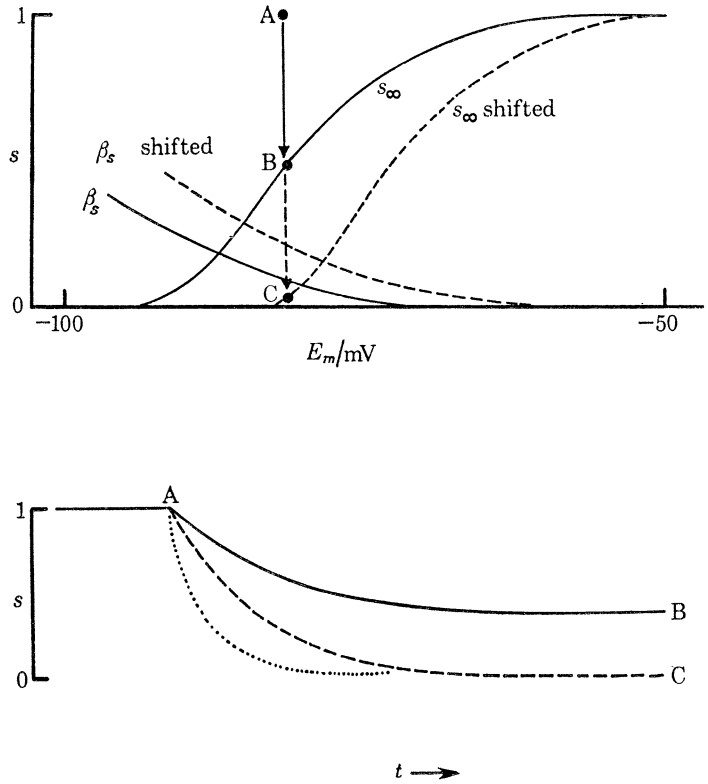

FIGURE 2. Diagram showing how a positive shift in the activation curve, s_∞, will accelerate the rate of decay of s. When curve is shifted, s falls from A to C. Even if τ_s is unaltered, the absolute rate of decay increases (– –). Since β_s increases (τ_s falls) the actual effect will be even more dramatic (. . .). (Noble 1974.)

Mechanism of acceleration in Purkinje fibres

The fact that the position of the activation curve for s limits the rate of decay of I_{K_2} suggests a way in which acceleration could be achieved. This is to shift the activation curve in a depolarizing direction, i.e. raise its threshold, so that the steady state value at potentials in the pacemaker range is reduced. As shown in figure 2 this would be a powerful way of producing acceleration. If the time constant of decay were unaltered, the decay would be more rapid from A to C than from A to B since a larger absolute change is involved; the degree of activation decays towards a lower value (interrupted curve). In fact, the mechanism of acceleration would be even more powerful since the time constant of decay becomes much shorter as the membrane is hyperpolarized. If the time constant curve is also shifted (as is likely since, in a Hodgkin–Huxley mechanism, the time constant and activation curves are closely related) the increase in rate of decay will be even greater (dotted line).

An experiment to test the possibility that a shift in the s activation curve occurs in the presence of adrenaline was performed by Hauswirth, Noble & Tsien and is illustrated in figure 3. Here it can be seen that the action of adrenaline does indeed shift the activation curve in a depolarizing direction and that this action may be reversed by the β-blocker pronethalol. The lower part of this figure shows the results of some computer calculations done to test whether the observed shifts are adequate to account for the acceleration produced. Curve N shows the pacemaker depolarization computed from a model of the ionic currents analysed by voltage-clamp techniques (see Hauswirth, McAllister, Noble & Tsien 1969; McAllister *et al.* 1975). The other curves show the pacemaker potentials computed after introducing various degrees of shift (in mV). It is clear that even quite small shifts in a depolarizing direction produce a marked degree of acceleration.

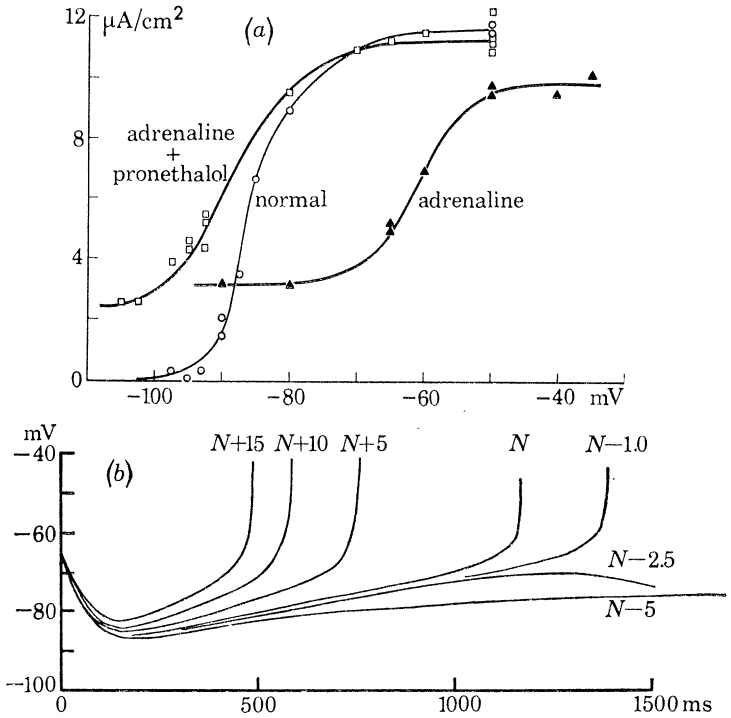

FIGURE 3. (*a*) Positive shift in activation curve for s produced by adrenaline (5×10^{-7} g/ml). The shift is reversed by the β-blocker pronethelol (10^{-6} g/ml). (Hauswirth, Noble & Tsien 1968). The change in amplitude of curve is not significant (see Tsien 1974*a*).

(*b*) Computed acceleration of pacemaker depolarization produced by shift of s activation curve by 5, 10 and 15 mV in a depolarizing direction. The negative chronotropic effects of negative shifts (−2.5 and −5 mV) are also shown. (Hauswirth *et al.* 1969.)

Pacemaker mechanism in atrial fibres

It is difficult to perform voltage-clamp experiments on the natural pacemaker, the sino-atrial node, and, as yet, only one report has appeared (Irisawa 1972). This suggests that the mechanism involved is similar to that in Purkinje fibres. In this section we shall explain the reasons against this view, although we agree with Irisawa that a potassium conductance decay is involved.

Quiescent frog atrial trabeculae (on which it is much easier to perform voltage-clamp experiments – Rougier, Vassort & Stämpfli 1968; Brown & Noble 1969) may often be made to

generate pacemaker activity very similar to that in the sinoatrial node by applying small steady depolarizing currents. This is illustrated in figure 4. When the current level indicated by the interrupted line is maintained the membrane fires repetitively. The major difference between the pacemaker activity in sinus and atrial tissue and that in Purkinje fibres lies in the fact that the potential range over which pacemaker activity occurs is quite different. As we have already noted, the pacemaker depolarization in Purkinje fibres runs from about -90 mV to about -60 mV (figure 1), whereas in atrial fibres the pacemaker range is usually positive to -60 mV (figure 4). Since s is fully activated in the steady state at potentials positive to -60 mV, one would not expect variations in a current controlled by s to be involved. Moreover, little or no delayed potassium current is activated in the s range in atrial fibres (Brown, Clark & Noble 1975*a*, *b*). It is quite possible, therefore, that the s mechanism does not exist in these fibres.

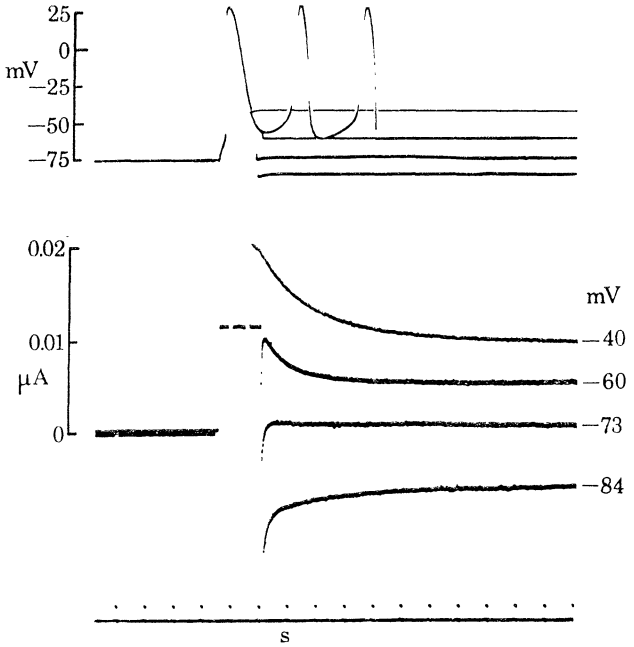

FIGURE 4. Currents recorded in frog atrial trabeculum when membrane potential is clamped to various values (-40, -60, -73 and -84 mV) after first action potential. Above -73 mV an outward current decay is recorded. Below -73 mV the decay tail is inward. (Brown, Clark & Noble 1972.)

Nevertheless, a potassium conductance of some kind is involved in pacemaker activity. This is illustrated by the experiment by Brown, Clark & Noble shown in figure 4. At the termination of the first action potential the voltage clamp was applied at various potentials. At potentials positive to -73 mV the result is a decaying outward current. At potentials negative to this value, a decaying inward current is observed. This suggests that a potassium current is involved but that its reversal potential is significantly positive to E_K, which is probably near -90 mV (Haas, Glitsch & Kern 1966). This contrasts with the properties of I_{K_2} in the Purkinje fibre which reverses very near to E_K (Noble & Tsien 1968; Peper & Trautwein 1969). The atrial pacemaker K^+ mechanism is therefore less highly selective than that in Purkinje fibres.

A less selective K current mechanism is in fact displayed by Purkinje fibres but the activation range lies substantially positive to that for I_{K_2} and it is not normally involved in pacemaker

activity. This mechanism was called I_x by Noble & Tsien (1969) to indicate that some ions other than potassium must also be transported to a small extent. The activation threshold for I_x lies at about -40 mV, which is 50 mV more positive than that for I_{K_2}.

The activation curve for the atrial potassium current also lies at similar potentials (see figure 5). These observations suggest that it is comparable to I_x in Purkinje fibres and not to I_{K_2}. We shall present further evidence for this view when the actions of adrenaline have been discussed.

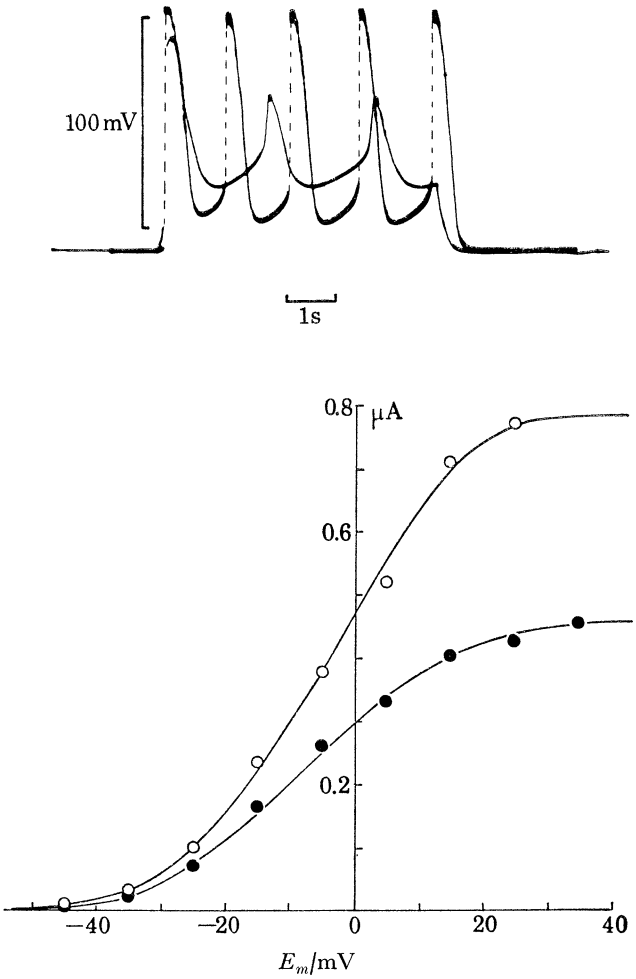

FIGURE 5. *Top.* Action of adrenaline (10^{-8} M) on induced pacemaker activity in frog atrium. The height and frequency of action potentials are increased and the maximum negative potential becomes more negative. (Brown & Noble 1974).

Bottom. Increase in amplitude of I_x current produced by adrenaline. Note that there is no shift of the activation threshold for I_x comparable to that seen in the case of I_{K_2} in Purkinje fibres (see figure 3). (Brown & Noble 1974.)

Mechanism of acceleration in atrial fibres

Figure 5 (top) shows induced atrial pacemaker potentials before and after the addition of adrenaline to the Ringer's solution. It can be seen that when adrenaline is present the action potential height is increased, the repolarization is more rapid, the potential then reaches a more negative level and the pacemaker depolarization which follows is more rapid. It is clear

from figure 3 that not all of these changes could be reproduced by a simple depolarizing shift of the potassium activation curve.

In fact, none of the changes seen in figure 5 (top) are attributable to a potassium threshold shift since the threshold for I_x is unaltered by adrenaline (figure 5 bottom). Instead, the *magnitude* of the current is increased. This contrasts very strongly with the action on the pacemaker K current, I_{K_2}, in Purkinje fibres, although it partly resembles the action of adrenaline on the current I_x in Purkinje fibres. Here also an increase in magnitude is seen (Tsien, Giles & Greengard 1972; P. A. McNaughton, unpublished).

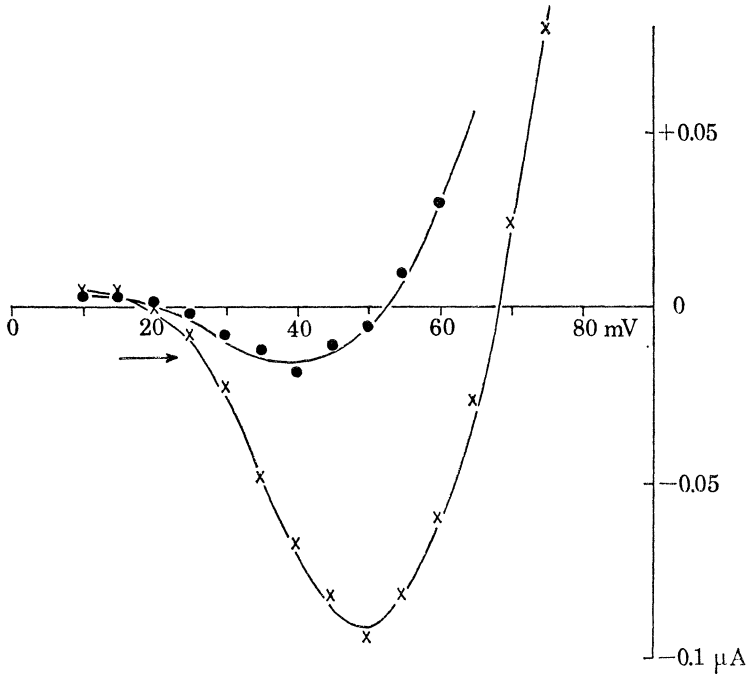

FIGURE 6. Increase in inward calcium current in atrial muscle produced by adrenaline (5×10^{-9} M). Tetrodotoxin (5×10^{-7} g/ml) was used to block the sodium current. (Brown & Noble 1974.) ●, 5×10^{-7} g/ml TTX only; ×, plus 5×10^{-9} M adrenaline.

These results further strengthen the identification of the atrial pacemaker current with I_x. They also go some way towards explaining the actions of adrenaline on atrial fibres. The increased rate of repolarization and the increased maximum negative potential are both explained by a larger K current activated during the action potential.

However, by themselves, these effects would have a decelerating effect since a larger K current will take longer to decay and should therefore lead to a prolonged interval between action potentials. There must therefore be an effect of adrenaline on another current component. The obvious choice is the calcium current, which is known to be increased by adrenaline (Rougier *et al.* 1969). If a large enough increase in inward calcium current were to occur, the pacemaker depolarization would be accelerated as it approaches the calcium threshold. As shown in figure 6, the increase in calcium current produced by adrenaline can sometimes be extremely large and is large enough to allow pacemaker activity and action potentials to occur when the sodium current is blocked by tetrodotoxin. It is certain therefore that the calcium current can be involved in pacemaker activity (see also Lenfant, Mironneau & Aka 1972).

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In view of the fact that normal repetitive activity in the sinoatrial node occurs over a potential range similar to that into which atrial preparations must be depolarized to produce pacemaker activity it seems likely that here also the pacemaker mechanism may be attributable to decay of I_x and that the mechanisms of actions of adrenaline resemble those in induced atrial pacemakers. In this connexion it is interesting to note that Purkinje fibres may also show pacemaker activity at less negative potentials than normal under many circumstances (e.g. anoxia, low $[K]_o$ and high doses of cardiac glycosides). In these cases the pacemaker activity resembles that of induced atrial activity and the potassium current involved is I_x rather than I_{K_2} (Hauswirth, Noble & Tsien 1969).

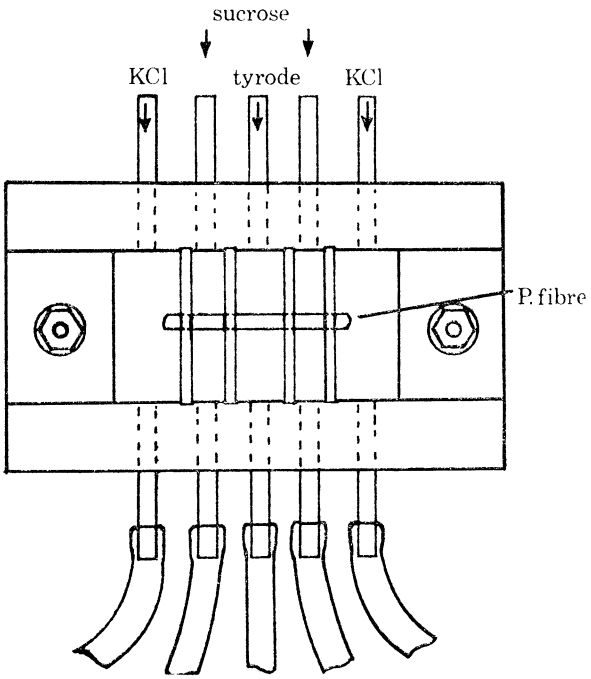

FIGURE 7. The sucrose gap bath viewed from above. The fibre is held between the two sections of the bath, the uppermost of which is shown here. Tight sealing between compartments is ensured by a liberal coating of Vaseline over the faces of the two sections prior to assembly. The fluid levels in each of the open compartments are carefully adjusted to be equal. (From McNaughton & Spindler 1973.)

Possible mechanisms of adrenaline action

The observation that adrenaline shifts the *s* activation curve in Purkinje fibres suggested a possible clue to its mode of action. Similar shifts in a variety of excitable cells are produced by agents, like Ca^{2+} and H^+, that alter surface charge on excitable membranes (Frankenhaeuser & Hodgkin 1957; Huxley 1959; Hille 1968; Drouin & Thé 1969; Gilbert & Ehrenstein 1970). Moreover, calcium ions do shift the *s* activation curve in a depolarizing direction (R. H. Brown & D. Noble 1975). Since adrenaline possesses a positive charge which is important for its physiological action, it might be postulated that adrenaline acts by adding positive charge (or neutralizing negative charge) to the outside surface of the membrane close to the I_{K_2} channels.

However, this simple hypothesis is incorrect. Thus, theophylline, which is neutral, may produce adrenaline-like shifts in the activation curve which are unlikely to be attributable to direct modification of external surface charge (Tsien 1973*b*). Since it is also possible to mimic

these shifts by injecting 3′5′-cyclic AMP inside the cells (Tsien 1973*a*, 1974*a*, *b*) the search for possible mechanisms has shifted to the inside of the Purkinje fibre.

We may, of course, resurrect the surface charge hypothesis by postulating that it is the alteration of *inside* surface charge that is involved. Thus, the removal of positive charge from the inside surface (e.g. the removal of calcium ions) would also produce a depolarizing shift in the activation curve.

The possibility that removal of calcium ions is involved is suggested by the observation that the adrenaline action is mediated by cyclic AMP (see above) since this compound is known to stimulate microsomal calcium uptake in cardiac muscle (Kirchberger, Tada, Repke & Katz 1972). A reduction in intracellular free calcium concentration might then lead to dissociation of calcium from membrane binding sites. Conversely, the addition of excess intracellular calcium ions might inhibit the action of adrenaline on the activation curve by preventing the calcium concentration from falling low enough for dissociation to occur.

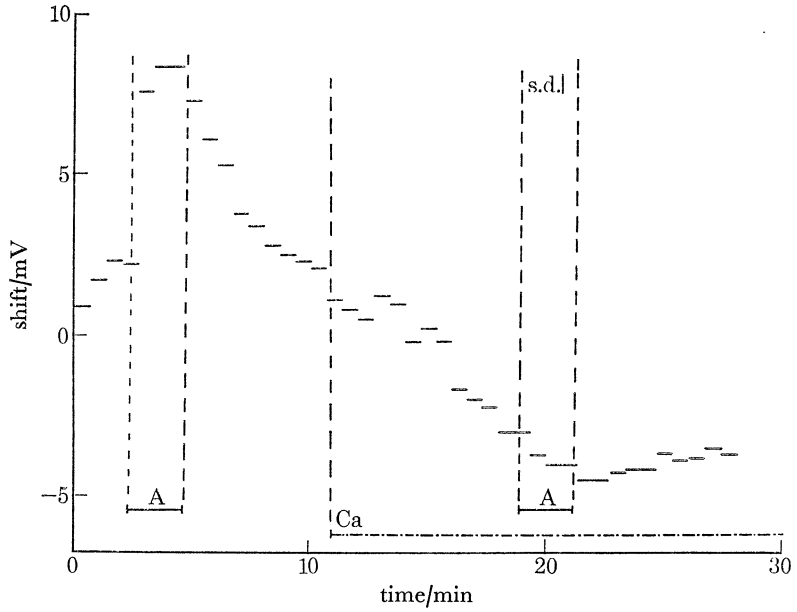

FIGURE 8. The location of the s_∞ half-activation point on the potential axis, plotted as a function of time. Shifts in the s_∞ curve have been followed by imposing a pattern of positive (to −52 mV) and negative (to −96 mV) voltage-clamp pulses from a holding potential of −80 mV, and comparing the magnitudes of the positive and negative current decay tails on return to the holding potential. Each horizontal bar is calculated from one positive and negative pulse pair. The estimated standard deviation of each measurement is indicated by the vertical bar at top right.

The duration of admission of adrenaline (A, 1 μg/ml) to the test chamber is indicated by the solid horizontal line (——). The broken line (Ca, —·—) indicates the admission of a depolarizing KCl solution containing 20 mM Ca^{2+} to the end compartments of the sucrose gap bath (see figure 7). The depolarizing solution had previously contained 10^{-3} M EGTA. Admission of calcium to the end compartments induces a delayed hyperpolarizing s_∞ shift of 4 mV, and abolishes the adrenaline-induced s_∞ depolarizing shift. (McNaughton 1974.)

Since the perfusion techniques that have been so successfully employed in squid nerve are not practical in cardiac muscle, it is difficult to alter intracellular ion concentrations directly. However, a possible way of doing this depends on the use of the sucrose gap technique. McNaughton & Spindler (1973) have developed a double sucrose gap technique for Purkinje fibres that allows attempts to be made at altering the intracellular calcium concentration by

varying the free calcium concentration at the cut ends in the end pools of the apparatus (see figure 7). Using this technique, McNaughton (1974) has shown that excess calcium does inhibit the action of adrenaline on the *s* activation curve and that this inhibition may be removed by exposing the fibre ends to a solution containing the calcium chelator EGTA (see figure 8).

It can also be seen from figure 8 that the effect of calcium ions themselves is to shift the activation curve in a hyperpolarizing direction. This is what one would expect if Ca^{2+} were to bind at inside surfaces near the I_{K_2} channels and the observation also excludes the possibility that significant quantities of calcium are leaking through the sucrose gap to increase the extracellular calcium in the test gap since, as noted above, extracellular calcium ions have the opposite action, i.e. they produce a depolarizing shift of the activation curve.

Nevertheless, it is probably too early to conclude that intracellular calcium binding sites are definitely involved in mediating the adrenaline-induced shift of the *s* activation curve since the observations could bear other interpretations. As suggested to us by R. W. Tsien, intracellular calcium ions might also control one or other of the reactions related to cyclic AMP metabolism and the final action on the membrane could be as easily attributed to the addition of negative charges (e.g. by phosphorylation) as to the removal of positive charges on the inside surface of the membrane.

Moreover, it is unlikely that the calcium dissociation hypothesis can account for the actions of adrenaline on I_x and I_{Ca} since the view that adrenaline *reduces* free intracellular calcium concentration is only plausible during diastole. During systole, when the contractile machinery becomes activated, the intracellular calcium concentration must increase. We might, of course, suppose that the systolic and diastolic calcium concentration changes occur in opposite directions (as is indeed suggested by the observation that adrenaline speeds relaxation and can, in some circumstances, relax K contractures (Morad & Rolett 1972) but since the effects of adrenaline on I_x and I_{Ca} are observed in the plateau range of potentials, when the intracellular calcium concentration is increased to produce contraction, they cannot be attributed to a decrease in free intracellular calcium. On the other hand they are mediated by cyclic AMP (Tsien *et al.* 1972). Further experiments are clearly required to clarify these problems further, although it is already evident that differences of this kind in the mechanisms of action of adrenaline are not at all unexpected given the quite different effects observed on I_{K_2} and on I_{x_1} and I_{Ca}.

We should like to acknowledge the support of the Medical Research Council.

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