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THE STIMULATORY ACTION OF ACETYLCHOLINE ON ISOLATED RABBIT ATRIA

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Quinidine (2.5 to 5×10^{-6} g./ml.) decreased the maximum rate of depolarization of the action potentials (AP) of both isolated rabbit atrial and ventricular fibres recorded with an intracellular microelectrode and finally abolished electrical excitability and spontaneous rhythmicity. Acetylcholine (ACh) 10^{-6} restored these properties in the atrium (but not ventricle) without changing the membrane resting potential (MRP). Adrenaline 10^{-6} to 10^{-4} at the same time was without effect. The maximum rate of depolarization of an AP in response to an extra shock interposed between the 8th and 9th driving stimuli was increased by ACh 10^{-6} in atria which had received quinidine to a greater extent than that of the normal AP. It is suggested that ACh restarted atria arrested by quinidine by an action on the sodium "carrying system" (excitatory mechanism) and this was not the result of an increase in MRP. An hypothesis is put forward to explain the stimulatory and inhibitory effects of ACh on rabbit atria.

Acetylcholine (ACh) restarts isolated rabbit atria which have been arrested by quinidine (Briscoe and Burn, 1954). Armitage (1957) suggested that quinidine decreases the potassium permeability of the cell membrane so that repolarization becomes less and less complete and that ACh restarts such atria by increasing potassium permeability. This obviously implies a concomitant restoration of the membrane resting potential (MRP).

However, this hypothesis has not been substantiated with electrophysiological evidence and the results do not exclude the obvious alternative that the interaction of quinidine and ACh is on the excitatory mechanism (Johnson and Robertson, 1957). Indeed this latter possibility is compatible with the findings of Weidmann (1955a), Johnson (1956) and Johnson and McKinnon (1957a) that the action of quinidine on other cardiac tissue is directly on the excitatory mechanism, for it predominantly affected the depolarization phase of the action potential without changing the MRP.

The experiments in the present paper were designed to show whether there was any change in MRP associated with the restarting of quinidinized atria by ACh and whether there was any evidence to suggest that ACh itself had an effect on the sodium carrying system, namely the excitatory mechanism (Hodgkin and Huxley, 1952; Weidman, 1955b).

METHODS

The apparatus used was basically similar to that described previously (Johnson, 1956). The salient features of the floating grid electrometer cathode follower input stage (Murray, 1955, personal communication) without microelectrode were: (1) the rise time was <1 μsec., (2) the input resistance was $>10^{16}$ ohms, (3) the input capacitance was $<10^{-2}$ $\mu\mu$F., (4) the current drawn from cell by the microelectrode was $<10^{-16}$ A., (5) the grid current with ± 10 volt grid swing was $<10^{-15}$ A. and the drift ± 2 mV/hr.

Important modifications and differences in apparatus and technique from those described previously are as follows: (1) The organ bath was redesigned with an improved temperature stabilization (better than $\pm$ 0.1°) and was equipped with a temperature measuring device of low heat capacity and rapid response time, which was sensitive to changes of temperature of the muscle of 0.01°. (2) A feedback differentiator was modified to be capable of registering rates of rise from 1.5 to 1,500 V./sec. (3) The microelectrodes were filled by boiling in 3 M-KCl under reduced pressure for 20 min.

Isolated rabbit right atria or ventricle in Krebs-Henseleit solution were used. The tissue was driven at a constant rate of approximately 150/min. using external electrodes. In some experiments an extra shock was interposed at variable times between the

8th and 9th driving stimuli. The "single cell technique" was used (Johnson and McKinnon, 1957a) by which any one set of observations was made from recordings of the one cell.

The drugs used were quinidine sulphate (Burroughs Wellcome), acetylcholine chloride (Roche), and hyoscyamine sulphate.

They were made up in Krebs-Henseleit, added directly to the organ bath and washed out by passing fresh Krebs-Henseleit solution through the bath.

RESULTS

Effect of ACh on the Membrane Resting Potential of Atria Arrested by Quinidine

The effects of ACh (10^{-6} g./ml.) on normal atria were identical to those described for isolated guinea-pig atrium (Johnson and McKinnon, 1957b), consisting of an increase in the rate of repolarization with no change in membrane resting potential (MRP), maximum rate of depolarization or amplitude of the action potential. When the tissue was exposed to quinidine 2.5 to 5×10^{-6}, the typical effects already described for Purkinje and ventricular fibres (Weidmann, 1955a; Johnson, 1956) were seen, namely a progressive decrease in the maximum rate of depolarization which was accompanied eventually by a fall in the amplitude of the action potential, and finally, after a period of approximately 10 to 20 min., the atrium failed to respond to electrical stimuli and lost its spontaneous rhythmicity. At this point ACh 10^{-6} was applied and in every case (10/10) there was a transient appearance of propagated action potentials of low voltage and low rate of depolarization. The maximum rate of depolarization and amplitude of these action potentials rose and fell during the period of their transient appearance (10 to 60 sec.). This restoration of electrical excitability produced by ACh was never accompanied by any detectable change in MRP. Further applications of ACh frequently restored electrical excitability for several minutes and also restored spontaneous rhythmicity. All these effects were abolished or prevented by hyoscyamine (10^{-6}). This "stimulating" action of ACh appears identical to that described by Briscoe and Burn (1954). In view of the failure to detect any change in the MRP, it appeared worth while to proceed with an investigation of the effects of ACh on the "sodium carrying system."

Effect of ACh on the "Sodium Carrying System" of Normal and Quinidinized Atria

The maximum rate of depolarization can be considered as proportional to the sodium current into the cell or the activity of the "sodium carrying system" (Hodgkin and Katz, 1949; Weidmann, 1955b). By observing the change in the maximum rate of depolarization of an action potential due to an extra shock interposed between the 8th and 9th driving stimuli, an index can be obtained of the effects of a drug on the rate of recovery of the "sodium carrying system."

In the normal fibre, it was frequently impossible to initiate an extra action potential of diminished rate of rise compared with the normal without evoking it during the latter part of the repolarization phase of the previous action potential (see Fig. 1*a*). This indicates that the rate of recovery

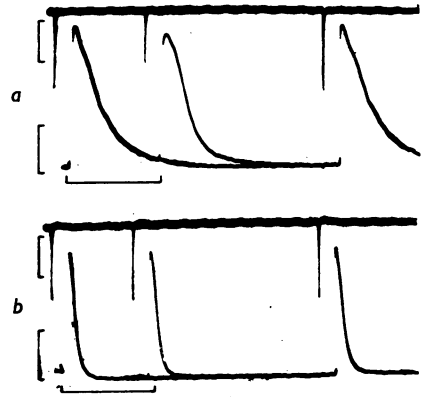

FIG. 1.—Effect of ACh on the action potential (AP) of the normal isolated rabbit atrium driven at 210/min. In both (*a*) and (*b*): upper trace, first differential of AP; lower trace, AP. In each trace, the first and last AP are in response to the 8th and 9th driving stimuli and the middle AP to the extra shock. Calibration: Upper ordinate, 100 V./sec.; lower, 30 mV.; abscissa, 100 msec. (*a*) Shows extra AP of diminished maximum rate of depolarization (1st differential) evoked during the repolarization phase of previous AP. (*b*) After 30 sec. exposure to ACh 10^{-6}. The maximum rate of depolarization of the extra AP has increased equalling those of the normal AP.

of the "sodium carrying system" of the normal fibre closely followed the repolarization phase of the action potential.

The application of ACh 10^{-6} increased the maximum rate of depolarization of the extra shock under these circumstances often to that of the "normal" action potential (see Fig. 1*b*). This is most likely due not to a direct effect on the rate of recovery of the "sodium carrying system" but to the accompanying increase in the rate of repolarization produced by ACh, since, after ACh, the extra action potential was now evoked at a time when the membrane potential had returned to normal.

If after ACh an extra action potential was evoked, especially during the latter part of the repolarization phase, repetitive firing in response

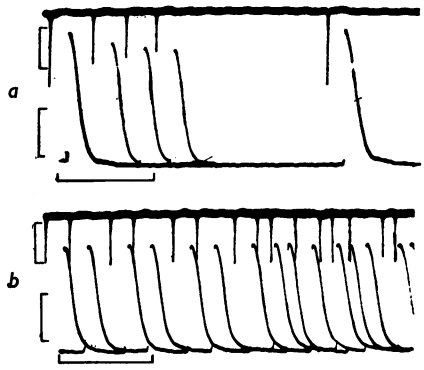

FIG. 2.—Effect of ACh on the action potential (AP) of the normal isolated rabbit atrium driven at 210/min. Same cell as in Fig. 1. In both (*a*) and (*b*): Upper trace, first differential of the AP; lower trace, AP. Calibration: upper ordinate, 100 V./sec.; lower, 30 mV.; abscissa, 100 msec. (*a*) After 60 sec. exposure to ACh 10^{-6}. The first and last AP are in response to the 8th and 9th driving stimuli. The second AP is in direct response to an extra shock; note repetitive firing following this extra AP. (*b*) After 75 sec. exposure to ACh 10^{-6} sustained repetitive firing originating as in (*a*). Record is of two consecutive traces superimposed with free running time base.

to this extra shock was often observed (see Fig. 2) (Hoffman and Suckling, 1953). Suggestion of a direct effect of ACh on the excitatory mechanism was afforded by the occasional observation that, in the untreated fibre with an unusually low rate of depolarization, ACh 10^{-6} increased the maximum rate of rise and amplitude of the action potentials (in response to the normal driving stimuli) with no detectable change in MRP.

During the early stages of treatment with quinidine, it was frequently possible to evoke, prior to arrest, an action potential of diminished rate of depolarization at a time when repolarization of the previous action potential was complete (see Fig. 3*a*). This indicates that quinidine can produce a dissociation between repolarization and restoration of the "sodium carrying system" as suggested previously by Weidmann (1955a) and Johnson and McKinnon (1957a). Application of ACh 10^{-6} under these conditions increased the maximum rate of depolarization of both the normal and extra action potentials. The latter was affected to a greater extent and frequently the two values became similar (see Fig. 3*b*).

It appears that ACh increases the maximum rate of depolarization of the extra and the normal action potentials by an effect on the "sodium carrying system" which is not the result of a change in MRP; for the extra action potential was evoked some time after repolarization of the previous action potential and ACh had no effect on the MRP.

Effect of Adrenaline in Atria Arrested by Quinidine

In the only experiment in which it was tested, adrenaline failed to restore electrical excitability of the atria arrested by quinidine 5×10^{-6}. However, the presence of adrenaline did not affect the restoration of electrical excitability by ACh 10^{-6} added immediately afterwards. This phenomenon was repeated several times.

Therefore it appears that the restarting action of ACh is not due to its causing the release of adrenaline nor does adrenaline affect this stimulatory action of ACh.

Effect of ACh in Normal and Quinidinized Ventricle

ACh 10^{-6} to 10^{-4} did not restore in 3/3 experiments the electrical excitability of isolated rabbit ventricle which had been arrested by cooling or quinidine. ACh had no effect on the maximum rate of depolarization of partially quinidinized

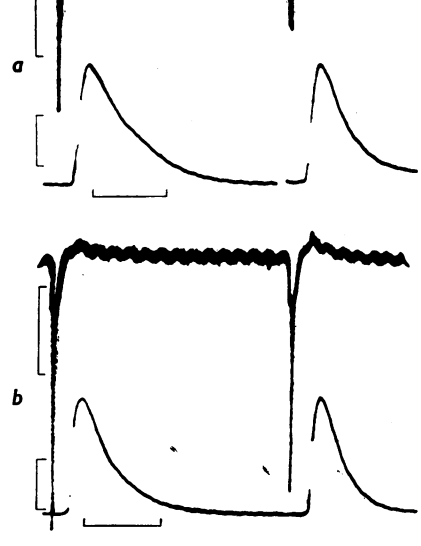

FIG. 3.—Effects of ACh on the action potential (AP) of the isolated rabbit atrium driven at 165/min. in the presence of quinidine. In both (*a*) and (*b*): Upper trace, first differential of AP; lower trace, AP. In each trace, the first AP is in response to the 8th driving stimulus and the second to an extra shock. The tissue did not respond to the 9th driving stimulus, for it fell in the refractory period of the extra AP. Calibration: upper ordinate 7.5 V./sec.; lower, 30 mV.; abscissa, 100 msec. (*a*) 10 min. exposure to quinidine 5×10^{-6}. Note that, although the extra AP is evoked some time after repolarization of the preceding "normal" AP is completed, its maximum rate of depolarization (1st differential) is less than that of the latter. (*b*) as in (*a*) but after 30 sec. exposure to ACh 10^{-6}. Both maximum rates of depolarization are increased, that of the extra AP more than the "normal."

ventricle or on that of an extra action potential evoked between the 8th and 9th driving stimuli in normal and quinidinized ventricle.

Discussion

The results of the present work clearly indicate that ACh can restart an atrium arrested by quinidine without raising the MRP. They show that ACh has a restorative effect on the "sodium carrying system." Previous work has shown that quinidine interferes with this system (Weidmann, 1955a; Johnson and McKinnon, 1957a) and this interference is sufficient to explain the ultimate arresting action of quinidine in rabbit atrium.

The hypothesis proposed by Armitage (1957) and supported by Burn (1956) that ACh restarts an atrium arrested by quinidine by increasing the potassium permeability of the cell membrane thus presumably restoring the MRP is not supported by our findings. Their hypothesis is based on the fact that by reducing the potassium concentration in the bathing medium (K_o) the rate and size of contractions of atria which have been depressed by quinidine were increased again. The most probable explanation for this effect is that the reduction in K_o to $\frac{1}{4}$ and $\frac{1}{2}$ normal would result in an increase in MRP. Judging from the results of Weidmann (1955b) this hyperpolarization would cause an increased availability of the "sodium carrying system" giving an increase in the number of resting sodium-carrying units and an increase in the maximum rate of depolarization of a subsequently evoked action potential.

We have recently obtained further evidence of an action of ACh on the excitatory mechanism of rabbit atrial cells, for, even though it reduces the membrane resistance, it decreases the amount of depolarization required to initiate an action potential (Johnson and Robertson, 1958). Marshall (1957) has shown that cooling causes a fall in the MRP of rabbit atrial fibres and the restarting action of ACh in these circumstances is accompanied by a rise in membrane potential. However, ACh can restart isolated rabbit atrial fibres arrested by cooling without altering the MRP (Johnson and Robertson, unpublished observations). Thus it appears that the excitatory actions of ACh on the heart described by Burn (1956) cannot be attributed entirely to ACh increasing the MRP and the rate of repolarization.

We reaffirm the following hypothesis (Johnson and Robertson, 1957) which takes into account the action of ACh on the rate of repolarization, the membrane potential and the excitatory mechanism. The usual inhibitory action of ACh on the rate of beat of pacemaker cells is probably due, as is well known, to an increase in potassium permeability which diminishes the rate of diastolic depolarization responsible for rhythmicity. The arrest of atria by quinidine, cooling and fatigue and the restarting by ACh are almost certainly the result of inactivation and restoration of the "sodium carrying system." Although the availability of this system is directly related to the membrane potential (Weidmann, 1955b), it can also be affected independently of it as in the case of quinidine. One or both of these two mechanisms are involved in the restoration by ACh of the availability of this system in arrested atria. Thus the effect of ACh on the MRP may be the more important in the case of fatigued fibres which are reported to have a low MRP (Weidmann, 1955a). On the other hand ACh can restart quinidinized atria by the more direct effect on the "carrying system" and finally both mechanisms could operate in the case of cooled fibres when a change in MRP occurs.

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