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THE EFFECT OF ADRENALINE ON THE VENTRICULAR MUSCLE OF TOAD

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Electrophysiological studies on action of adrenaline on cardiac muscle have already been reported by some investigators (1, 9), and recently the experiments on the same subjects by means of the microelectrode technique also appeared in various papers (4, 6, 8). But these results seem insufficient to clarify its action and effecting mechanism. Especially, influences on the slow repolarization phase were not studied in detail. This paper describes experiments bearing on these points.

METHOD

All the experiments were made on the ventricular muscle of Japanese toad. The heart was excised and dissected complete at the annulus fibrosus. The ventricular muscle was also cut into two portions and the lower portion (apex side) was used as the experimental material.

The preparation was fixed by small needles on the cork plate in the moist chamber made from acrylite and immersed in Ringer solution. A pair of needles fixing the preparation was coarted with the insulating paint except for two millimeter from the tip and was used as the stimulating electrodes.

Care was taken to keep constant the amount of Ringer solution in the moist chamber, into which Ringer solution containing adrenaline with concentration from 1×10^{-5}g/ml to 1×10^{-4}g/ml was introduced.

The measurement of the membrane potential was performed through a d-c amplifier with the cathode follower input and 3M-KCl filled capillary glas microelectrode of resistance in range between 10 and 30 megohm. The conventional method described by previous workers (13, 16) was adopted in order to measure the changes of membrane resistance. Both electrode for this purpose were inserted with the distance of 0.1 mm.

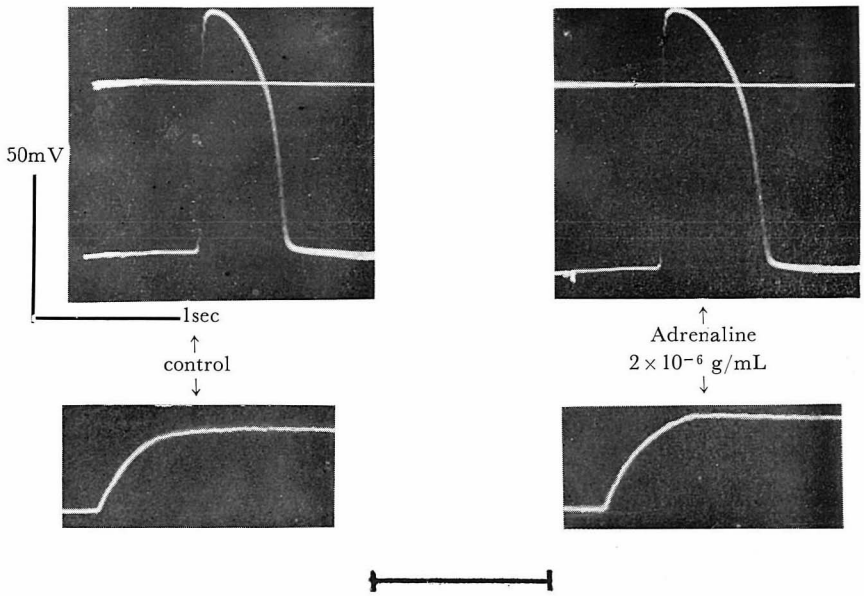
RESULTS

Effects of adrenaline on the still-standing heart muscle are illustrated in the records of Fig. 1. These preparation were arrested by applying the stimulus of 100 c.p.s.. When the concentration of adrenaline is low ($2-6 \times 10^{-6}$g/ml), both

resting and action potential of the still-standing heart muscle was slightly elevated. In addition to these changes, worthy to note, the slow repolarization phase is rather prolonged. On the other hand, when adrenaline in same concentration was applied to the spontaneously contracting heart muscle no detectable change could be observed.

With increasing the concentration of adrenaline (8×10^{-6}g/ml-2×10^{-5}g/ml), both resting and action potential show the lower value than the former case: these values lie under the level of normal state (resting potential; 53mV, action potential; 75mV, in average).

In such a concentration, it is not seldom to recover the spontaneous activity, but the heart rate reaches only several beat a minute at most, though both resting and action potential are increased in height with the lapse of time. When the repetitive stimulation with the frequency of 1.5 c. p. s. is applied to such a preparation, it is possible to recover its spontaneity in almost all case. In the recovered heart muscle, both resting and action potential are silghtly increased and



ADRENALINE ON STANDING HEART MUSCLE

Fig. 1. Effects of adrenaline (5 10^{-6}g/ml) on the still-standing heart muscle
Upper; the action potential evoked by artifical stimulation.
Note the increase of membrane potential and on the prolongation of plateau (see DISCUSSION).
Lower; the charging curves obtained in the same period corresponding to the left.

slow repolarization phase is shortened, as already described by *Otsuka* (8).

When adrenaline is more concentrated ($2-5\times10^{-5}$g/ml), the changes of electrical activity became more evidently. In early stage, the slow repolarization

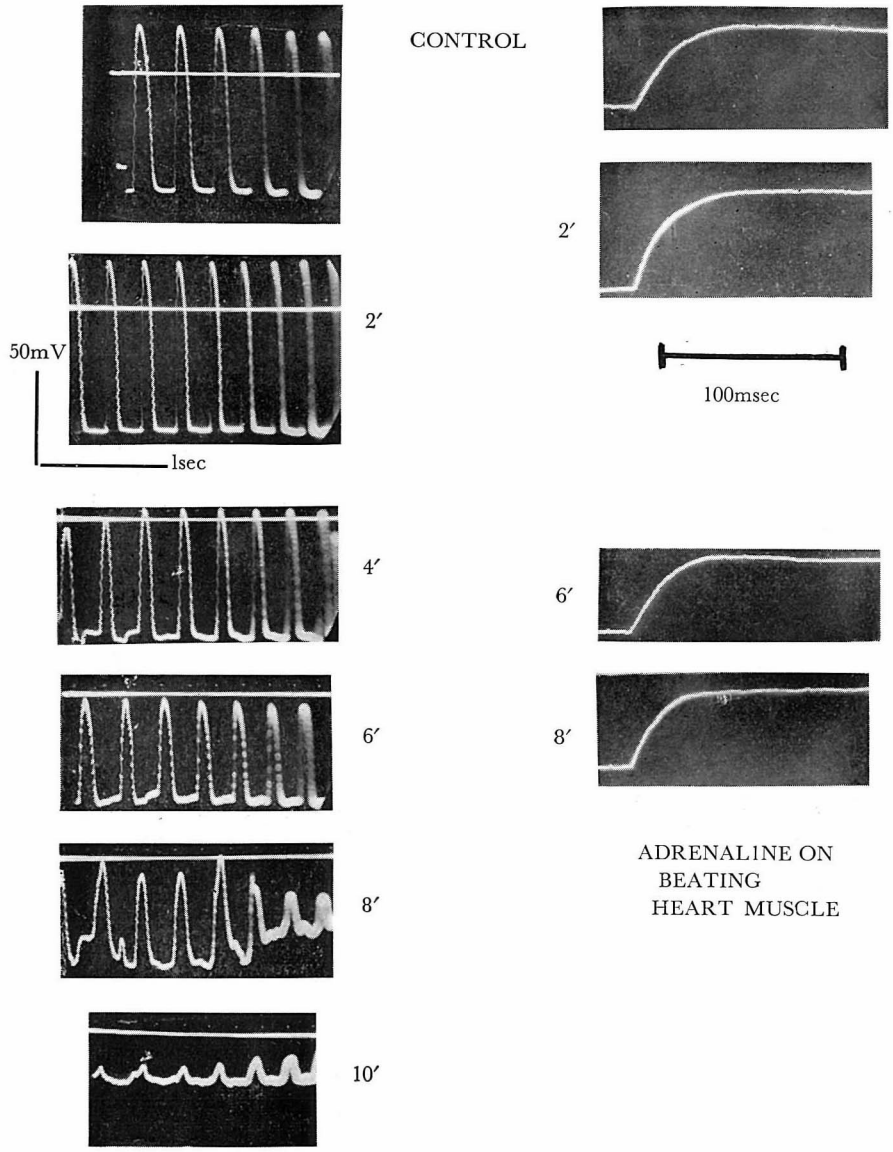
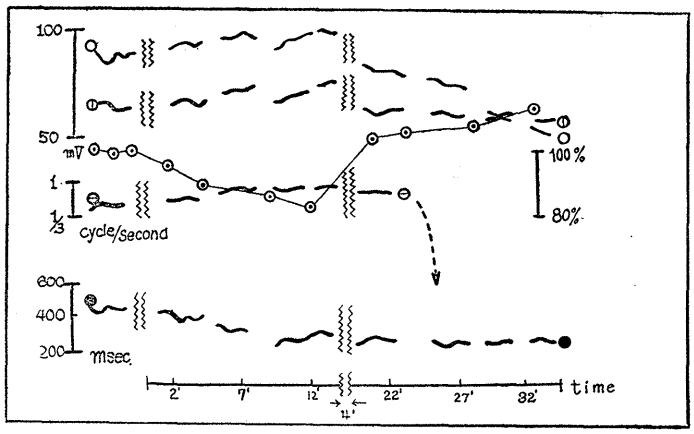


Fig. 2. The successive records of effects of adrenaline (3×10^{-5}g/ml) on the spontaneously contracting heart muscle.
Left; the action potential evoked spontaneously.
Right; the charging curves obtained in the same period corresponding to the left.
Numbers show the time in minute after the administration of drug.

phase disappeared by and by. When the preparation was left without any treatment in this case, both resting and action potential are decreased in amplitude and the duration of slow repolarization phase is completely disappeared. Especially the decrease in height of action potential is remarkable and the overshoot could not be recognized (Fig. 2). Similar features were encountered because of the destruction of electrode or the unsuitable insertion. But the records in Fig. 2. were obtained by the repetition of the selection of electrodes and the careful insertion. In addition to these changes mentioned above, moreover, the lengthening of rising phase was observed. It is reported by Johnson (7) that the similar results were obtained by application of cardiac stabilizing agents, for example, procaine or quinidine.

The arresting of the heart beat owing to the administration of large dosage of adrenaline may be called as the systolic arrest. In this state, the resting potential remained at the level ranging between 25 mV and 30 mV. And even if the careful washing with fresh Ringer solution was repeated, complete recovery could be hardly obtained. Generally speaking, the effects of adrenaline seems to be continued during several hour after administration in such a concentration.

The records in the right raws in Fig. 1 and Fig. 2 illustrate changes of membrane resistance during the administration of adrenaline. These exponential curves were obtained by charging the mambrane with the square wave of 300 msec. duration. The time constant of the curve obtained by such a method are approximately 14–18 msec., and this may be reasonable value refering to these of previous papers (5, 13, 15, 18). When the dosage of adrenaline is small the membrane resistance decreases to 70%–80% of mormal value approximately. However, by



○ ACTION POTENTIAL ⊖ BEATING FREQUENCY
① RESTING POTENTIAL ● PLATEAU DURATION
⊙ MEMBRANE RESISTANCE

Fig. 3. Effects of adrenaline (2×10^{-5} g/ml) on spontaneously contracting heart.

the administration of large quantity of adrenaline, the membrane resistance increases rather to 120–150% of control value. As shown in Fig. 3, both resting and action potential are lowered in this stage. In later stage after the administration of large dosage, the membrane resistance is again lowered. When the heart muscle falls in the state of systolic arrest, the lowering of membrane resistance was clearly recognized.

Discussion

It has been already reported that the application of adrenaline to the excised beating heart musle produces the increase of membrane potential and the rate of rise of action potential (8). The elevation of resting potential is brought about by the increase of K-conductance and the shortening of rising phase depends upon the function of sodium carring system (15, 16, 18, 19). Moreover the increase of K-conductance must cause the shortening of slow repolarization phase, because the slow repolarization phase is affected through the K-ouflow, and by increasing K-conductance its duration must be shortened (12, 13).

In the present experiment, the duration of slow repolarization phase of beating heart is typically shortened, and the resting potential is increased. But the elevation of resting potential is observed only when the dosage is fairly small or in the initial stage of dosing of large quantity. In the later stage after the administration of high concentration, though the slow repolarization is shortened, the resting potential is lowered to 25mV–35mV and the spontaneous activity is ceased at last. From these results the intoxication by the large dosage of adrenaline must be considered.

By applying the small dosage on the still-standing heart muscle, beside the increase of resting potential, the prolongation of slow repolarization phase is observed. These changes may be not conceivable from the viewpoint that adrenaline increases the K-conductance. On the other hand, the membrane resistance is lowered by the application of small dose, but contrary to our expectation, with the increasing of dosing quantity its value rather increases.

If the membrance resistance depends only on the K-conductance, these results are difficult to explain reasonably. Similar changes of membrane resistance caused by strophanthine have been demonstrated by *Dudel* and *Trautwin* (5). Hence these positive inotropic agents can be generally assumed to decrease the membrane resistance in large quantity reversively.

The difference of electrical activity between the standing heart muscle and beating one e.g. the prolongation of plateau in the former and the shortening in the latter, might be not so resonably interpreted. It is likely assumed that the quality of membranes of still standing heart muscle differs from that of beating heart muscle.

Considering the fact that the resting potential of standing heart muscle tends to decrease, the properties of membrane would be changing with the progress of time. Moreover the decrease in membrane potential is often regained to the original value through stimulation artiffcially. The possible explanation for a series of question mentioned above may be hardly obtained by basing on the results observed in the present experiment. Perhaps the chnges of metabolic state of heart muscle affect seriously the slow repolarization phase. In fact, its disappearance under the unfavorable condition of metabolic state has been described by many authors(2, 3, 10, 11). As already suggested by some authors (5, 8), the critical relationship to the activation and the inactivation of sodium carring system are regarded to play the main part under these condition.

SUMMARY

1. The effects of adrenaline in various concentration (1×10^{-6}g/ml-1×10^{-4} g/ml) are studied on the still-standing and beating heart musles of japanese toads.

2. In low concentration of the drug or in early stage in high concentration, the action potential of standing heart muscle, especially the slow repolarization phase, is slightly prolonged. On the contrary, its duration of beating heart muscle is shortened by same procedure.

3. In later stage of large dosing of adrenaline, heart muscle ceases to beat in the systolic state and the resting potential shows a very low value (25mV–35mV).

4. The relation between the ionic conductance of membrane and the action potential by the administration of adrenaline was discussed.

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