EFFECTS OF ADRENALINE, NORADRENALINE AND RESERPINE ON THE TRANSMEMBRANE POTENTIALS IN BOTH PACEMAKER AND NON-PACEMAKER FIBERS OF THE RABBIT ATRIUM

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Some years ago, Bulbring and Burn (1) showed that the choline acetylase activity in aceton-dried powder from the atrium which had ceased to beat was low, but the activity was increased when acetylcholine was added before incubation and the increase was proportional to the amount of acetylcholine added. Thus, they considered that the endogenously liberated acetylcholine sustained the building up of excitement in the pacemaker and as a result automatic contractions were initiated and maintained. Kottegoda (2) reported that a high concentration of acetylcholine had a stimulant action on the rabbit atrium in the presence of atropine.

On the other hand, much information about the electrophysiology and pharmacology of the cardiac cells has been obtained by the use of the intracellular microelectrode. The effects of neurohumors such as acetylcholine and sympathomimetic amines on the transmembrane potentials of the atrial fibers were studied by many workers. Acetylcholine produces a marked acceleration of the repolarization in atrial muscle of the dog (3), cat (4), rat (5) and rabbit (6). Acetylcholine causes a slight increase in steepness of the depolarization in the dog atrium (3); carbamylcholine does the same in the cat atrium (4). In the atrium of the dog (3), cat (4) and rat (5) acetylcholine slightly increases the resting potential. Adrenaline lowers the resting potential of the rat atrium (5), while in the dog atrium the resting potential is elevated by adrenaline and noradrenaline (7). In the dog auricular tissue (8) and chick embryo heart (9), the amines increase the rate of repolarization and shorten the total duration of action potential without significantly affecting the resting potential. Recordings of the transmembrane potential from the specific pacemaker area gave light on the origin of impulse formation in the heart. In the sinoatrial node of the isolated rabbit atrium (6, 10), acetylcholine slows the atrial rate and decreases the slope of diastolic depolarization, and increases the resting potential in pacemaker fibers. On the contrary, adrenaline applied to the same region accelerates the heart rate, and increases the slope of diastolic depolarization of the pacemaker fibers. In Purkinje fibers of the sheep heart, rate acceleration produced by adrenaline is mainly due to an increase in slope of the prepotential (11).

The present experiment deals with the effects of catecholamines and their potent releaser, reserpine, on the transmembrane potentials of pacemaker and non-pacemaker
fibers in the isolated spontaneously beating atria of rabbits. It was hoped to find out whether catecholamines, which are continuously synthesized and liberated in the cardiac tissue, are concerned with the initiation and maintenance of the atrial rhythmicity.

METHODS

Rabbits, weighing 1.8 to 2.2 kg, were sacrificed by bleeding from the both common carotid arteries. The experiments were carried out on isolated spontaneously beating atria according to the technique described by Matsumura and Takaori (12). However, in this case 40 ml of nutrient solution was used to immerse the atrium. The pacemaker region of the sinoatrial node was localized as previously reported by West (10).

The transmembrane potentials of pacemaker and non-pacemaker fibers were recorded on a double-gun cathode ray oscilloscope and on the ink-writing oscillograph, being amplified by means of d.c. amplifier.

The drugs employed were l-adrenaline hydrochloride, dl-noradrenaline hydrochloride, reserpine (in ascorbic acid) and atropine sulfate. They were added into the side chamber which was conducted to the atrial bath. The drug concentrations were expressed in terms of g/ml.

RESULTS

1. Effects of adrenaline

The effects of adrenaline and noradrenaline on the atrial transmembrane potential were studied in the concentration which exhibited positive inotropic and chronotropic actions on the atrial preparation of the rabbit.

a) Pacemaker transmembrane potential

The typical effects of adrenaline in concentrations of $10^{-7}$ to $10^{-4}$ on the pacemaker potential are illustrated in figures 1A and 1B. Owing to a lowering in the level of depolarization threshold, the duration of the pacemaker prepotential was shortened, and the atrial rate was accelerated. Significant changes were not observed in the slope of prepotential or, if any, only a minor decrease. The magnitude of the action potential was also not significantly affected in most cases, but some preparations exhibited a slight increase. The rate of repolarization was somewhat increased, and thus the total duration of the action potential was shortened. Higher concentrations of adrenaline produced further shortening in the duration of both prepotential and action potential, and hence marked acceleration of the atrial rate.

In a few cases in the experiment, the application of adrenaline in the same dose ranges caused a diminution in magnitude of the action potential and a disappearance of the overshoot. Two to three minutes after the adrenaline application a number of small depolarizations, which might be due to occurrence of irregular potentials in the pacemaker fibers, were observed in the repolarization phase (Fig. 1C). Such effects of adrenaline were sometimes observed in the preparation previously treated with reserpine.
b) Non-pacemaker transmembrane potential

The application of adrenaline above the concentration of $10^{-3}$ increased the rate, and shortened the total duration of action potential in non-pacemaker fibers, accelerating the rate of repolarization (Fig. 2). When applying higher concentrations of adrenaline, these changes became more apparent.
2. Effects of noradrenaline

The application of noradrenaline above the concentration of $2 \times 10^{-4}$ revealed similar effects on the transmembrane potentials in the pacemaker and non-pacemaker fibers to those observed after the application of adrenaline. Noradrenaline used throughout the present experiment was of the $dl$-form. Hence, the concentrations of noradrenaline were double those of adrenaline in order to compare the effects of both amines in corresponding amounts of $l$-form.

3. Effects of reserpine

Reserpine was used in concentrations of $10^{-5}$ to $10^{-6}$, which were considered to deplete 90 to 97% of the normal amount of the catecholamines of the heart. Ten to twenty minutes after the reserpine application, the following changes of the transmembrane potential appeared gradually in the pacemaker and non-pacemaker fibers.

\textit{a) Pacemaker transmembrane potential}

As illustrated in figures 3A and 3B, the application of reserpine in the above-mentioned concentrations exhibited an increase in the total duration of the action potential which
was mainly ascribed to a lengthening of the repolarization phase. Despite of a lowering of the threshold potential, reserpine prolonged the duration of the diastolic prepotential due to a considerable decrease in slope of the prepotential. This resulted in retardation of the heart rate. The rate of rise of the action potential was not altered, but the magnitude of depolarization was reduced, and frequently the overshoot disappeared. The reduction in magnitude of the depolarization was approximately proportional to the time elapsed after the reserpine application. Finally, the disappearance of the pacemaker transmembrane potential was obtained at one to two hours after the reserpine application.

b) Non-pacemaker transmembrane potential

When reserpine was applied in the

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**FIG. 3A.** The effect of $10^{-4}$ g/ml of reserpine on the pacemaker potential.

**FIG. 3B.** Gradual changes in the pacemaker potential 12, 18, 25, 38, 65 and 95 minutes after reserpine, $10^{-4}$ g/ml, are seen. Ninety-six minutes after reserpine the application of adrenaline $10^{-4}$ g/ml, suddenly restored the magnitude of action potential, but not the atrial rate.
above-mentioned concentration, a lengthening in the total duration of the action potential accompanied by a retardation of the rate, and a slight reduction in the overshoot were seen in the non-pacemaker fibers. These changes are seen in Fig. 4. Even at a stage of marked reduction in magnitude of the depolarization of the pacemaker potential and a considerable slowing of the atrial rhythm, only a slight reduction in magnitude of the non-pacemaker potential was observed. The disappearance of the non-pacemaker potential was subsequent to that of the pacemaker potential.

\[\text{Reserpine } 10^{-4}\]

\[\text{20 min after}\]

\[\text{40 min after}\]

\[\text{60 min after}\]

\[\text{90 min after}\]

**Fig. 4.** The effect of $10^{-4}\text{ g/ml of reserpine on the non-pacemaker potential.}\]

4. **Irreversibility of reserpine action**

After the effects of reserpine on the transmembrane potential had fully developed, repeated replacement of the reserpine contained Tyrode solution by a reserpine-free one could not restore the normal potential and rhythm. On the contrary, reinforcement of the rate retardation and further reduction in magnitude of the depolarization of the pacemaker fibers were frequently observed on replacement of the nutrient solution. However, if the alteration of the potential following the reserpine application for a short period...
was not fully developed, replacement of the nutrient solution usually restored it to almost normal potential.

5. Effects of adrenaline and noradrenaline on the atrium in the presence of reserpine

When the atrial preparation exhibited a marked rate slowing and other signs following the reserpine application, the addition of adrenaline or noradrenaline into the reserpine contained nutrient solution restored the normal rhythm and configuration of the potentials in the pacemaker and non-pacemaker fibers. However, the concentration of the amines required to reverse the reserpine action was usually 10 to 100 times higher than that of the amines which affected the action potential before reserpine. Figure 5 illustrates the antagonistic effects of $2 \times 10^{-4}$ of noradrenaline on the pacemaker potential treated by previous administration of $10^{-4}$ of reserpine. The total duration of the potential in the pacemaker fibers was shortened, and the atrial rhythm was increased. In the control experiment before the application of reserpine, the effective concentration of noradrenaline required to produce the rate acceleration in the same preparation was $2 \times 10^{-4}$. The antagonistic effect of adrenaline against reserpine did not significantly differ from that of noradrenaline (Fig. 3B).

![Reserpine 10^{-4}](image)

Noradrenaline $2 \times 10^{-4}$

1 min after noradrenaline

**FIG. 5.** The effect of $2 \times 10^{-4}$ g/ml of noradrenaline on the pacemaker potential 19 minutes after the application of $10^{-4}$ g/ml reserpine.

6. Effects of adrenaline and noradrenaline on the reserpinized preparation from which reserpine was washed-out

As mentioned in section 4, the effects of reserpine on the transmembrane potentials in the pacemaker and non-pacemaker fibers were usually irreversible even on repeated
washing of the preparation. Therefore, it was necessary to test whether adrenaline and noradrenaline could reverse the delayed action of reserpine which was preserved after washing the preparation.

The amines again restored the normal potential, but the concentration required to reverse the reserpine action varied somewhat with the time course after the washing. Twenty to twenty-five minutes after washing the preparation, a relatively high concentration of adrenaline (10^-4) was required to antagonize the reserpine action (Fig. 6 & 7). In some cases, the antagonistic effect of the amine was transient and the potential was reduced in magnitude again (Fig. 6). However, the same concentration of the amine sometimes

Adrenaline 10^-4

![Graph showing the effect of adrenaline on the pacemaker potential](image)

Fig. 6. The effect of 10^-4 g/ml of adrenaline 20 minutes after washing-out of 10^-4 g/ml of reserpine.

A

Adrenaline 10^-4

B

C

Fig. 7. The effect of 10^-4 g/ml of adrenaline on the pacemaker potential 25 minutes after washing-out of 10^-4 g/ml of reserpine applied for 27 minutes. The gradual increase of the height of the depolarization and of the slope of prepotential without significant changes in the rate is seen.

A : Before reserpine.

Reserpine in the concentration of 10^-4 g/ml was applied for 27 minutes between A) and B).

B : The alterations soon after adrenaline addition.

C : Three minutes after adrenaline.
resulted in long-lasting restoration to almost normal potential (Fig. 7). After repeated application and washing of the preparation with the amine, eventually lower concentration ($10^{-4}$) of the amine was enough to reverse the reserpine action. The increase in concentration of adrenaline ($10^{-4}$) produced a more conspicuous effect. Thus obtained preparations fully sensitized to react to low concentrations of the amines responded with both increased rhythm and magnitude of the potential (Fig. 8). Whereas, the restoration of the reduced potential to normal magnitude following the amine applied at an early stage of the washing-out of reserpine was not accompanied by an increase in rate (Fig. 6 & 7).

Corresponding concentrations of noradrenaline produced similar results to adrenaline (Fig. 9).

\[\text{Adrenaline } 10^{-4}\]
\[\downarrow\]
\[\text{2 min after Adrenaline } 10^{-7}\]
\[\downarrow\]
\[\text{4 min after}\]

Fig. 8. The effect of adrenaline on the pacemaker potential three hours after washing-out of reserpine applied for 25 minutes.

Three hours later, $10^{-4}$ g/ml of adrenaline was added. The height of the depolarization, the slope of prepotential and the frequency of rhythm gradually increased. Adrenaline was increased up to $10^{-7}$ g/ml five minutes later. More marked increase occurred.

\[\text{Noradrenaline } 2 \times 10^{-7}\]
\[\downarrow\]
\[\text{3 min after}\]

Fig. 9. The effect of $2 \times 10^{-7}$ g/ml of noradrenaline on the pacemaker potential 45 minutes after washing-out of $10^{-4}$ g/ml of reserpine applied for 20 minutes.
7. Restoration of the abolished transmembrane potential

Occasionally, the application of $10^{-7}$ of reserpine for a long period to the atrial preparation completely abolished the transmembrane potentials in the pacemaker and non-pacemaker fibers. Macroscopically visible movement of the atrium disappeared before the manifestation of marked reduction in height and rate of the potential. The replacement of the nutrient solution at this stage of the reserpine action occasionally rather reinforced the depressant action of reserpine. When the reserpine action further advanced and the potential changes were completely abolished, the replacement of the nutrient solution could not restore the potential changes or, if any, in small magnitude. On the contrary, the application of adrenaline or noradrenaline restored the potential changes (Fig. 10). For the restoration of the abolished transmembrane potential a threshold concentration of the amines was required. This is shown by figure 11. The application of $10^{-6}$ of adrenaline reproduced the potential. But a lower concentration of the amine was without effects. On replacement of the nutrient solution the reappeared potential changes disappeared again, but in a few cases, the potential changes were preserved in a long sequence.

8. Effects of atropine

The application of $10^{-4}$ to $10^{-3}$ of atropine to the atrial preparation did not significantly modify the transmembrane
potentials in both pacemaker and non-pacemaker fibers. The same concentrations of atropine did not affect the reserpine effect exhibited on the rate and configuration of the potential.

DISCUSSION

The results described in this report deal with the effects of adrenaline, noradrenaline and reserpine on the transmembrane potentials in the atrial pacemaker and non-pacemaker fibers. West et al. (6) showed that adrenaline increased the rate of diastolic depolarization (prepotential slope) and accelerated the atrial rate when applied to the sinoatrial node of the isolated rabbit atrium. The evidence was established in the Purkinje fiber pacemaker from sheep hearts (11). The present results somewhat differed from these results in that there were not significant changes in slope of the prepotential but the threshold potential was lowered following adrenaline and noradrenaline. Results concerning the action of adrenaline on the atrial pacemakers might be complicated by a frequent shift of the pacemaker site after application of this agent. Apart from this, both adrenaline and noradrenaline accelerated the repolarization of action potential and shortened the duration of the potential, particularly in non-pacemaker fibers. Acetylcholine also does the same in the atrial muscles (3-6). There is another similarity between the effects of the amines and acetylcholine. The administration of the latter to isolated atrial preparation results in the appearance of sustained spontaneous activity at a very high rate. Following the increase of the spontaneous activity, arrhythmia, often of long duration is observed (3). Similar sequences were obtained by the application of either amine. These data may suggest that adrenaline and noradrenaline, as well as acetylcholine, play some part in initiation and maintenance of the potentials in the atrial pacemaker and thus in spontaneous rhythmicity of the atrium.

Concerning the rhythmicity of the atrial pacemaker, Bulbring and Burn (1) concluded that it is initiated and maintained by the endogenously liberated acetylcholine. Their conclusion was derived from the experimental results that the amount of acetylcholine and the choline acetylase activity in atrial preparation were proportional to the rhythmic activity of the atrium, and that the atrium which had ceased to beat contained less acetylcholine and less activity of choline acetylase than fresh atrium, and further that the rhythmic activity was restarted by the addition of acetylcholine in an optimal concentration. On the other hand, adrenaline and noradrenaline were found to be contained in amounts of 1.2 to 3.0 μg/g in hearts of various animals, being for the most part noradrenaline (13-16). The auricle contains much larger amounts of noradrenaline than the ventricle (15). Thus, experiments on the atrial preparation under depletion of the catecholamines might provide a clue to know whether they are concerned with initiation and maintenance of the atrial activity. Since reserpine above the dose of 1.0 mg/kg depletes more than 90% of the normal content of the catecholamines in the heart of cat (17), dog (18, 19), rabbit (13, 20) and rat (21), and moreover the depletion of catecholamines from
the extirpated organ occurs following in vitro administration of reserpine, the effects of reserpine on the action potential of isolated atrial fibers were studied when it was applied into the atrial chamber.

The concentration of $10^{-4}$ of reserpine was considered to be sufficient to deplete a considerable part of the heart catecholamines. The results obtained following this concentration of reserpine are as follows: 1) Lengthening in duration of the repolarization phase in both pacemaker and non-pacemaker fibers, 2) reduction in magnitude of the depolarization, 3) reduction in slope of the diastolic prepotential in pacemaker fibers and 4) retardation of the atrial rate. The final effect after reserpine was the arrest of the atrium without manifesting significant changes in the resting potential and the non-pacemaker action potential. It is unlikely that these reserpine effects are derived from its direct action on the tissue, because of slow onset of action and irreversibility on repeated washing of the preparation. The alteration of the atrial potential obtained following the application of reserpine may have been correlated with the depletion of the catecholamine from the atrium. The evidence that the effects of adrenaline or noradrenaline on the atrial potentials are approximately the opposite of those of reserpine may also suggest the depletion of both amines in the tissue by reserpine. It is probable that the former effects are due to uptake of the amines into the cell membrane, while the latter effects are due to the liberation of the amines from the membrane. The atrial potentials affected by the application of reserpine were usually reversed following adrenaline or noradrenaline. However, in the presence of reserpine the atrial preparation revealed less sensitivity to both amines and 10 to 100 times higher concentrations of the amines were required to exhibit their effects than in reserpine-free preparations. This may allow the assumption that reserpine not only liberates the catecholamines from the membrane but also prevents the uptake of the amines into the membrane, and hence that higher concentrations of the amines are required to enter the membrane for antagonizing the reserpine action. The reserpinized atrium gradually increased its sensitivity to the catecholamines with the time elapsed after washing the preparation. Rather higher sensitivity to the amines was obtained in the preparation two to three hours after the washing than in the normal one. Small amounts of the amines which were inactive on the normal preparation could easily antagonize the reserpine effect and hence restore normal pattern of the potentials. It has already been shown by Burn and Rand (22), and Shimamoto and Nakamura (23) that the reserpinized animal responded to the catecholamine with an increased sensitivity as in the denervated structure. They concluded that the increased sensitivity of the reserpinized animal to adrenaline or noradrenaline might result from the depletion of the amines in tissue. The increased sensitivity to the amines of in vitro reserpinized atrium may also be correlated with the depletion.

Occasionally, the application of reserpine completely abolished the action potential in both pacemaker and non-pacemaker fibers without affecting the resting potential. Macroscopically visible movement of the atrium disappeared before abolishment of the potential
changes. The action potential could not be restarted by the replacement of the nutrient solution, but restored by the addition of optimal amounts of adrenaline or noradrenaline. When the concentration of amines applied was not high enough to preserve for a long period, the restarted potential changes gradually disappeared again. It is possible that there is a difference between the concentrations of the amine required to initiate and to maintain the action potential. Even in fully restored cases, a removal of the amines from the nutrient solution frequently resulted in a reduction and finally disappearance of the potential changes again. These findings may suggest that the restarting and hence the initiation and maintenance of the potentials in atrial fibers are concerned with the presence of a certain amount of the catecholamines in the nutrient solution. The uptake of the amines into the atrial fibers is supposed to be performed only in the presence of optimal amount of the amine. On the other hand, after repeated application and washing of the preparation with the amine, the restarted action potential was preserved even in absence of the amines in the nutrient solution. From this, it seems likely that the catecholamines when applying repeatedly over a long period restore the endogenous production of the amines in the atrium.

Although Bülbbring and Burn (1) showed that the liberation of acetylcholine from the tissue conditioned the initiation of the heart rhythm, the results in the present experiment indicate that the endogenously liberated adrenaline and noradrenaline may also be concerned with the initiation of the atrial rhythm. The atrium receives dual innervation as in other autonomic structures. It is unlikely that only acetylcholine, the parasympathetic transmitter, is an initiator of the atrial rhythmicity, while adrenaline and noradrenaline, the sympathetic transmitters, are not the initiators and rather modulators of the atrial rhythmicity. Hukovic (24) showed that electrical stimulation of the sympathetic nerve of the reserpinized atrium induced negative inotropic and chronotropic effects, which were potentiated by eserine and antagonized by atropine. Burn and Rand (25) also showed that the positive inotropic and chronotropic effects of the reserpinized atrium in response to stimulation of the vagus nerve were cholinergic in origin. It is worth while to mention that the reserpine effects on the atrial transmembrane potential shown in the present experiment were not affected by the addition of atropine. It seems that the effects of reserpine have no correlation with the liberation of acetylcholine from the atrium.

**SUMMARY**

Using the intracellular microelectrode technique, the effects of adrenaline, noradrenaline and reserpine on the transmembrane potentials in the pacemaker and non-pacemaker fibers of isolated rabbit atria were studied.

The results obtained are as follows:

1. Adrenaline or noradrenaline in the concentration, which revealed positive inotropic and chronotropic effects on the atrial preparation of the rabbit, shortened the total duration of action potentials in both pacemaker and non-pacemaker fibers and accelerated the
atrial rate.

2. Reserpine, on the contrary, lengthened the duration of action potentials in both fibers. In the pacemaker fiber reserpine reduced the magnitude of depolarization and decreased the slope of prepotential, and hence retarded the atrial rate. Finally, one to two hours after the reserpine application, pacemaker and then non-pacemaker potentials disappeared without significant changes in the resting potential.

3. The above-mentioned effects of reserpine were usually irreversible even after repeated washing.

4. The effects of reserpine were antagonized by the addition of adrenaline or nor-adrenaline. However, relatively high concentrations of the amines were required to reverse the reserpine action in the presence of reserpine or at an early stage of the washing-out of reserpine. After repeated application and washing of the preparation with the amines, the atrial preparation became more and more sensitive to the amines and responded to lower concentrations with a returning of the potentials.

5. Physiological role of adrenaline and noradrenaline in initiating and maintaining atrial rhythmicity was discussed in correlation with the reserpine action.

REFERENCES

4) BURGEN, A.S.V. AND TERROUX, K.G. : J. Physiol. 120, 449 (1953)
7) TRAUTWEIN, W. : Experientia 12, 396 (1955)
8) SIEBENS, A.A., HOFFMAN, B.F., ENSON, Y., FARRELL, J.E. AND BROOKS, C. MCC. : Am. J. Physiol. 175, 1 (1953)
11) OTUKA, M. : Pflügers Arch. 266, 512 (1958)
12) MATSUMURA, M. AND TAKAORI, SH. : J. Pharmacol. 104, 103 (1952)
13) BERTLER, A., CARLSSON, A. AND ROSENGREN, E. : Naturwissenschaften 43, 521 (1956)