THE ANTAGONIZING MECHANISM OF ACETYLCHOLINE ON THE ATRIAL NON-PACEMAKER POTENTIALS DEPRESSED BY ADRENOLYTICS

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The depressive effects of adrenolytics on the transmembrane potentials of isolated atria in the intact and reserpinized rabbits have been described in the previous reports (1, 2). The heart which had ceased to beat by the application of the adrenolytics was easily restarted by the addition of adrenaline or noradrenaline. The recovery of the configuration of atrial non-pacemaker potentials by either amine was usually transient and incomplete, though almost full recovery of the repolarization phase of the potentials was observed. In the reserpinized atria, the action potentials depressed by the adrenolytics also restarted and recovered by the addition of acetylcholine except the rate and repolarization phase. The recovering effects of acetylcholine on the resting potential and the amplitude and depolarization phase of the action potentials were complete and long-lasting. These results suggested that endogenous acetylcholine maintained the depolarization phase, while endogenous noradrenaline regulated the repolarization phase of the transmembrane potentials.

The stimulating effects of acetylcholine on the spontaneous contraction of the heart have been reported by many investigators (3-8). These effects are likely to derive from endogenous noradrenaline released by acetylcholine since the stimulating effects of acetylcholine are prevented or blocked by the application of dichlorisoproterenol (DCI) or the full reserpinization (3-5 and 9). However, the restarting effects of acetylcholine on the atria of reserpinized rabbits which had been depressed by the adrenolytics were confirmed in the previous report (2). Moreover, it will be shown that the depressed heart by DCI can be restarted by the addition of acetylcholine but not of catecholamines (10). These evidences suggest that acetylcholine stimulates the heart directly but not indirectly through endogenously released noradrenaline.

On the other hand, the adrenolytics have been reported to affect the effects of acetylcholine in the various structures. Benfey et al. (11) have shown that the adrenolytics block parasympathetic effects of the vagus nerve in the isolated guinea-pig's atria. The blocking effects of dibenamine on the pharmacological action of acetylcholine in the aorta and strips of the stomach of rabbits and the isolated auricles of rats have
been reported by Furchgott (12). The anti-acetylcholine effects of chlorpromazine have been widely confirmed (13).

The present investigations are attempted to correlate depressive effects of the adrenolytics on the transmembrane potentials of the isolated rabbit's atria with endogenous role of acetylcholine.

METHODS

Seventy albino rabbits, weighing 1.8 to 2.3 kg and of either sex, were used in the experiments. Twenty rabbits were reserpinized by two daily successive intraperitoneal injections of 0.5 mg/kg before the day of isolation of the heart. The isolation of the atrial preparation and the recording of the transmembrane potentials were followed to the methods described in the previous report (1, 2). The transmembrane potentials were recorded from the atrial appendages. The parameters of the transmembrane potentials affected by acetylcholine were measured and compared at the peak effect between 1 to 5 minutes after the application of acetylcholine according to the same method as that in the previous report (2). The concentration of the drugs in the bath fluid was expressed in g/ml.

RESULTS

I. Recovering Effects of Acetylcholine on the Transmembrane Potentials of the Isolated Rabbit's Atria Abolished by Dibenamine

The application of dibenamine affected the transmembrane potentials of the atrial non-pacemaker fibers. As shown in Fig. 1-A to -D, 5×10^{-5} of dibenamine prolonged markedly the depolarization time, depressed the amplitude of the action potentials and decreased slightly the resting potentials. The effects progressed gradually until the total abolition of the action potentials about 90 minutes after the application of dibenamine. Thereafter, the addition of 10^{-5} of acetylcholine restarted the action potential within 1 to 2 minutes (Fig. 1-E). The addition of acetylcholine reversed
gradually and simultaneously the decreased resting potential, the prolonged depolarization phase and the depressed amplitude of the action potentials in all of 6 atria tested. The optimal concentration of acetylcholine required to reverse the dibenamine effects was $10^{-7}$ to $10^{-6}$. The peak effect of acetylcholine in the concentration of $10^{-4}$ was usually observed 3 to 5 minutes after the addition. The concentration below $10^{-7}$ was ineffective, while the concentration above $10^{-3}$ resulted in the manifestation of several action potentials 1 to 2 minutes after the addition. The higher concentration reduced markedly the rate of the potentials. Fig. 1-F shows the transmembrane potential 4 minutes after the second addition of $10^{-6}$ of acetylcholine after washing the preparation. The comparison of Fig. 1-E with Fig. 1-F shows that the repetition of the additions of acetylcholine and the washing-out of the nutrient solution resulted in the progressive recovery of the depressed transmembrane potentials. The recovering effects of acetylcholine on the configuration of the depressed potentials were more completely and longer-lasting than those of adrenaline or noradrenaline. Fig. 1-G shows the transmembrane potential 30 minutes after the second washing-out. The almost complete recovery to the level before the application of dibenamine is shown in the potential 5 minutes after the third addition of acetylcholine (Fig. 1-H). However, the rate of the potentials was more easily restored by the addition of adrenaline or noradrenaline than by that of acetylcholine.

The prolonged repolarization phase of the action potential of the atrial non-pace-maker fibers in the reserpinized rabbit was not fully recovered by the additions of acetylcholine (2), while the same phase of the action potentials of the intact atria was readily recovered by the addition of acetylcholine.

II. Recovering Effects of Acetylcholine on the Transmembrane Potentials of the Isolated Rabbit's Atria Abolished by Chlorpromazine

The application of $10^{-4}$ of chlorpromazine affected the atrial transmembrane potentials similarly to dibenamine. The addition of acetylcholine restarted the action potentials of the atria which had been abolished by chlorpromazine. Fig. 2 shows the details. The atrial non-pace-maker potential at 40 minutes after the application of chlorpromazine was depressed in amplitude and prolonged markedly in duration of the de- and repolarization phases (Fig. 2-B). At 70 minutes, thereafter, the action potentials disappeared totally. In 4 out of 5 preparations the depressed atrium by chlorpromazine was restarted by the concentration of $10^{-6}$ of acetylcholine. The depressed amplitude and the prolonged de- and repolarization phases of the action potentials were progressively restored by acetylcholine and the peak effect was observed 3 to 5 minutes after the addition. The repetition of the addition of acetylcholine and the washing of the preparation activated the recovery of the potentials. The restarted but still markedly depressed potential at 5 minutes after the second addition of acetylcholine is shown in Fig. 2-C. The relatively restored potentials recovered gradually and progressively without further
additions of acetylcholine. Fig 2-D shows the potential at 50 minutes after the second addition of acetylcholine, which exhibits marked recovery of the resting and action potentials compared with the potential in Fig. 2-C. Further increase of the overshoot of the action potential was observed 3 minutes after the third addition of acetylcholine (Fig. 2-E). The configuration of the potential at 30 minutes after the second washing of the preparation was fully recovered to the level before the application of chlorpromazine.

The addition of adrenaline or noradrenaline restarted easily the action potentials which had been abolished by chlorpromazine. However, the recovery was incomplete except the restoration of the repolarization phase, and was usually transient. The depressed rate of the potentials was also reversed by the addition of either amine, though the duration of the recovery was transient (1, 2). On the other hand, the addition of acetylcholine did not fully increase the rate of the potentials to the level before the application of chlorpromazine. However, the recovering effects of acetylcholine was long-lasting. The further increase of the concentration of acetylcholine resulted in the decrease of the rate.

In one preparation, the action potentials were abolished by chlorpromazine and failed to restart by the addition of $10^{-8}$ of acetylcholine. But the decreased resting potential by chlorpromazine increased markedly in voltage by the addition of acetylcholine without firing of action potential. Further addition of $10^{-8}$ of noradrenaline to the same preparation resulted in the decrease of the resting potential but not in the firing of action potential similarly to acetylcholine.

III. Recovering Effects of Acetylcholine on the Transmembrane Potentials of the Isolated Rabbit's Atria Abolished by Yohimbine

The application of yohimbine abolished the spontaneous rhythmic manifestation of the atrial non-pacemaker potentials, and the depressed potentials were restarted and recovered by the addition of acetylcholine. Fig. 3 shows the details of the results. Fig.
3-B and -C show the depressed potentials 45 and 70 minutes after the application of $10^{-5}$ of yohimbine, respectively. The depressed potentials show the marked prolongation of the depolarization phase accompanied with the depression of the amplitude and the manifestation of the small inflection or notch in the depolarization phase. About 100 minutes after the application of yohimbine the atrial non-pacemaker potentials disappeared totally. Thereafter, the addition of $10^{-6}$ of acetylcholine restarted the action potentials (Fig. 3-D). The recovery of the configuration of the potential was markedly seen in the shortening of the depolarization phase, accompanied with increase in the amplitude of the resting and action potentials. The peak effect of the recovery by the first addition of acetylcholine was observed 3 to 5 minutes after the addition. In 3 out of 5 preparations the first addition of acetylcholine restarted the atrial potentials sustainedly and in other 2 preparations the restarted potentials were observed during 5 to 10 seconds at 1 to 2 minutes after the addition of acetylcholine.

Though the restarted potentials by adrenaline or noradrenaline lasted for a relatively short period of time (1, 2), the potentials recovered to a certain extent of its configuration by acetylcholine were maintained for a considerably long period, and recovered spontaneously and progressively. Fig. 3-E shows the potential at 3 minutes after the second addition of $10^{-6}$ of acetylcholine. The amplitude of the action potential was still lower than that before the application of yohimbine. Fig. 3-F shows the potential at 3 minutes after the third addition of $10^{-6}$ of acetylcholine. The amplitude of the resting and action potentials and the duration of the de- and repolarization phases were almost the same as those before the application of yohimbine. The recovered rate of the potentials by acetylcholine was incomplete but relatively long-lasting, though the further increase of the concentration of acetylcholine resulted in the decrease of the rate.

![Fig. 3. Effects of yohimbine on the transmembrane potentials of the atrial fibers in rabbit (A-C). The recovery courses after the addition of acetylcholine (ACh) are shown in D-F.](image)
IV. Influences of Atropine on the Restarting Effect of Acetylcholine

The influences of $10^{-6}$ of atropine on the restarting effect of acetylcholine were observed in the atria, of which action potentials disappeared by the application of $5 \times 10^{-5}$ of dibenamine and were confirmed to be restarted by acetylcholine in all cases. Atropine was added 5 to 10 minutes before the expected time when the action potentials disappeared. The addition of $10^{-6}$ of acetylcholine restarted the action potentials only in one out of 4 preparations. Moreover, the restarted potentials in one preparation were small in amplitude of the action potential, though the effect of acetylcholine was considerably long-lasting. The higher concentration of acetylcholine restarted the action potentials in one out of other 3 preparations which had not restarted by the additions of $10^{-6}$ of acetylcholine. Though 2 preparations were not restarted even by the higher concentration of acetylcholine, the washing of the preparations resulted in the gradual and progressive recovery of the transmembrane potentials.

V. Effects of the Repetitive Administrations of Acetylcholine on the Responses of the Atrial Transmembrane Potentials to Adrenolytics

The prolongation of the depolarization phase of the non-pacemaker potentials in the un-treated atria produced by the adrenolytics was a sharp contrast to the prolongation of the repolarization phase in the reserpine-treated rabbit's atria by the same procedure (1, 2). In order to confirm the mechanism of the excitatory effect of acetylcholine, acetylcholine was added repeatedly at intervals of 10 to 15 minutes during the progression of the effects of the adrenolytics in the un-treated atria.

The repeated administrations of acetylcholine modified the responses of the atrial transmembrane potentials to the adrenolytics, as shown in Figs. 4-6. The arrows in these figures show the numbers of the repeated administrations of acetylcholine. The concentrations of acetylcholine are $10^{-8}$ (↑), $10^{-7}$ (↑↑) and $10^{-6}$ (↑↑↑).

Fig. 4 shows the modification of the atrial responses to dibenamine by the repetitive administrations of acetylcholine. Compared to the actions of application of dibenamine alone (Fig. 1-B and C), the prolongation of the depolarization phase of the action potentials was extremely diminished by the repetitive administrations of acetylcholine. However, the prolongation of the repolarization phase was similarly marked to that by the applica-
tion of dibenamine alone in the reserpinized atria (Fig. 4-B and -C). The resting potential was little affected. The prolongation of the repolarization phase was markedly observed even at the time-period when the amplitude of the action potential showed a profound depression (Fig. 4-D). The concentrations of acetylcholine which required to modify or reverse the actions of dibenamine were in ranges from $10^{-7}$ to $10^{-6}$.

The repetitive administrations of acetylcholine modified the atrial responses to chlorpromazine similarly to dibenamine. Fig. 5 shows the details of the results. The prolongation of the depolarization phase of the action potentials which was usually observed in the intact atria (Fig. 2-B) was prevented by the repetitive administrations of acetylcholine (Fig. 5-B and -C). On the other hand, the same procedure prolonged markedly the repolarization phase (Fig. 5-D). The concentrations of acetylcholine to produce these effects were in ranges from $10^{-8}$ to $10^{-7}$. The preparation shown in Fig. 5 was not depressed by chlorpromazine until the disappearance of the action potentials even 140 minutes after the application of $10^{-5}$ of chlorpromazine. The resting potential was little affected (Fig. 5-B to -F). Moreover, as shown in Fig. 5-F, the transmembrane potential could be recovered to the level before the application of chlorpromazine without washing out of the nutrient solution. From the results it is likely that the repetitive administrations of acetylcholine prevent the decrease of the amplitude of the resting and action potentials by chlorpromazine.

The effects of yohimbine on the atrial transmembrane potentials were also modified by the repetitive administrations of acetylcholine similarly to those of dibenamine or chlorpromazine, as shown in Fig. 6. Without treatment of acetylcholine, the application of $10^{-5}$ of yohimbine produced the prolongation of the depolarization phase accompanied with the marked depression of the amplitude of the action potential (Fig. 3-B and -C). On the other hand, the application of yohimbine followed with the repetitive administrations of acetylcholine produced the prolongation of the repolarization phase even before the marked manifestation of the depression of the amplitude and the prolongation of the depolarization phase (Fig. 6-B and -C). The concentrations of acetylcholine to modify the action of yohimbine were usually in ranges from $10^{-8}$ to $5 \times 10^{-8}$. 

- Fig. 5. Effects of chlorpromazine modified by the repeated administrations of acetylcholine (ACh).
  - A: Before, B: 10 min after the application of chlorpromazine ($10^{-5}$), C: 35 min after, D: 50 min after, E: 60 min after, F: 140 min after.
  - $\uparrow$: The administration of $10^{-8}$ of ACh, $\uparrow\uparrow$: The administration of $10^{-7}$ of ACh, $\uparrow\uparrow\uparrow$: The administration of $10^{-6}$ of ACh.
  - Time calibration shows interval of 100 msec. Vertical bar is a voltage calibration of 100 mV (See text).
The administration of acetylcholine in the concentration of $10^{-5}$ resulted in the shortening of the repolarization phase accompanied with the increase of the overshoot (Fig. 6-D). In the presence of acetylcholine, yohimbine did not produce the marked prolongation of the depolarization phase, though the marked depression of the amplitude and the marked prolongation of the repolarization phase were steadily observed (Fig. 6-E and -F).

### VI. Antagonistic Effects of Adrenolytics on the Responses of the Atrial Transmembrane Potentials to Acetylcholine

The effects of the adrenolytics on the action of acetylcholine were observed in the concentrations which did not abolish the atrial non-pacemaker potentials. The adrenolytics were applied 5 minutes before the addition of $10^{-8}$ to $10^{-6}$ of acetylcholine. It was clear that the higher concentrations of the adrenolytics could block or antagonize the decreasing effects of acetylcholine on the rate and duration of the action potentials, as shown in chapter V.

### Table 1. Antagonistic Effects of Adrenolytics on the Parameters of the Atrial Transmembrane Potentials to Acetylcholine

<table>
<thead>
<tr>
<th>Adrenolytic</th>
<th>Receptor potential (mV)</th>
<th>Duration of action potentials (msec)</th>
<th>Repolarization time (msec)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>83</td>
<td>144 ± 5.9</td>
<td>139.2 ± 5.1</td>
<td>-43.5</td>
</tr>
<tr>
<td>Acetylcholine $10^{-6}$</td>
<td>10</td>
<td>94 ± 1.2</td>
<td>144.1 ± 1.2</td>
<td>94.1</td>
</tr>
<tr>
<td>Acetylcholine $10^{-5}$</td>
<td>11</td>
<td>95 ± 3.5</td>
<td>145 ± 3.5</td>
<td>95.8</td>
</tr>
<tr>
<td>Yohimbine $5 \times 10^{-4}$</td>
<td>8</td>
<td>96 ± 4.3</td>
<td>146 ± 4.3</td>
<td>96.2</td>
</tr>
<tr>
<td>Chlorpromazine $10^{-7}$</td>
<td>7</td>
<td>95 ± 3.9</td>
<td>145 ± 3.9</td>
<td>95.5</td>
</tr>
<tr>
<td>Dibenamine $10^{-6}$</td>
<td>7</td>
<td>95 ± 4.2</td>
<td>145 ± 4.2</td>
<td>95.0</td>
</tr>
</tbody>
</table>

* Standard error: ↑ The pre-treatment of adrenolytics, 5 minutes before the addition of acetylcholine.
As shown in Table 1, the mean reduction of the total duration of the atrial non-pacemaker potentials in response to $10^{-6}$ of acetylcholine was 60.5%. The reduction by the same dose of acetylcholine in the atria treated with $10^{-6}$ of dibenamine was 28.3%. Fig. 7 shows the differences in the configuration of the atrial transmembrane potentials produced by $10^{-6}$ of acetylcholine between the un-treated and dibenamine-treated atria. In the un-treated atrium $10^{-6}$ of acetylcholine produced a marked shortening of the total duration and slight increases of the amplitude of the resting and action potentials (Fig. 7-C and -D). On the other hand, the shortening of the total duration was not observed in the atrium treated with $10^{-6}$ of dibenamine, although the slight increase of the amplitude of the action potential by acetylcholine was still detected in the presence of dibenamine (Fig. 7-c and -d). The decrease of the heart rate in response to $10^{-6}$ of acetylcholine is shown in Fig. 8. Each point in the figure represents the mean value in 7 to 12 rabbits.

![Fig. 7. Effects of acetylcholine (ACh) on the transmembrane potentials of the un-treated (A-D) and dibenamine-treated (a-d) atria.](image)

A : Before, B : 1 min after the application of $10^{-6}$ of ACh, C : 3 min after, D : 5 min after.

a : Before, b : 5 min after the application of $10^{-6}$ of dibenamine, c : 3 min after the application of $10^{-6}$ of ACh, d : 5 min after.

Time calibration shows interval of 100 msec. Vertical bar is a voltage calibration of 100 mV (See text).

![Fig. 8. Effects of $10^{-7}$ of acetylcholine on the spontaneous rate of the un-treated, chlorpromazine-, yohimbine-treated and reserpinized atria. Each point in the figure represents the mean value in 7 to 12 rabbits.](image)
Acetylcholine was blocked manifestedly by $10^{-6}$ of dibenamine, as shown in Fig. 9.

Similarly, the presence of $10^{-6}$ of chlorpromazine, or $5 \times 10^{-6}$ of yohimbine prevented the shortening of the total duration and the decrease of rate of the action potentials in response to $10^{-7}$ of acetylcholine. The results are shown in Table 1 and Fig. 8.

**VII. Differences of the Effects of Acetylcholine on the Transmembrane Potentials in the Un-treated and Reserpinized Atria**

The effects of $10^{-8}$ to $10^{-6}$ of acetylcholine on the rate and configuration of the non-pacemaker potentials were compared in the un-treated and reserpinized atria. As was described in the previous report (2), the reserpinization of the animal decreased the rate and prolonged the repolarization time, especially the phase 3 of the repolarization. The time course of the mean reduction of the rate by $10^{-8}$ to $10^{-6}$ of acetylcholine did not differ markedly on the average of 9 to 12 preparations between both intact and reserpinized atria, as shown in Figs. 8 and 9.

![Figure 9](image.png)

**FIG. 9.** Effects of $10^{-8}$ and $10^{-6}$ of acetylcholine on the spontaneous rate of the un-treated, dibenamine-treated and reserpinized atria. Each point in the figure represents the mean value in 7 to 12 rabbits.

The comparative changes of the parameters of the non-pacemaker potentials in response to $10^{-8}$, $10^{-7}$ and $10^{-6}$ of acetylcholine in the un-treated and reserpinized atria are shown in Table 2. Though the amplitude of the resting and action potentials were not affected by acetylcholine, the percentage changes of the shortening of the total duration and the repolarization time were significantly more marked in the reserpinized atria than in the un-treated ones.
DISCUSSION

The cholinergic mechanism of the adrenolytics in the isolated atria of rabbits was studied by observing the mode of the restarting and maintaining effects of acetylcholine on the non-pacemaker potentials depressed or abolished by the application of the adrenolytics. In addition, the effects of acetylcholine on the non-pacemaker potentials of the intact and reserpinized atria were observed comparatively.

The atrial potentials which had been abolished by the application of dibenamine, chlorpromazine and yohimbine were restarted by the concentration of $10^{-7}$ to $10^{-6}$ of acetylcholine. The atrial action potentials were restarted 1 to 2 minutes after addition of acetylcholine. The peak effect of the recovery was obtained 3 to 5 minutes after the addition. The recovering effects of acetylcholine were activated by the repetitive additions and the washing of the preparations. The decreased amplitude of the resting and action potentials as well as the prolonged total duration and especially the prolonged depolarization phase were gradually recovered to the level of those before the application of the adrenolytics. The recovering effects of acetylcholine were complete in the configuration of the transmembrane potential and long-lasting, compared with the recovering effects of adrenaline or noradrenaline (1, 2). On the other hand, acetylcholine was less effective in recovering the decreased rate by the adrenolytics, and the higher concentration of acetylcholine decreased the rate profoundly. The intensity of the restarting effects of $10^{-6}$ of acetylcholine differed considerably according to adrenolytics and it was in the order of the following description: dibenamine, chlorpromazine and yohimbine.
Though the depolarization phase of the action potential was depressed by the adrenolytics in the intact atria (1), the repeated administrations of $10^{-8}$ to $10^{-4}$ of acetylcholine at the interval of 10 to 15 minutes modified the atrial responses to the adrenolytics. The prolongation of the depolarization phase was diminished extremely, while that of the repolarization phase was augmented by these procedures. The reduction of the resting potential was lessened. The concentration of acetylcholine to produce these modifications of the atrial responses to the adrenolytics was highest against dibenamine and least yohimbine. Further, the decrease of the rate and the shortening of the total duration of the atrial potentials caused by acetylcholine were confirmed to be antagonized by the adrenolytics in the concentration which did not abolish the atrial non-pacemaker potentials. The antagonistic effect was strongest in dibenamine and least in yohimbine. The results described above show that acetylcholine exhibited strongest restarting effect on the action potentials which had been abolished by dibenamine and dibenamine was most powerfully antagonistic against the inhibitory effect of acetylcholine.

The excitatory effect of acetylcholine on the atria has been concluded to derive from the endogenously released catecholamine (3-5 and 9), since 1) the effect manifests in the atria treated with atropine, 2) the effect is blocked by the treatment of the atria with ganglion blocking agents and DCI, and 3) the effect does not manifest in the reserpine-pretreated atria. It has been reported that the similar stimulating effect of acetylcholine was eliminated by phentolamine, an adrenergic blocking agent (6). This suggests that the stimulating effects of acetylcholine derive from the adrenergic mechanism. On the other hand, it has been reported that the electrical excitability of the atria depressed by the application of quinidine is not restored by adrenaline but restored by acetylcholine, and that the restoring effect of acetylcholine is prevented by hyoscymamine (7).

The addition of adrenaline or noradrenaline restarted the non-pacemaker action potentials which had been abolished by the adrenolytics, but the recovery of the configuration of the potentials was incomplete and transient (1). On the other hand, the recovering effects of acetylcholine on the similarly depressed atria were shown to be complete and long-lasting in the present experiments. The similar restarting effects of acetylcholine have been shown in the reserpinized atria by the adrenolytics (2). Further, it will be described that the action potentials which are abolished by DCI are restarted by acetylcholine but not by adrenaline or noradrenaline (10). These evidences support the conclusion that the restarting and recovering effects of acetylcholine originate from some direct effect and not from the endogenously released catecholamine by acetylcholine.

The blocking effects of the adrenolytics on the negative chronotropic and inotropic actions of acetylcholine in the heart were shown by several investigators (11, 12) and were confirmed in the present experiments. The order of intensity of the adrenolytics in blocking the negative responses of the atria to acetylcholine accorded well with that
of the antagonizing effect of acetylcholine to reverse the atrial depression by the adrenolytics. These evidences suggested that the reversing effect of acetylcholine might not be blocked by atropine. However, the pretreatment of the atrial preparation with atropine lessened the restarting effect of acetylcholine on the arrested atria by dibenamine. Takaori et al. (14) have shown that high concentration of atropine causes marked irregularities of the atrial action potentials accompanied with reduction of the rate and amplitude but the followed addition of acetylcholine restores the almost normal potentials. It has been reported that the reduction of the rate and the shortening of the total duration caused by acetylcholine are blocked by atropine (15). Therefore, it is likely that atropine interferes with the restarting effect of acetylcholine related with the blockade of some effects other than the muscarinic effect. Since Johnson et al. (7) have shown that the restarting effect of acetylcholine on the depressed atria by quinidine is prevented by hyoscyamine, the more detailed observations on the changes of the configuration of the potentials caused by acetylcholine and the adrenolytics in the intact and reserpinized atria should be needed.

The assumption presented by Burgen and Teroux (16) that acetylcholine increases the permeability of the cardiac cell membrane toward potassium ion was supported electrophysiologically (17, 18) and radioisotopically (19, 20). Armitage (8) has shown that the antagonizing action of acetylcholine against the atrial depression caused by quinidine results from the restoration of the permeability of the cell membrane toward potassium ion and the concomitant increase of the resting potential. The assumption was supported by Burn (3) and Trautwein (9). However, Johnson et al. (7) have concluded that the restarting effect of acetylcholine derives from the direct mechanism on the sodium carrying system of the atrial fiber and not from the increase of the resting potential by demonstrating the recovering effect of acetylcholine on the reduced maximum rate of depolarization of the action potential in the atria of which electrical excitability and spontaneous rhythmicity are abolished by quinidine without changing the resting potential.

A definite decrease of the resting potential was observed in the atria at the time when the action potentials were abolished by the adrenolytics (1). The restarted transmembrane potentials low in height of the amplitude and slow in rate of the depolarization showed a gradual but not prompt recovery of the resting potential by the addition of acetylcholine. The evidence indicates the rationality of the assumption that the mechanism of restarting effect of acetylcholine consists in the increased permeability of the cell membrane toward potassium ion. However, some atria depressed by the adrenolytics were not restarted by acetylcholine inspite of the clear-cut hyperpolarization, and the repeated administrations of acetylcholine blocked the prolongation of the depolarization phase by the adrenolytics. These evidences suggest the restarting mechanism of acetylcholine other than the hyperpolarization origin. The possibility of the direct affection on the sodium carrying system could not be excluded from the present experiments.
The possible mode of mechanism that acetylcholine regulates and maintains the depolarization phase of the action potentials, while adrenaline and noradrenaline do so the repolarization phase has been described in the foregoing report (2). The results shown in the present experiments may serve to substantiate the possibility. The results that the percentage changes of the shortening of the repolarization time were more marked in the reserpinized atria than in the un-treated ones may be an indirect evidence to support the possibility.

**SUMMARY**

The interrelations of effects of acetylcholine and adrenolytics such as dibenamine, chlorpromazine and yohimbine on the transmembrane potentials of the isolated rabbit's atria were studied with the microelectrode technique and effects of acetylcholine on the non-pacemaker potentials of the intact and reserpinized atria were observed comparatively.

1. The action potential which had been abolished by the application of the adrenolytics was restarted after the addition of $10^{-7}$ to $10^{-6}$ of acetylcholine. The recovering effects of acetylcholine were activated by the repetition of its addition and the washing of the preparation. The decreased amplitude of the resting and action potentials and the prolonged de- and repolarization phases were gradually recovered to the level of those before the application of the adrenolytics. The recovery of the transmembrane potentials by acetylcholine was complete and long-lasting in the intact atria. However, the restoration of the decreased heart rate was incomplete.

2. The intensity of the restarting effects by $10^{-6}$ of acetylcholine differed considerably according to the adrenolytics and it was in the order of the following description: dibenamine, chlorpromazine and yohimbine.

3. The pretreatment with atropine ($10^{-4}$) lessened the restarting effect of acetylcholine on the arrested atria by dibenamine.

4. The repeated administrations of $10^{-8}$ to $10^{-6}$ of acetylcholine in the course of action of the adrenolytics modified the atrial responses to the adrenolytics. The prolongation of the depolarization phase was diminished extremely, while that of the repolarization phase was augmented by these procedures. The reduction of the resting potential was lessened. The concentration of acetylcholine to produce these modifications was highest against dibenamine and least against yohimbine.

5. The decrease in heart rate and total duration of the action potential caused by acetylcholine was inhibited by the adrenolytics. The antagonistic effect of the adrenolytics on acetylcholine was strongest in dibenamine and least in yohimbine.

6. The percentage changes of the shortening of the total duration by acetylcholine were significantly more marked in the reserpinized atria than in the un-treated ones, though the decreasing effect of acetylcholine on heart rate did not show a significant difference between them.

7. It is discussed that the restarting and recovering effects of acetylcholine originate
from some direct effects and not from endogenous catecholamine released by acetylcholine, and that acetylcholine regulates and maintains the depolarization phase of the action potential, while catecholamine does so the repolarization one.

REFERENCES

1) MISU, Y.: This Journal. 13, 167 (1963)
2) MISU, Y.: Ibid. 14, 43 (1964)
3) BURN, J.H.: Function of Autonomic Transmitters Williams & Wilkins Company, Baltimore (1956)
5) LEE, W.C. AND SHIDEMAN, F.E.: J. Pharmacol. 126, 239 (1959)
6) ELIASIM, M., BELLET, S., TAWIL, E. AND MULLER, O.: Circulation Res. 9, 1372 (1961)
8) ARMITAGE, A.K.: Ibid. 12, 74 (1957)
10) MISU, Y., TANAKA, C. AND TAKAORI, S.: This Journal, to be published
14) TAKAORI, S., INOKI, R., TODA, N. AND TACHI, S.: This Journal 10, 137 (1961)
16) BURGEN, A. S.V. AND TERRONX, K.G.: J. Physiol. 120, 449 (1953)
18) FATT, P. AND KATZ, B.: J. Physiol. 115, 329 (1951)
19) HARRIS, E.J. AND HUTTER, O.F.: Ibid. 133, 58p (1956)