EFFECTS OF DOPAMINE AND DOPA ON THE ISOLATED RABBIT'S ATRIUM TREATED WITH RESERPINE

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In the foregoing report Matsuo and Tachi (1) showed that though the isolated atrium of rabbit which was beating in vitro took up considerable amounts of noradrenaline added to the bath fluid, the content of noradrenaline of the restarted atrium by addition of noradrenaline did not significantly differ from the reduced content of noradrenaline of the atrium which was ceased to beat by addition of 10^-7 of reserpine. From the results the authors suggested that the restarting effect derived from the pharmacological action of the catecholamine and not from the biochemical binding of catecholamine to the storage site. The role of the dopa-decarboxylase in noradrenaline synthesis was substantiated with the demonstration of the conversion of dopa and of dopamine to noradrenaline (2-3). In this report the effects of dopa and dopamine were studied on the spontaneous contraction, transmembrane potentials and content of noradrenaline in the isolated rabbit's atrium which had been depressed or abolished the spontaneous activity and depleted the content of noradrenaline by reserpine.

METHODS

Albino rabbits, weighing 1.5 to 2.5 kg, were used. The animal was killed through hemorrhage by cutting both common carotid arteries. The heart was immediately extirpated and the atrial preparation was made following the technique described elsewhere (4). The movement of the preparation was recorded on the smoked drum and the rate of the contraction was counted every five minutes. The recording of the transmembrane potential of the right atrium was followed the technique described by Toda (5). The drugs used in the experiment were administered to the bath fluid in the organ bath. After the incubation of the drugs with the beating atrium in the organ bath the atrium was taken up and the content of noradrenaline was measured fluorophotometrically as described before (6).

The drugs employed were dl-dopa, dl-dopamine hydrochloride and reserpine which was dissolved in the mixture solution of phosphoric acid, propylene glycol and glucose. The concentrations of the drugs were expressed in terms of g/ml.
RESULTS

I. Effects of Dopamine

The addition of dopamine in the concentration above $10^{-6}$ to the isolated atrium of rabbit increased the frequency and the amplitude of the rhythmic contraction. As is shown in Fig. 1, the increase in the amplitude of contraction in response to $10^{-5}$ of dopamine manifested immediately and reached its peak effect from three to five minutes after the administration. The increasing effect decreased gradually until the constant amplitude was obtained for a while, and thereafter the amplitude reincreased gradually and progressively. The length of the time of the secondary increase in the amplitude was more than thirty minutes. The effect of dopamine on the rate of the rhythm did not usually go along with that on the amplitude. The maximal increase in the rate was usually obtained at about ten minutes after the application of $10^{-5}$ of dopamine and thereafter the rate decreased gradually. However, even at thirty minutes after the application of dopamine there was still an increase in the rate which was about ninety per cent of the maximal rate.

Fig. 1. Effects of $10^{-5}$ of dopamine on the rate and amplitude of spontaneous contraction of the normal isolated atrium. The figures on the top are the counts of rate.

The rate of the transmembrane potentials of the atrium increased and the configuration of the potentials was also affected by the addition of $5 \times 10^{-8}$ to $10^{-5}$ of dopamine. As is shown in Fig. 2, the application of $10^{-5}$ of dopamine induced a slight and transient decrease in the rate both in pacemaker and atrial non-pacemaker potentials followed by a marked increase. The increase in the rate was long-lasting and still observed at twenty minutes after dopamine. In accordance with the manifestation of the increase in the rate the slope of the prepotential in the pacemaker potential increased and the duration of the action potential decreased. However, the threshold potential,
Fig. 2. Effects of $10^{-3}$ of dopamine on the transmembrane pacemaker potential.

I: control, II: 1 minute after the addition of dopamine, III: 4 minutes after the addition of dopamine.
the maximal diastolic potential and the magnitude of the potential were not affected. In the atrial potentials only the decrease in the duration was observed.

As in shown in Table 1, the administration of $10^{-5}$ of dopamine to the normal atrium for two hours did not significantly affect the content of noradrenaline in the right and left atria. In the left atrium some reduction of the content of noradrenaline was observed, but the reduction was statistically not significant.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>No. of Experiments</th>
<th>Right Atrium</th>
<th>Left Atrium</th>
<th>Whole Atrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>1.32±0.04</td>
<td>0.83±0.10</td>
<td>1.09±0.09</td>
</tr>
<tr>
<td>Dopamine ($10^{-5}$, 2 hrs)</td>
<td>5</td>
<td>1.32±0.16</td>
<td>0.75±0.02</td>
<td>1.00±0.07</td>
</tr>
<tr>
<td>Dopa ($10^{-5}$, 2 hrs)</td>
<td>5</td>
<td>1.35±0.16</td>
<td>0.88±0.07</td>
<td>1.11±0.10</td>
</tr>
</tbody>
</table>

II. Effects of Dopamine on the Isolated Atrium of which Spontaneous Contraction and Transmembrane Potentials were Abolished by Reserpine

As was reported before (4, 5), the administration of adrenaline or noradrenaline restarted the spontaneous contraction and the transmembrane action potentials of the atrium, spontaneous rhythmicity of which had been abolished by reserpine. The administration of $10^{-5}$ of dopamine revealed the similar effects. However, as is shown in Fig. 3, the restarting effects of dopamine were somewhat different from those of adrenaline or noradrenaline. The administration of the effective concentration of adrenaline or noradrenaline restarted the rhythm dramatically and the peak effect of the amines was obtained about five minutes after the application and thereafter the restarted contraction was depressed again. When the preparation was left without further addition of the amines or was washed by fresh Ringer solution, the rhythmic contraction disap-

![Fig. 3](image-url)
Fig. 4. Effects of $10^{-8}$ and $10^{-4}$ of dopamine and $10^{-7}$ of adrenaline on the atrium of which S-A nodal transmembrane potential had been abolished by $10^{-5}$ of reserpine.

I: Dopamine $10^{-4}$, II: Dopamine $10^{-3}$, III: Adrenaline $10^{-7}$.
peared again. When the application of the amines and the washing-out of the preparation were repeated, there occurred the rhythmic contraction spontaneously and the contraction could be maintained even after the washing-out of the preparation. On the other hand, the restarting effect of dopamine manifested gradually and progressively. When the restarted preparation was washed by Ringer solution, the spontaneous contraction of the preparation sometimes disappeared again. Two thirds of the reserpinized preparation, which had been restarted by dopamine and washed by fresh Ringer solu-

![Diagram]

**FIG. 5.** Effects of $10^{-5}$ of dopamine on the S-A nodal transmembrane potential markedly depressed by $10^{-5}$ of reserpine.

I : control, II : 3 minutes after the addition of dopamine, III : 4 minutes after the addition of dopamine, IV : 5 minutes after the addition of dopamine.
tion repeatedly, was restarted spontaneously without the further application of dopamine within two hours after the disappearance of rhythmic contraction by reserpine. The restarted contraction of the atrium increased in rate and amplitude gradually and progressively.

The similar restarting effects of dopamine on the transmembrane potentials were observed in the pacemaker and atrial non-pacemaker potentials. In half of the preparations of which action potential had been completely abolished by $10^{-6}$ of reserpine, the action potentials were restarted by the administration of $10^{-4}$ to $10^{-5}$ of dopamine. In the other half of the preparations the repeated application of the same concentration of the amine and the repeated washing-out of the preparation also restarted the action potentials. As is shown in Fig. 4, the restarted action potential recorded from S-A node was usually small in height. The height of the small potentials was increased slightly by the further addition of $10^{-4}$ of dopamine, while the rate of the potentials was markedly increased. In this case the addition of $10^{-2}$ of adrenaline increased the height of the action potentials without affecting the rate. On the other hand, the partially depressed potential and the decrease in rate during the early stage of reserpine action were restored almost completely by the addition of $10^{-3}$ of dopamine (Fig. 5). The fact that the full recovery of the action potentials in response to any concentration of dopamine used were not obtained in the preparation of which action potentials had been completely abolished by reserpine was the main difference of action between dopamine and adrenaline or noradrenaline.

The chemical assay studies of catecholamine revealed that the content of noradrenaline in the heart of which spontaneous contraction had been abolished by reserpine did not significantly differ from the content of the amine in the heart of which spontaneous contraction was restarted by dopamine, as is shown in Table 2. The similar results that the content of noradrenaline of the restarted atrium by adrenaline or noradrenaline was almost the same as the content of the amine of the atrium of which spontaneous contraction had been abolished by reserpine were already reported by Matsuo and Tachi (1).

<table>
<thead>
<tr>
<th>Drugs</th>
<th>No. of Experiments</th>
<th>Noradrenaline in the Whole Atrium (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0.90±0.11</td>
</tr>
<tr>
<td>Dopamine</td>
<td>3</td>
<td>0.81±0.01</td>
</tr>
<tr>
<td>Dopa</td>
<td>3</td>
<td>0.79±0.09</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>5</td>
<td>0.90±0.10</td>
</tr>
</tbody>
</table>

Control: Noradrenaline content when the preparations were abolished its spontaneous contraction by the administration of $10^{-6}$ to $5\times10^{-6}$ of reserpine.

III. Effects of Dopa

The application of $10^{-2}$ to $10^{-4}$ of dopa to the normal atrial preparation did not affect the rate and amplitude of the contraction as well as the slope of the transmembrane potentials. The single application of the same concentration of dopa to the atrium of which spontaneous contraction was abolished by $10^{-4}$ to $5\times10^{-4}$ of reserpine did not
restart the contraction. However, the repeated application of the same concentration of dopa and the repeated washing-out of the preparation at the interval of thirty minutes restarted the contraction within two hours in two thirds of the preparations (Fig. 6). The mode of the restarting effect of dopa was similar to that of dopamine. When the preparation was treated with dopa and washed by the fresh Ringer solution repeatedly, the spontaneous contraction developed gradually and progressively within thirty minutes after the last washing-out of the preparation. When once the spontaneous contraction was restarted, then the rhythmic contraction could be maintained without further addition of the drug. The abolished action potential after reserpine was not restarted by addition of $10^{-6}$ to $10^{-4}$ of dopa, but the effects of the further repeated application of dopa and the washing-out of the preparation was not tested. The partially depressed action potential after by reserpine was also not affected by application of dopa.

The chemical assay of the content of noradrenaline showed that the application of $10^{-6}$ of dopa to the normal atrium for two hours did not increase the content of noradrenaline in the atrium (Table 1). Furthermore, the content of noradrenaline of the atrium restarted by the repeated application of dopa and the repeated washing-out of the preparation did not significantly differ from that in the heart of which spontaneous contraction was totally abolished by addition of $10^{-2}$ of reserpine (Table 2).

**DISCUSSION**

Though the spontaneous contraction (4) and the transmembrane potentials (5) of the isolated atrium of rabbit were abolished by reserpine, the reduction of the content of noradrenaline (6) was only about twenty to thirty per cent in contrast to the over ninety per cent depletion of noradrenaline in the atrium from rabbit which had received 1.0 mg/kg of reserpine ten hours before the isolation of the heart. The content of noradrenaline of the latter atrium was almost completely depleted (7, 8). The isolated atrium of which
spontaneous contraction and transmembrane potentials had been abolished by reserpine in vitro was not restarted by the repeated washing-out of the preparation, while it was restarted by the addition of adrenaline or noradrenaline (4, 5). It has been shown that the circulating noradrenaline is taken up, bound and retained at or near the sympathetic nerve endings (9, 10). The uptake of noradrenaline or adrenaline by the heart in response to the intravenous injection of the amine has been shown by Strömblad and Nickerson (11) and Hertting et al. (12). Though the normal isolated atrium took up considerable amounts of noradrenaline added to the bath fluid, the content of noradrenaline in the restarted heart by noradrenaline did not differ significantly from that in the heart of which spontaneous contraction was totally abolished by the addition of reserpine. From the results Matsuo and Tachi (1) and Toda (13) suggested that the abolishment of the spontaneous contraction and of the transmembrane potentials derived from some mechanism of action of reserpine other than the catecholamine-depleting effect, and the restarting effect of adrenaline or noradrenaline derived from the pharmacological effect of the amine and not from the biochemical effect such as binding or retaining of the amine at the storage site.

In the normal atrial preparation the addition of dopamine induced positive inotropic and chronotropic effects. The time courses of both effects were somewhat different between each other. The increase in the rate was maximum at about ten minutes after the addition of $10^{-4}$ of dopamine and decreased gradually thereafter, while the increase in the amplitude was biphasic, the first peak of the increase being obtained immediately after the application and the second increase in the amplitude manifested gradually and progressively about ten minutes after the addition. The second increase in the amplitude could not be observed in the low but effective concentration of dopamine. From the results Tachi (4) suggested that the first positive inotropic effect derived from the pharmacological action of dopamine and the second inotropic effect derived from the biochemical action of dopamine, i.e. the conversion of dopamine to noradrenaline.

The effects of dopamine on transmembrane potentials were the increase in the rate and some changes of the configuration of the potentials. The effects of dopamine on the membrane potentials were more marked in the pacemaker than in the atrial non-pacemaker fibers. The increase of the prepotentials and the decrease of the total duration induced by dopamine were closely similar to the effects of adrenaline or noradrenaline. The decrease in the rate manifested immediately before the manifestation of the increase but was short lasting. This initial rate-decreasing effect of dopamine resembled to the effect of adrenaline and noradrenaline reported by West (14) and Toda (13).

Though the isolated atrium took up noradrenaline when the amine was added to the bath fluid, the addition of dopamine or dopa to the bath fluid did not increase the content of noradrenaline even if the positive inotropic and chronotropic effect had been observed. The results may show that dopamine or dopa was not taken up by the atrium, or if dopamine and dopa had been taken up, the conversion of dopamine or dopa to noradrenaline did not occur in the tissues. However, the retarded second positive
inotropic effect of dopamine might have derived from the minute amount of noradrenaline which had been converted from dopamine and had escaped from the chemical assay.

The addition of dopamine restarted the rhythm in the atrium of which both spontaneous contraction and transmembrane potential had been abolished totally by reserpine. The mode of the restarting effects of dopamine differed from that of adrenaline or noradrenaline in the following points. 1) When the contraction of the atrium was restarted by dopamine, the rate and amplitude increased gradually but progressively, while the increase in the rate and amplitude caused by adrenaline or noradrenaline was abrupt and often transient. 2) The restarted S-A nodal action potential by dopamine was small in height and the height of the potential increased very slowly, while the height of the restarted potential by adrenaline or noradrenaline was as high as that of the normal potential before reserpine. In the previous report Tachi (4) suggested that the initiation of the rhythmicity, i.e. the quick restoration of the rate and amplitude caused by adrenaline or noradrenaline derived from the pharmacological action of the amine, and the maintenance of the spontaneous contraction from the biochemical effects of the amine. He further suggested that because of the weak pharmacological effect, dopamine could not quickly initiate or restore the spontaneous contraction and the transmembrane potential, but because of considerably potent biochemical effect, i.e. the conversion of dopamine to noradrenaline and the binding to the storage site, the spontaneous contraction and the transmembrane potential caused by dopamine increased gradually and progressively.

However, the biochemical studies revealed that the addition of dopamine or dopa to the atrium of which spontaneous contraction and the transmembrane potential had been abolished by reserpine did not increase the content of noradrenaline even when the rhythm of the preparation was restored. From the results it was likely that the minute amount of dopamine which could be converted to noradrenaline might play a great role in the maintenance of the rhythmicity of the atrium. In this report the measurement of dopamine or dopa in the atrium was not tested. If the uptake of dopamine or dopa does not occur in the atrium, the conversion of dopamine or dopa to noradrenaline should be denied at least in the atrium of rabbit.

The single addition of dopa to the atrium of which spontaneous contraction and the transmembrane potential had been abolished by reserpine did not restart the rhythm. But when the addition of dopa and the washing-out of the preparation were repeated, some of the preparation was initiated the spontaneous contraction, and the rate and amplitude of the contraction increased gradually but progressively. These results also show the weaker pharmacological effect and the considerably stronger biochemical effect of dopa than those of dopamine.

SUMMARY

The effects of the addition of dopamine and dopa were studied on the spontaneous contraction, the transmembrane potential and content of noradrenaline in the isolated rabbit's atrium of which spontaneous contraction and transmembrane potential had been
abolished by the addition of reserpine. The results obtained are summarized as follows.

1. The addition of dopamine in the concentration of above $10^{-6}$ to the isolated rabbit's atrium increases markedly the rate and amplitude of the spontaneous contraction, and the effects last more than thirty minutes. The rate of the atrial transmembrane potential and the slope of the prepotential of the pacemaker increase and the total duration of the action potential decreases. The chemical assay of noradrenaline shows that the addition of $10^{-6}$ of dopamine or doppa for two hours does not affect the content of noradrenaline in the atrium.

2. The repeated administration of $10^{-6}$ of dopamine restarts the spontaneous contraction and the transmembrane potential abolished by the addition of $10^{-6}$ to $5 \times 10^{-4}$ of reserpine, about two hours after the disappearance of the rhythm. The restarting effects of dopamine differs from those of noradrenaline and adrenaline in that the former effect develops gradually but progressively, while the latter effect is abruptly and decreasingly. The content of noradrenaline in the atrium restarted by the addition of dopamine does not significantly differ from that of noradrenaline in the atrium of which spontaneous contraction has been abolished by reserpine.

3. The administration of $10^{-6}$ to $10^{-4}$ of doppa does not affect the content of noradrenaline, the spontaneous contraction and the transmembrane potential of the isolated rabbit's atrium. However, the repeated administration of doppa to the atrium of which spontaneous contraction has been abolished by reserpine restarts the rhythmicity in the similar way to dopamine, while the content of noradrenaline is not affected.

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