THE EFFECTS OF RESERPINE ON THE RESPONSES OF THE NICTITATING MEMBRANE IN THE CAT

KEIJI NAKAMURA AND KIRO SHIMAMOTO

Department of Pharmacology, Faculty of Medicine, Kyoto University, Kyoto

Received for publication December 8, 1959

Bein (1) was the first to describe that the administration of reserpine potentiated the pressor responses of the experimental animals to adrenaline and noradrenaline. The discharge of the catecholamines from the adrenal glands and from the other sympathetic structures such as the heart, liver, spleen and hypothalamus have been shown by Holzbauer and Vogt (2), Carlsson and Hillarp (3), Shore et al. (4) and others (5-8). Although the animals which have received reserpine, did not exhibit very obvious signs of an increased sympathetic activity such as would be expected during a discharge of adrenal medullary amines, Everett et al. (9) showed that the mice revealed a transient phase of piloerection, Kuschke and Franz (10) demonstrated a hyperglycemic effect in the rabbit. Rises of blood pressure in the rat and the spinal dog, the contraction of the denervated nictitating membrane in the cat have been reported following the injection of reserpine by de Jongh and van Proosdij-Hartzema (11) and Maxwell et al. (12). Torii (13) also showed that the carotid injection of reserpine (0.1 mg/kg) to intact rabbit or the intravenous injection of reserpine (1 to 3 mg/kg) to the iproniazid-treated rabbit induced increased spontaneous movements and the signs of the increased sympathetic activity. Within 30 minutes after the intravenous injection of reserpine the pressor responses of the animal to stimulation of the thalamic and hypothalamic nuclei had been rather potentiated. Burn et al. (14, 15) confirmed that the contractile response of the spiral strips of the thoracic aorta of the reserpinized rabbit to adrenaline and noradrenaline was much sensitive than that of the normal one and that the contractive responses of the nictitating membrane of the cat to the same amines were also potentiated by the successive dose of reserpine.

Burn and Rand (14) discussed the mechanism of the supersensitivity of these structures by reserpine concluding that the depletion of the catecholamines in the tissues by reserpine might be responsible. It would be expected to elucidate the mechanism of reserpine hypersensitivity to catecholamines by studying the effect of reserpine on the responses of the normal and denervated nictitating membranes to sympathomimetic stimuli. The effect of reserpine on the membranes was also compared with those of the denervation, cocainization, ephedrinization, iproniazidization and noradrenaline infusion.

METHODS

Cats, weighing 2.5 to 3.5 kg of body weight, were anesthetized with 30 mg/kg of
nembutal (intraperitoneal) and were used under an artificial respiration. In many of the
experiments the spinal cord was sectioned between C7 and C11. Ten to 14 days previously
the left superior cervical sympathetic ganglion was extirpated following the technic of
Kosterlitz et al. (16). The contraction of the nictitating membrane was recorded on the
kymograph via an isotonic lever, magnified 7.2 times. The carotid blood pressure was
also recorded occasionally via Hg-manometer in the hapanirized animal. The pregang-
lionic fibre of the superior cervical ganglion was stimulated with square waves, delivered
20/sec in frequency, 1 msec in duration and submaximal in intensity for 5 seconds. For
the continuous infusion of noradrenaline the dose of 100 mµ/kg/min was selected, because
though Stjärne et al. (17) showed that the amounts of catecholamines liberated in the
venous flow of the adrenals after the administration of reserpine was 10 mµ/kg/min at
the maximal level, the normal amount of noradrenaline was determined as 37 µµ/kg/min.

As the sympathomimetics, l-adrenaline hydrochloride, dl-noradrenaline hydrochloride,
dl-tyramine hydrochloride, l-ephedrine hydrochloride, d-amphe:amine hydrochloride and
dl-serotonin creatine sulfate were used. They were all injected into the femoral vein.

RESULTS

1. The Effect of Reserpine on the Responses of the Normal and Denervated Membrane

The intravenous injection of 0.1 to 3.0 mg/kg of reserpine usually did not reveal any
response of the normal membrane. But it was occasionally observed that membrane
contracted in response to large doses of reserpine (1 to 3 mg/kg). The contraction by
reserpine developed very slowly and the maximal contraction was obtained 1 to 1.5 hours
after the injection. Since then the contraction relaxed gradually. Ten to 20 hours there-
after the membrane became fully relaxed.

The denervated membrane contracted usually in response to reserpine above the dose
of 1 mg/kg. The contraction of the denervated membrane revealed gradually and progres-
sively and the maximal contraction was also obtained 1 to 2 hours after the injection.
The height of the contraction of the denervated membrane by reserpine was in propor-
tion to the dose administered. Thereafter the membrane began to relax and after 10 to
20 hours it was fully relaxed as was the normal membrane.

II. The Effect of Reserpine on the Response of the Normal Membrane
to Preganglionic Stimulation of the Cervical Sympathetic Nerve

Small doses of reserpine did not affect the contractive response of the normal mem-
brane to preganglionic cervical sympathetic stimulation. As was stated above, when the
membrane contracted in response to reserpine, slight and transient depression of the
response of the membrane to nerve stimulation was observed. Two to 3 hours after the
injection of 1 mg/kg of reserpine the response of the membrane to nerve stimulation was
gradually depressed in accordance with the manifestation of the relaxation of the mem-
brane. But 10 to 20 hours after the injection the fully relaxed membrane usually
responded to the nerve stimulation. But the administration of 1 to 2 mg/kg of atropine abolished the response.

**III. Effect of Reserpine on the Responses of the Normal and Denervated Membrane to Adrenaline, Noradrenaline, Tyramine and Serotonin**

Table 1 indicates the minimal contractive doses of adrenaline, noradrenaline, tyramine, and serotonin on the normal and the denervated membrane in the normal and the reserpine-treated (1 mg/kg) animals. The denervated membrane of the intact cat revealed always a marked increased sensitivity to all these amines. The contractive ratios between the normal and the denervated membranes in response to 2 µg/kg of adrenaline, 2 µg/kg of noradrenaline, 10 µg/kg of serotonin, 50 µg/kg of tyramine and 50 µg/kg of ephedrine are listed in Table 2. Because of the confirmation that the increased response of the denervated structure was much markedly shown by smaller doses of the amines, the ratios were obtained by use of the submaximal doses.

**TABLE 1.** The minimal contractive doses of adrenaline, noradrenaline, tyramine and serotonin on the normal, denervated and reserpine-treated nictitating membranes in the spinal cats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adrenaline</th>
<th>Minimal dose (mg/kg)</th>
<th>Tyramine</th>
<th>Serotonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.0005</td>
<td>0.002</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Denervated</td>
<td>0.0001</td>
<td>0.0002</td>
<td>0.01</td>
<td>0.002</td>
</tr>
<tr>
<td>Reserpine-treated</td>
<td>0.0002</td>
<td>0.0005</td>
<td>0.01</td>
<td>0.002</td>
</tr>
</tbody>
</table>

**TABLE 2.** The contraction-ratios between the denervated, acute reserpinized, chronic reserpinized and normal membranes during the infusion of noradrenaline and the normal nictitating membranes in the spinal cats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adrenaline 2 µg/kg</th>
<th>Contraction-ratio Noradrenaline 2 µg/kg</th>
<th>Tyramine 50 µg/kg</th>
<th>Serotonin 10 µg/kg</th>
<th>Ephedrine 50 µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Denervated</td>
<td>5.0</td>
<td>28.7</td>
<td>3.2</td>
<td>4.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Acute reserpinized</td>
<td>2.2</td>
<td>4.8</td>
<td>2.8</td>
<td>1.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Chronic reserpinized</td>
<td>4.9</td>
<td>13.4</td>
<td>0.17</td>
<td>0.26</td>
<td>0.1</td>
</tr>
<tr>
<td>Noradrenaline infusion</td>
<td>2.3</td>
<td>4.3</td>
<td>2.6</td>
<td>3.4</td>
<td>1.7</td>
</tr>
</tbody>
</table>

These results obtained in this experiments concerning the increased responses of the denervated membrane to the sympathomimetic amines well agreed with the results showed by Trendelenburg (18). It was reported that the response of the nictitating membrane to tyramine was decreased by the denervation (19). The difference between their results and the present results may be supposed to consist in the dose of tyramine, because the dose of tyramine in this experiment was one-tenth of their dosage.

Within 2 to 3 hours after the injection of reserpine in the doses of 0.5 mg to 3.0 mg/kg the responses of the normal membrane to the sympathomimetic amines were all
potentiated. The potentiating ratio of the normal membrane to the reserpine-treated one are listed in Table 2. The potentiating effects of reserpine revealed rather quickly and progressively, and were proportional to the dose injected. The maximal potentiating effects of reserpine on the response of the normal membrane to adrenaline and noradrenaline were obtained at 10 to 20 hours after the injection, whilst the same effects of reserpine on the responses of the membrane to tyramine, serotonin and ephedrine were obtained at 2 to 3 hours after the injection. Thereafter, the increased sensitivity of the normal membrane to tyramine, serotonin and ephedrine decreased gradually, and at 10 to 20 hours after the injection a steady diminution of the responses to these amines were seen. When the decreased responses of the reserpine-treated membrane to tyramine and serotonin were manifested, the continuous infusion of noradrenaline in the rate of 100 \(\mu\text{g/kg/min}\) restored the membrane to normal or increased responses, but several minutes after the interruption of the infusion the restored responses of the membrane to tyramine and serotonin fell again.

Because of the sustained contraction of the denervated nictitating membrane by reserpine which revealed gradually and progressively, the potentiating effects of reserpine on the responses of the membrane to adrenaline, noradrenaline, tyramine, and serotonin were not marked or rather depressed during 2 to 3 hours after the injection of reserpine. Ten to 20 hours after the injection of reserpine the responses of the fully relaxed denervated membrane to adrenaline and noradrenaline did not show marked difference compared with the same responses of the normal membrane, but the contraction of the denervated membrane in response to tyramine and serotonin was always smaller than that of the normal membrane. The continuous infusion of noradrenaline at the same rate, as was mentioned above, restored the both membrane to increased response, in which the denervated membrane showed always a marked contraction as was seen in an intact animal.

IV. Effects of Monoamine Oxidase Inhibitors on the Responses of the Nictitating Membrane

The effects of the monoamine oxidase inhibitors such as cocaine, iproniazid, ephedrine and amphetamine on the responses of the normal and the denervated membranes to the sympathomimetic amines were compared with the similar effect of reserpine. The administration of 0.2 mg/kg of cocaine potentiated the responses of the normal membrane to adrenaline, noradrenaline and serotonin, but not the same responses of the denervated membrane. The responses of both membranes to tyramine were rather depressed. Cocaine have a different effect on the responses of the membrane in this point. It could not hardly be concluded that the potentiating effect of cocaine was derived from its monoamine oxidase inhibition only, because tyramine was supposed to be an optical substrate for the enzyme.

Iproniazid was shown to have a powerful monoamine oxidase inhibitory effect \textit{in vitro} (20) and \textit{in vivo} (21, 22). But the intravenous injection of 5 to 50 mg/kg of iproniazid never potentiated or rather depressed the responses of both membranes to adrenaline, noradrena-
line, tyramine and also serotonin.

The administration of ephedrine in a dose of 0.5 mg/kg potentiated the responses of both membrane to adrenaline, noradrenaline and serotonin as well as the same response of the normal membrane to tyramine. The same dose of amphetamine showed neither significant potentiation nor depression on the responses of both membranes to these amines. Table 3 summarizes the effects of the monoamine oxidase inhibitors on the responses of both membranes to the sympathomimetic amines (Table 3).

The administration of cocaine, ephedrine and amphetamine in the above-mentioned poses respectively all potentiated the responses of the normal membrane to stimulation of the cervical sympathetic nerve.

Table 3. Effects of cocaine (0.2 mg/kg), iproniazid (50 mg/kg), ephedrine (0.5 mg/kg), and amphetamine (0.5 mg/kg) on the responses of the normal and the denervated nictitating membranes in the spinal cats.

<table>
<thead>
<tr>
<th></th>
<th>Cocaine</th>
<th>Amine oxidase inhibitors</th>
<th>Amphetamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenaline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Denervated</td>
<td>None</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Denervated</td>
<td>None</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>Serotonin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Denervated</td>
<td>None</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>Tyramine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Denervated</td>
<td>-</td>
<td>+</td>
<td>None</td>
</tr>
</tbody>
</table>

Potentiation, Depression

Table 4. The contraction ratios of the normal and the denervated membranes before and during the infusion of noradrenaline.

<table>
<thead>
<tr>
<th></th>
<th>Contraction ratio</th>
<th>Normal Denervated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenaline (2:kg)</td>
<td>1.6</td>
<td>2.8</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>1.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Serotonin</td>
<td>1.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Tyramine</td>
<td>1.0</td>
<td>2.1</td>
</tr>
</tbody>
</table>

V. Effects of Adrenalectomy and Infusion of Noradrenaline on the Reserpine Action

The bilateral adrenalectomy of the animal did not affect significantly the effects of reserpine on the responses of the normal and the denervated membranes to adrenaline, noradrenaline, serotonin and tyramine. The decreased responses of the normal membrane of the animal to tyramine and serotonin, which had received reserpine 10 to 20 hours previously, were potentiated by the continuous infusion of noradrenaline at the rate of 100 mg/kg/min, as was mentioned above. The infusion of noradrenaline also potentiated the responses of the normal membrane to adrenaline. The similar effects were also obtained on the responses of the normal membrane to tyramine.
and serotonin. Table 4 shows the potentiation ratios of the infusion of noradrenaline on the responses of the normal nictitating membrane to the sympathomimetic amines (Table 4).

VI. Effects of the Other Alkaloids of Rauwolfia serpentina on the Responses of the Normal Nictitating Membrane to the Sympathomimetic Amines

The effects of 2 mg/kg of reserpine, rescinamine, reserpinine, serpentine, serpentinine and ajimaline on the responses of the normal membranes to adrenaline, noradrenaline, serotonin and tyramine were tested within 3 or 4 hours after their injection. The results are summarized in Table 5. Rescinamine was only an alkaloid which potentiated the responses of the normal membrane to adrenaline, noradrenaline, serotonin and tyramine as reserpine did. But the potentiating effects of rescinamine were markedly weaker than those of reserpine (Table 5).

| Table 5. Effects of the alkaloids of Rauwolfia serpentina on the responses of the normal nictitating membranes of the spinal cats. |
|---|---|---|---|---|
| **Dose (mg/kg)** | **Blood pressure** | **Adrenaline** | **Noradrenaline** | **Serotonin** | **Tyramine** |
| Reserpine | 2.0 | Fall | - | - | - | + |
| Rescinamine | 2.0 | Fall | - | - | - | + |
| Reserpinine | 2.0 | None | - | - | + | - |
| Serpentine | 5.0 | None | - | - | - | - |
| Serpentinine | 5.0 | None | - | - | - | - |
| Ajimaline | 5.0 | None | - | - | - | - |

+ Potentiation, - No effect.

VII. Effects of Reserpine on the Responses of Blood Pressure to the Sympathomimetic Amines

The effects of 0.5 to 1.0 mg/kg of reserpine on the responses of the carotid blood pressure in the cat anesthetized with nembutal to adrenaline, noradrenaline, tyramine, ephedrine and serotonin were studied. The effects of reserpine were classified into two groups, namely, at 1 hour after the injection (acute effects) and at 10 hours after the injection (chronic effects).

After the injection of reserpine the pressor responses of the animals to adrenaline, noradrenaline, ephedrine and tyramine were significantly increased after 1 hour. Ten hours after the injection the further increase of the pressor response to adrenaline and noradrenaline were obtained, while the same response to ephedrine and tyramine were significantly decreased. The depressor response of the animal to serotonin was decreased 1 hour after injection, and a marked pressor response was observed after 10 hours.

**DISCUSSION**

It has been postulated by several authors that the sedative and hypotensive effects of reserpine were concerned with the release of serotonin or catecholamines and therefore...
with the depletion of these amines from the tissue. There are also some evidences that the administration of reserpine to a variety of experimental animal induce a sympathomimetic effect, as was mentioned above.

In the present experiments large doses of reserpine did occasionally contract the normal nictitating membranes of the cats and almost always contracted the denervated membranes. The contraction of the denervated membranes developed slowly and progressively and reached their maximum 2 to 3 hours after the injection. Thereafter, in accordance with the manifestation of the sedation the contracted membrane began to be relaxed gradually and during 10 to 20 hours after the injection of reserpine the denervated membrane were fully relaxed as were the normal membranes.

The contraction of the normal membranes in response to stimulation of the cervical sympathetic nerve was not markedly affected by reserpine, except a slight initial inhibition of the response. This inhibition was supposed to result from the weak adrenolytic effect of reserpine, which was showed by Shimamoto et al. (23) in the perfused vessels of rabbit’s ear. But with the lapse of time after the injection of reserpine the same response was markedly inhibited, though it was not abolished even after 10 to 20 hours. The administration of 1 to 2 mg/kg of atropine abolished the inhibited response of the membrane to nerve stimulation. Consequently, the contraction of the normal nictitating membranes of the fully iproniazidized animals to nerve stimulation was supposed to be cholinergic origin, which was confirmed by Bacq and Fréderic (24), Shimamoto and Inoue (25), and Burn and Trendelenburg (26). If this abolishment of the response of the membrane to nerve stimulation is the case, it may be concluded that the sympathetic response of the membrane is gradually depressed and finally is abolished during 10 to 20 hours after the injection of reserpine. Carlsson et al. (27), and also Torii (13) in this laboratory showed that the hypertensive response of the cat and rabbit to stimulation of the splanchnic nerve was abolished during the sedative phase of reserpine action, which manifested most markedly between 10 to 20 hours after the administration of reserpine. The authors agreed in the conclusion that the diminution of the pressor response to stimulation of the splanchnic nerve was induced by the release of the catecholamines present in the adrenal glands, that is, the depletion of the medullary hormones in the tissues. The depletion of catecholamines from the adrenals (6-8) and from the heart (5) by reserpine revealed its maximal effect concurred with that of the sedative effect. It was supposed that the same sequences could occur in the nictitating membrane.

In the acute phase of reserpine action (from 30 minutes to 2 hours after reserpine) the responses to adrenaline, noradrenaline, serotonin and tyramine were all potentiated. The potentiating effect of reserpine revealed most marked to noradrenaline, and next was to adrenaline. In the chronic phase a further potentiation was obtained concerning with the responses of the membrane to adrenaline and noradrenaline, in which the increase of both responses amounted almost the same pattern, as that of the denervated membrane. In contrast to the increased responses of the denervated membrane to tyramine and sero-
tonin the reserpine-treated membrane showed decreased responses to the same amines. The increased responses of the nictitating membrane to adrenaline and noradrenaline and the decreased responses of the membrane to tyramine and serotonin agreed well with the results of Burn and Rand (14). But they did not describe the acute effects of reserpine on the action of these amines. They expressed the opinion that tyramine and serotonin normally act on the membrane by releasing the catecholamines from the store in the arterial wall. This was attributed to the reversal of action of these amines by the continuous infusion of noradrenaline into the blood stream in the reserpinized animals. If the suggestion of Burn and Rand may be adjustable, the increased response of the membrane to tyramine and serotonin in the acute phase of reserpine action is the sequence of the increase of the circulating noradrenaline, while the depression of the same response in the chronic phase is the sequence of the decrease of the circulating noradrenaline. In the denervated membrane the response to both amines may be increased, because the circulating noradrenaline is not affected. The difference of the response of the membrane between the chronic phase and acute phase of reserpine action was supposed to consist in the difference of the amounts of circulating noradrenaline, which was normal in the latter and much reduced in the former.

The responses of the nictitating membrane in the chronic phase of reserpine action to adrenaline, noradrenaline, tyramine and serotonin under noradrenaline infusion were similar to the effects of the denervation and cocainization. The increased response of the reserpine-treated animals might be supposed to originated from the same mechanism, namely, the depletion of the catecholamines from the tissues, as was in the denervated membrane. The mechanism why cocaine mimic the effect of the denervation, was not elucidated from the present experiment.

Among the alkaloids of Rauwolfa serpentina tested in this experiment only reserpine and rescinamine increased the response of the normal nictitating membrane to the sympathomimetic amines. The pressor response of the cats to these amines were similarly affected as the nictitating membrane.

**CONCLUSION**

The acute and chronic effects of reserpine (0.5 to 3.0 mg/kg) on the responses of the normal and the denervated nictitating membrane were investigated in the spinal and nembutalized cats. The results obtained were as follows:

1. The administration of reserpine contracted the denervated membrane, in which the contraction reached its maximal effect 2 to 3 hours after the injection and thereafter the contraction subsided until the full relaxation of the membrane between 10 to 20 hours after the injection. On the contrary the normal membrane showed none response initially, but after 10 to 20 hours of the injection it was also fully relaxed.

2. The contractile response of normal membrane to stimulation of the cervical sympathetic nerve was depressed gradually, but abolition of the response was not obtained.
even 10 to 20 hours after the injection of reserpine. But the depressed response to nerve stimulation 10 to 20 hours after reserpine was abolished by the administration of atropine.

3. In acute phase of reserpine action (30 minutes to 3 hours after reserpine) the responses of the normal membrane to adrenaline, noradrenaline, tyramine, serotonin and ephedrine were moderately enhanced, while in chronic phase the same responses to adrenaline and noradrenaline were markedly enhanced, but the same responses to tyramine, serotonin and ephedrine were moderately depressed. The continuous infusion of noradrenaline decreased the responses of the reserpine-treated membrane to adrenaline and noradrenaline, and inverted the decreased responses of the same membranes to tyramine and serotonin.

4. The effects of the sympathetic denervation, cocainization, iproniazidization, ephedrinization and amphetaminization on the response of the normal nictitating membrane to the sympathomimetic amines were compared with the similar effects of reserpine and the mechanism of action of reserpine was discussed.

REFERENCES

8) de Schafjorpyver, A.F. and Pfeifpost, P.: Arch. int. Pharmacodyn. 120, 177 (1959)
13) Torii, H.: Folia pharmacol. japen. 55, 1227 (1959)
19) Fleckenstein, A. and Burn, J.H.: Ibid. 8, 69 (1953)
20) Zeller, E.A.: Experientia 8, 347 (1952)
23) Shimamoto, K. et al.: To be published.