ACTION OF SEVERAL ANTI-HISTAMINICS ON THE ISOLATED RAT PHRENIC-DIAPHRAGM PREPARATION

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Since Fourneau and Bovet (1) reported 2-isopropyl-5-methyl phenoxy-ethyl-diethylamine as a potent anti-histaminic, many publications on the central action of anti-histaminics have appeared during the last three decades. However, no report on the action of anti-histaminics to the neuromuscular junction has appeared, despite the fact that listlessness of the limbs occurs when a small dose of anti-histaminics is administered, as in the case of succinylcholine administration.

In the present paper, the neuromuscular blocking property of several anti-histaminics was first screened, and then its mechanism of action was studied using rat phrenic-diaphragm preparation.

MATERIALS AND METHODS

Rat phrenic-diaphragm preparations were used throughout the experiment by a conventional modification of Bulbring's method (2). Direct stimulation to the muscle was recorded, as the control, based on the same principle used by Ing and Wright (3) on the frog sartorius. The responses elicited by maximal direct and indirect stimulation were recorded alternately.

1. Preparation

The diaphragm muscles were cut about 1.5 cm wide as far along the direction of the muscle fibers as possible. The strip of diaphragm was taken from the left side and cut so that the phrenic nerve entered it at the junction of its lateral three quarters and its medial quarter. The phrenic nerve was taken about 3 cm long with connective tissue in order to avoid any damage. It was placed on an indirect electrode which was supported on an upright plastic rod and seated just clear of the meniscus.

At the top of the strip of the diaphragm, which was placed just below the meniscus of the fluid in the bath, the musculotendinous junction was connected by a string to the spring-loaded light straw lever. At the lower end of the preparation, the costal margin was attached by two ligatures to the plastic direct electrode holder (Fig. 1).

2. Stimulator

The stimuli were applied alternately to the nerve and muscle every 15 sec by two electrically separated and mechanically coupled stimulators, each consisted of a three
contact rocking mercury switch and a condensor. When the switch tilted one way it discharged the condensor through the preparation and when it rocked the other way it charged the condensor again (Fig. 2). In case of the nerve the discharge from a 0.1 mF condensor, charged to about 6 volts, was used while a 1 mF condensor, charged to about 45 volts, was used to stimulate the muscle directly. Both the nerve and muscle electrodes have a 3,000 ohms resistance in parallel with them.

3. Organ bath and fluid

The Tyrode solution (0.8% NaCl, 0.1% NaHCO₃, 0.005% Na₂HPO₄, 0.2% KCl, 0.005% MgCl₂, 0.2% CaCl₂, 0.2% glucose) was used throughout the experiment. Prior to each experiment, the fluid was gassed for about 30 minutes by passing the gas mixture (95%
O$_2$+5% CO$_2$). The very fine bubbles of the same gas mixture were also supplied through a fine plastic tube in the organ bath throughout the experiment. The temperature of fluid in the organ bath was maintained at 38°C by pumping water from a thermoregulator through the water jacket. The standard volume of fluid in the bath was 25 ml.

**RESULTS**

1. **Diphenhydramine hydrochloride**

Fig. 3 shows the effect of diphenhydramine on the rat phrenic-diaphragm preparation. By administering 2.5–20 µg/ml of diphenhydramine, a gradual increase of responses to both direct and indirect stimuli was registered simultaneously. By administering 20–40 µg/ml of diphenhydramine, both responses decreased simultaneously after a transitory increase and finally ceased. However, when the preparation was washed thoroughly with fresh Tyrode solution, both responses recovered completely to the control level.

![Diphenhydramine graph](image_url)

Fig. 3. Effect of diphenhydramine on the rat phrenic-diaphragm preparation. Effects of indirect nerve stimulation and direct muscle stimulation were recorded alternately.

At W, preparation was washed with fresh Tyrode solution. The transversal line indicates the base line. Muscle contractions downwards. The upper side from the base line is rebound of the lever.

2. **Chlorpheniramine hydrochloride**

Fig. 4 shows the effect of chlorpheniramine on the rat phrenic-diaphragm preparation. Almost the same results as the case of diphenhydramine were obtained. By ad-
Temporarily increases of responses both to direct and indirect stimuli were registered. Then the responses to indirect stimuli decreased, followed by a decrease of the responses to direct stimuli. Finally, in 2 cases out of 7, both responses stopped completely. However, when the preparation was washed thoroughly with fresh Tyrode solution, the responses recovered completely to the control level.

3. Thonzylamine hydrochloride

Fig. 5 shows the effect of thonzylamine on the rat phrenic-diaphragm preparation. By administering 40–80 μg/ml of thonzylamine, the responses to indirect stimuli decreased immediately while the responses to direct stimuli decreased gradually after a temporary increase. However, after a thorough wash with fresh Tyrode solution, the responses recovered completely to the control level.

4. Tripelennamine hydrochloride

Fig. 6 shows the effect of tripelennamine on the rat phrenic-diaphragm preparation. By administering 10–20 μg/ml of tripelennamine, the responses to both direct and indirect stimuli increased simultaneously. By administering 20–40 μg/ml the responses to direct stimuli increased temporarily, while the responses to indirect stimuli decreased gradually.
and finally ceased. However, when the preparation was washed thoroughly with fresh Tyrode solution, both responses recovered completely to the control level.

5. Promethazine hydrochloride

Fig. 7 shows the effect of promethazine on the rat phrenic-diaphragm preparation. By administering 10–20 μg/ml of promethazine, the responses to indirect stimuli decreased immediately and ceased completely after a certain time period, while few effects were observed on the responses to direct stimuli. When the preparation was washed thoroughly with fresh Tyrode solution, both responses recovered completely to the control level.


Fig. 8 shows the effect of Pandryl-p on the rat phrenic-diaphragm preparation. The initial administration of 2.5 μg/ml of Pandryl-p showed no effect on both responses to direct and indirect stimuli. By the second administration of the same dose of Pandryl-p, the responses to indirect stimuli decreased gradually and finally ceased, while the response to direct stimuli showed no effect.

Washing the preparation with fresh Tyrode solution at the point W in the figure it caused a slight temporary recovery, but even more than ten times of washing failed to regain any responses to indirect stimuli. However, when the preparation was washed thoroughly during the indirect block by Pandryl-p, the responses to indirect stimuli showed a tendency to revive. No complete recovery was observed in the tested cases.
7. Eserine-antagonism

Fig. 9-A shows the typical antagonistic action of d-tubocurarine (1.2 μg/ml) against eserine (0.4 μg/ml). By the administration of eserine, both responses to direct and indirect stimuli increased markedly and simultaneously. In this case, the subsequent administration of d-tubocurarine caused an immediate decrease of their responses returning to the control level. Exactly the same result was obtained as in the case of tripelennamine (Fig. 9-B). In cases of promethazine and Pandryl-p, the typical antagonistic action against eserine was not observed (Fig. 10-A and -B). The effects of promethazine and Pandryl-p following the increase of responses to direct and indirect stimuli by...
the administration of eserine were almost the same as the effects of their single administration. Whereas the responses to indirect stimuli decreased markedly, those to direct stimuli decreased gradually.

8. Potassium-antagonism

Fig. 11-A shows a typical antagonistic action of potassium against d-tubocurarine. The subsequent administration of KCl (0.16 mg/ml) during the progress of indirect block by d-tubocurarine exerted a marked antagonistic action. Almost the same antagonistic action was observed in the case of tripelennamine administration (Fig. 11-B). However, in cases of promethazine and Pandryl-p, unlike in the case of d-tubocurarine, the typical antagonistic action against potassium was not observed (Fig. 12-A and -B).

DISCUSSION

Table 1 summarizes the effect of anti-histaminics on the responses to direct and indirect stimuli of the rat phrenic-diaphragm preparation. Curare-like action, which shows the block only on the responses to indirect stimuli and no effect on the responses

<table>
<thead>
<tr>
<th>No. tested</th>
<th>Stim.</th>
<th>μg/ml</th>
<th>1.25</th>
<th>2.5~5</th>
<th>5~10</th>
<th>10~20</th>
<th>20~40</th>
<th>40~80</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Tubocurarine</td>
<td>5</td>
<td>Direct</td>
<td>No Effect</td>
<td>No Resp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>8</td>
<td>Direct</td>
<td>Inc.</td>
<td>Inc.</td>
<td>No Resp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thonzylamine</td>
<td>3</td>
<td>Direct</td>
<td>Inc.</td>
<td>Inc.</td>
<td>Inc.</td>
<td>Inc.</td>
<td>Inc.</td>
<td>No Resp.</td>
</tr>
<tr>
<td>Promethazine</td>
<td>3</td>
<td>Direct</td>
<td>Inc.</td>
<td>Inc.</td>
<td>No Resp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pandryl-p</td>
<td>3</td>
<td>Direct</td>
<td>No Effect</td>
<td>No Resp.</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Fig. 12. A: the antagonistic action of promethazine towards potassium. B: the antagonistic action of Pandryl-p towards potassium.
to direct stimuli, was observed in cases of thonzylamine, tripelennamine, promethazine and Pandryl-p administration.

Thonzylamine and tripelennamine are almost similar in chemical structure. They have two carbon atoms on their inter-nitrogen bridge. However, they are not methonium-type compounds (4). Tripelennamine showed the antagonism towards eserine and potassium as in the case of \( d \)-tubocurarine (5, 6). The most probable interpretation of their mechanism of action on the neuromuscular junction is to be explained as the similar mechanism to the phase II type block of \( C_{16} \) as mentioned by Jenden, Kamijo and Taylor (7). The alteration in responses to direct stimuli which is not seen in case of phase II type block of \( C_{16} \) would be explained by a pharmacologically complex action of weak phase I type block. On the other hand, promethazine which also possesses two carbon atoms on its inter-nitrogen bridge, showed no antagonistic action towards eserine and potassium. Furthermore it showed increasing responses to direct stimuli. This phenomenon is similar to what was observed in case of the phase I type block of \( C_{18} \) (7). The chemical structure of Pandryl-p is completely different from the others tested. However, in the present study using rat phrenic-diaphragm preparation, Pandryl-p showed a typical curare-like block to responses to both direct and indirect stimuli. However thorough washes of the preparation with fresh Tyrode solution failed to recover response to indirect stimuli. Furthermore no antagonism towards eserine and potassium was observed. The above-mentioned results indicate the difference of the mechanism of neuromuscular blocking action from that of \( d \)-tubocurarine. The mechanism of action of irreversible neuromuscular blocking property of Pandryl-p will be explained in further investigation. In other cases, such as diphenhydramine and chlorpheniramine administration, both responses to direct and indirect stimuli were decreased simultaneously. In other words, they did not show any curare-like activity. These two anti-histaminics are almost similar in chemical structure and both of them possess a nitrogen atom. The complete paralysis due to direct and indirect stimuli caused by a large dose of them (20-40 \( \mu g/ml \)) may be explained by quite different mechanisms, such as direct action on the nerve or muscle cells.

All anti-histaminics tested are shown to have a marked neuromuscular blocking property. It is difficult to explain clearly from the present experiments why refractriness occurs when a clinical dose of anti-histaminics is administered. This may not only be due to the peripheral, but also the central action of anti-histaminics.

**SUMMARY**

The neuromuscular blocking property of several anti-histaminics was screened and its mechanism of action was studied using the rat phrenic-diaphragm preparation.

1. Tripelennamine and thonzylamine have been found to possess a neuromuscular blocking action. The characteristics of these blocking action are same as those of phase II of \( C_{18} \).
2. Promethazine produced a similar effect on neuromuscular junction. The characteristics of the blocking action are same as that of phase I of C12.

3. Diphenhydramine and chlorpheniramine caused depression on the responses to both direct and indirect stimuli.

4. Pandryl-p showed the most potent neuromuscular blocking property. The characteristics of the blocking action are now under investigation.

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