MECHANISM OF SENSITIZATION TO THE LETHAL 
EFFECT OF HISTAMINE IN RATS INDUCED 
BY BORDETELLA PERTUSSIS VACCINE 

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Since the observation of Parfentjev and Goodline (1948) that the injection of B. pertussis vaccine renders mice highly sensitive to the lethal effect of histamine, evidence has been accumulated that vaccinated rats and mice are not only sensitive to histamine and serotonin, but also susceptible to other stresses, such as endotoxin, irradiation, anoxia and cold, just as the case with adrenalectomized animals (Kind, 1958). One of the attractive hypotheses to explain these effects of pertussis vaccine is that B. pertussis interferes with adrenal function, since Kind (1953) has reported the inhibition of histamine death in pertussis-inoculated mice by cortisone. However, the possibility that pertussis vaccine exerts its effect through injury to the adrenal gland has been denied by several authors in that adrenal function is not impaired by the vaccine. It has been suggested that pertussis may act at a level beyond the adrenal glands by either interfering with the utilization of steroids, enhancing their destruction, or increasing the tissue requirements of steroids. As the increased responsiveness to histamine is of great interest to many workers in various fields, it was intended here to settle the problem by determining whether there exist differences in the lethal mechanism of histamine intoxication in pertussis-inoculated and adrenalectomized rats. 

METHODS 

Experiments were done primarily on rats of both sexes weighing about 150 g. For blood pressure determinations, animals weighing more than 200 g were used. They were bred and raised in this laboratory from rats of a Wistar strain. The B. pertussis vaccine (approximately $10^{10}$ organisms per ml) that was used was kindly supplied by Dr. Sachio Ogata (Medical Research Institute, Tokyo University). Sensitization with vaccine was accomplished by an intraperitoneal injection of 0.2 ml vaccine per animal. Susceptibility to histamine was examined during the period of maximum sensitivity, i.e. 4-6 days following the vaccination. This dose of the vaccine was 100% lethal to
Sensitization to the lethal effect of histamine induced by pertussis inoculation.
Almost all normal rats tolerated well a histamine dose of 500 mg/kg (i.v.),
despite an immediate intense dyspneic response which subsided within 15 minutes. Modes of sensitization to histamine by pertussis vaccination and
adrenalectomy are illustrated in Fig. 1. The vaccinated group was more sensitive to histamine than the adrenalectomized group. Moreover, pertussis vaccine sensitized animals even in the absence of the adrenals. These facts might indicate that the sensitization to histamine by pertussis vaccine is independent of the adrenals.

Protective effect of glucocorticoid on the lethal effect of histamine. The effects of
glucocorticoid, epinephrine and endotoxin-conditioning upon the lethal effect of histamine in both adrenalectomized rats and pertussis-inoculated rats were
examined. As will be noticed in Fig. 2, complete protection was afforded by glucocorticoid only in the adrenalectomized group. Kind\(^6\) has reported that cortisone can inhibit the lethal effect of an LD\(_{50}\) dose of histamine in pertussis-inoculated mice, but this dose was much larger (3.0 mg/body). In rats a dose of physiological quantity of cortisone or even Decadron (1 mg/kg, i.m.) could not sufficiently protect pertussis-inoculated animals from the LD\(_{100}\) of histamine. Moderate protection was afforded by epinephrine in the adrenalectomized group, but not in the pertussis-inoculated groups. The endotoxin-conditioning was slightly effective in both groups. These results would indicate that the mechanism of sensitization to the lethal effect of histamine induced by pertussis-inoculation is independent of adrenal function.

Mechanisms of the lethal effect of histamine. The effects of histamine upon blood pressure were examined in adrenalectomized rats and pertussis-inoculated rats. Under the condition of blood pressure measurements with nembutal anesthesia a histamine dose of 10 mg/kg given intravenously was not tolerated by either group. But all the animals tolerated a dose of 1 mg/kg although they all showed a transient blood pressure drop. The threshold dose of histamine inducing a blood pressure fall was 0.1 mg/kg in both groups and also in the normal group. The increased sensitivity to the lethal effect of histamine, thus, seems not to be due to an increased sensitivity to the immediate hypotensive effect of histamine. With 10 mg/kg, as shown in Fig.
an immediate rapid drop in blood pressure occurred in all cases. A slow recovery from the initial drop ensued in intact rats and in adrenalectomized rats pretreated with cortisone. In pertussis-inoculated or adrenalectomized, but not cortisone-treated, rats a progressive hypotension followed with a diminution of pulse pressure. In the pertussis-inoculated group very often a sudden fall of blood pressure occurred reaching the zero line within several minutes after the injection associating with cardiac irregularities. Otherwise the blood pressure fall proceeded until respiratory arrest ensues, just as in the case with adrenalectomized rats, as illustrated. The critical level of blood pressure for the respiratory arrest was about 20 mmHg.

![Blood pressure changes after intravenous histamine injection (10 mg/kg).](image.png)

**Fig. 3.** Blood pressure changes after intravenous histamine injection (10 mg/kg). BP: systolic and diastolic arterial blood pressure. RR: respiratory rate. CR: cardiac rate. Observations on anesthetized animals.

The common change in ECG induced by histamine in rats was a tendency to an enlargement of the T waves. As shown in Fig. 4, this was extremely prominent in pertussis-inoculated rats and cardiac irregularities of various forms began to appear within several minutes. Unless these were fatal, the enlargement of T waves tended to subside after about 10 minutes and the incidence of the cardiac irregularities was rare. The occurrence of lethal cardiac irregularities, i.e. long continued ventricular flutter, was found to be
**TABLE 1.**

ECG changes after intravenous histamine injection (10 mg/kg) in pertussis-vaccinated rats. (Anesthetized with Nembutal.)

<table>
<thead>
<tr>
<th>ECG changes</th>
<th>Conditions</th>
<th>Cannulated</th>
<th>Non-cannulated</th>
<th>Non-cannulated + Heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cases</td>
<td></td>
<td>16</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>T-wave enlargement</td>
<td></td>
<td>16</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>Ventricular extrasystoles</td>
<td></td>
<td>12</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Ventricular tachycardia</td>
<td></td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Ventricular flutter</td>
<td></td>
<td>8</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>
markedly influenced by the experimental conditions. As shown in Table 1, in animals in which the carotid was cannulated for blood pressure measurement or for blood sampling, ventricular flutter occurred in about 50%, while in merely nembutalized animals ventricular extrasystoles or ventricular tachycardia were the main irregularities observed. These differences did not seem to be related to the body weight or the sex of the animals. Since heparin was used in the cannulated group, the effect of the same dose of heparin (100–200 U, i.v., 10 minutes before) on the histaminic reaction was determined. As shown in the Table the incidence of ventricular flutter markedly increased. Thus heparin was found to promote the appearance of a ventricular flutter if a state of repeated ventricular extrasystoles was present.

The cardiac irregularities occurred even in glucocorticoid-pretreated vaccinated rats. Thus, glucocorticoid is apparently ineffective in preventing the cardiac irregularities. This might be the reason why glucocorticoid was not fully effective in preventing histamine death in vaccinated rats. At any rate the appearance of cardiac irregularities after histamine injection was the characteristic feature in pertussis-vaccinated rats and was not observed in normal or adrenalectomized rats, even with larger doses of histamine (100 mg/kg). The presence of intact adrenals seemed to be necessary for the induction of the cardiac irregularities by histamine in vaccinated rats, since in vaccinated, adrenalectomized rats this could not be observed unless pretreated with glucocorticoid and epinephrine (Refer to Table 2).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Number of experiments</th>
<th>Cases of cardiac irregularities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenx. + GC.</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Adrenx. + Epi.</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Adrenx. + GC. + Epi.</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

Adrenx.: adrenalectomized rats.
GC.: pretreatment with glucocorticoid. Cortisone: 5 mg/kg/day (i.m.) for 3 days.
Epi.: pretreatment with epinephrine. Epinephrine: 100 μg/kg (i.m.) 5 min. before histamine injection.

Observations on anesthetized animals.

In vaccinated rats dyspnea was pronounced immediately after histamine injection, while in adrenalectomized rats no such a response occurred. But in both groups respirations became weak with the progress of hypotension and ceased before cardiac arrest. No death due to asphyxia was observed in either group. Autopsy findings were almost the same in both groups. The most prominent feature was a marked dilatation of the both ventricles. The
lungs were emphysematous especially in case of a protracted lethal course.

**Hemoconcentration and hyperkalemia.** Since the hematocrit and the serum potassium level have been known to elevate in histamine intoxication in rats, these changes were examined in relation to the lethal effect of histamine. As shown in Fig. 5, the hematocrit value rose early after the histamine injection in all the groups. But this was much pronounced in the pertussis-vaccinated group and the adrenalectomized group, while the rise in hematocrit in the normal and the glucocorticoid-pretreated adrenalectomized group was rather temporary. The main cause of the progressive hypotension may probably be due to hemoconcentration. Hyperkalemia was also prominent only in the pertussis-vaccinated and the adrenalectomized groups, but this does not seem to be related to the appearance of the cardiac irregularities observed in the pertussis-vaccinated group. Since at the time of the onset of the cardiac irregularities the rise in serum potassium level was immature and since no cardiac irregularities appeared in the adrenalectomized group despite a marked rise in the serum potassium level. The rise in the serum potassium level might be due to circulatory disturbances since it tends to recover with the recovery of circulation.

![Fig. 5. Changes in serum potassium level and hematocrit after intravenous histamine injection (10 mg/kg). On anesthetized animals.](image-url)
DISCUSSION

The mechanisms of sensitization to the lethal effect of histamine in rats induced by pertussis vaccine may be different from that induced by adrenalectomy. Symptoms in the vaccinated rats were characterized by an immediate dyspnea followed by cardiac irregularities. Pretreatment with glucocorticoid could not fully reduce the mortality rate in histamine intoxication in vaccinated rats, remaining the cardiac irregularities unaltered. BERGMAN and MUNOZ\textsuperscript{1,9} reported that a sufficient quantity of epinephrine protected in some strain of mice pretreated with “histamine sensitizing factor” (HSF) from histamine death. They inferred that the basic mechanism of histamine sensitization might be due to a $\beta$-adrenergic blocking action induced by HSF.

In our experiments on rats, epinephrine did not afford any protection against histamine death in the vaccinated animals (Refer to FIG. 2). There was no difference in the severity of hemoconcentration by histamine between pertussis-vaccinated rats and adrenalectomized rats. The hemoconcentration could be ameliorated by glucocorticoid pretreatment. Adrenalectomized rats in which cardiac irregularities did not occur survived, as HALPERN et al.\textsuperscript{5} noticed.

BOVET et al. (1958) considered that the mechanisms of death in histamine intoxication may be different between normal and pertussis-vaccinated rats. The death of normal rats induced by a massive dose of histamine was immediate and in a state of convulsion, while the death of vaccinated rats with a far less dose of histamine occurred during progressive deterioration ensuing with the initial hypotension from which normal rats recovered. In two experiments with vaccinated rats, BOVET et al. also observed that simultaneously with the appearance of a grave alteration in the ECG (ventricular flutter) a sudden fall in the blood pressure ensued, and death followed shortly afterwards. They inferred that an elevation of serum potassium level in histamine intoxication\textsuperscript{8} might be responsible for these events. However, this inference could not be substantiated. The characteristic ECG change preceding the appearance of cardiac irregularities was a marked enlargement of the T waves which started within one minute following intravenous histamine injection. BOVET et al. suggested this was a sign of coronary insufficiency, but it was quite similar to that observable in the state of mild hypercalcemia induced by intraperitoneal injection of isotonic CaCl$_2$ solution in rats, as will be shown in a following paper\textsuperscript{4}. Detailed mechanisms of the induction of the cardiac irregularities are now under investigation in our laboratory.

In passing it may be mentioned here that the fact that conditioning with endotoxin of gram-negative bacteria which makes adrenalectomized rats tolerate an endotoxin dose of 1 mg/kg (i. v.)\textsuperscript{3} did not interfere with the development of sensitization to histamine induced by pertussis-vaccine. This would indicate that the “histamine sensitizing factor” in the vaccine does not
concern with the ordinary endotoxin reactions.

SUMMARY

The mechanisms of sensitization to the lethal effects of histamine induced by B. pertussis vaccine were found to be different from that induced by adrenalectomy. The frequent appearance of cardiac irregularities which occurred several minutes after the histamine injection was a characteristic feature in the vaccinated rats. In the vaccinated rats glucocorticoid did not afford a complete protection against the lethal effect of histamine; the cardiac irregularities remained unchanged. This is in contrast to the resuscitating effect of the corticoid in the adrenalectomized animals. For the induction of cardiac irregularities presence of both adrenal cortex and medulla were found to be necessary. The cardiac irregularities were not due to hyperkalemia.

REFERENCES

6) KIND, L.S. Inhibition of histamine death in pertussis-inoculated mice by cortisone and neoantergan. J. Allergy, 24: 52, 1953.