Factors Influencing Fibrin-Induced Pulmonary Edema

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Abstract—Effects of depth of anesthesia, pH of fibrinogen and thrombin, and interventions in the vagus nerves on the development of fibrin-induced pulmonary edema were examined in the rat. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium, 25 or 50 mg/kg. Solutions of fibrinogen and thrombin at the same pH were separately injected into the cisterna magna. The pH values were adjusted to 6.5 or 8.5 with Tris buffer. Interventions in the vagus nerves, which consisted of atropine administration at a dose of 1 mg/kg, i.v. or bilateral vagotomy, were performed before the intracisternal injection of fibrinogen and thrombin. Animals in which no interventions in the vagus nerve was performed were designated as intact rats. Lung-water ratio was calculated as a ratio of the difference between wet and dry lung weight to dry lung weight. Incidences of pulmonary edema and lung-water ratios were lower under deep anesthesia than under light anesthesia. Both parameters were low in the vagotomized rats treated under deep anesthesia with fibrinogen and thrombin at a pH of 8.5, as compared to those treated similarly at pH 6.5. This phenomenon was not observed under light anesthesia. Interventions in the vagus nerves influenced the development of pulmonary edema to various degrees, depending on the pH values of the injected fibrinogen and thrombin. As suggested from these results, well-defined, specific conditions are required for investigating the mechanism triggering the development of fibrin-induced pulmonary edema.

Cerebral insults such as epileptic seizures and subarachnoid hemorrhage are often accompanied by rapid flooding of airways with a protein-rich edema fluid such as found in permeability edema. The mediator which elevates the pulmonary vascular permeability is still obscure (1). Experimental models for neurogenic pulmonary edema have been reported elsewhere (2–4). Fibrin-induced pulmonary edema, caused by intracisternal injections of fibrinogen and thrombin, is thought to be a possible model system for neurogenic pulmonary edema (5). A difficulty encountered in the course of our study using this model was that the incidence and severity of fibrin-induced pulmonary edema varied from animal to animal.

In this study, effects of various experimental conditions on the development of pulmonary edema in rats were examined in order to obtain a well-defined model in which fibrin-induced pulmonary edema may be reproducibly induced. We studied the effects of depth of anesthesia, pharmacological and surgical interventions in the vagus nerves, and the pH of the solutions of fibrinogen and thrombin on the incidence and severity of pulmonary edema. As to the last factor, it has been reported that fibrin formation could be affected by a change in pH (6). We will discuss the contributions of these factors to edema formation.

Materials and Methods

Seventy-two rats of either sex, weighing...
from 190 to 350 g, were anesthetized with intraperitoneal injection of pentobarbital sodium at the doses described below. A tracheal tube was inserted following tracheostomy. The right femoral artery was cannulated for measurement of systemic arterial pressure (SAP) and heart rate (HR). After completion of the surgical procedure, rats were fixed in a prone position with a stereotaxic instrument. SAP was measured with a pressure transducer (Nihon Kohden, MPU-0.5) and HR determined by a tachometer (Nihon Kohden, RT-5) which was triggered by an SAP signal. Respiratory movement was also monitored by a force-displacement transducer (Nihon Kohden, TB-611T), the lever of which was connected to the dorsal back thoracic skin.

Animals were divided into twelve groups, in terms of two doses of pentobarbital, two pH values of thrombin and fibrinogen solutions and three kinds of interventions in the vagus nerves (see Table 1). Doses of pentobarbital were 25 mg/kg for light anesthesia and 50 mg/kg for deep anesthesia. Thrombin and fibrinogen solutions were diluted before use at concentrations of 200 units/ml and 100 mg/ml, respectively, with a Tris buffer solution of 6.5 or 8.5 pH. Vagus nerves were left intact, severed on both sides or animals were given an intravenous injection of 1 mg/kg of atropine. Such maneuvers enabled us to compare the vagal functions between efferent and afferent nerves.

Solutions of fibrinogen and thrombin at the same pH, 0.05 ml per solution, were successively injected into the cisterna magna of individual rats. These procedures were performed 40 min after the administration of anesthetics. After intracisternal injections of solutions, 10 min were allowed to elapse, unless edema froth appeared in the tracheal tube. Then, the lungs were excised. When froth appeared in the tracheal tube within 10 min, the experiment was stopped and animals were quickly sacrificed to excise the lungs. Wet lung weight was obtained, and the lungs were dried at 70°C for two days to obtain the dry lung weight. We calculated the ratio (lung-water ratio) of the difference between the wet and dry lung weights (lung-water weight) to the dry lung weight. When edema froth was obtained, blood was also collected before killing the animal, to calculate the protein-ratio of edema froth to blood. Protein concentrations were measured by the Biuret method.

Degree of pulmonary edema was classified into four groups as reported previously (7): Grade 0, no change; Grade 1, the group in which small amounts of edema froth were recognized in the bronchi with compression of the removed lungs; Grade 2, the group in which froth ran off spontaneously from the trachea upon thoracotomy; Grade 3, the group in which froth ran off from the trachea within 10 min after the intracisternal injection of fibrinogen and thrombin.

Statistical significance of differences between two means was evaluated by analysis of variance and Student’s t-test. For nominal parameters such as grade and incidence of pulmonary edema, a median test was performed. Level of significance was taken as 0.05.

Results
Relation between lung-water ratio and incidence of pulmonary edema: Figure 1 shows cumulative distribution curves of lung-water ratio obtained from edematous lungs (grades 2 and 3, PE(+)) and nonedematous lungs (grades 0 and 1, PE(-)). The PE(-) curve was plotted in the same manner as the PE(+) curve, except in the figure, the curve for PE(-) was depicted up-side-down to more clearly demonstrate the relationship of the data. The distribution curves crossed at 4.6 on the abscissa and 15% on the ordinate: If we judge the results from a critical lung-water ratio of 4.6, the probability that edematous lungs may be mistaken for nonedema and vice versa is 0.15 (8).

Effects of anesthesia on development of pulmonary edema: Incidence of pulmonary edema of grades 2 and 3 was larger under light anesthesia (24/28) than under deep anesthesia (16/44) (P<0.05).

At a pH of 8.5, incidence was significantly larger under light anesthesia than deep anesthesia in the vagus-nerve intact and vagotomized rats (P<0.05) (Table 1). Mean values of lung-water ratio in the vagus-nerve intact rats and vagotomized rats were signifi-
significantly larger under light anesthesia than under deep anesthesia (P<0.01) (Table 2).

At a pH of 6.5, incidences were not different between light and deep anesthesias (Table 1), whereas lung-water ratios obtained in the vagotomized rats were significantly larger under light anesthesia than under deep anesthesia (Table 2).

**Effects of pH on the development of pulmonary edema:** Incidence for edema formation of grades 2 and 3 was larger at a pH of 6.5 (29/46) than at 8.5 (11/26) (P<0.05). In the vagotomized rats under deep anesthesia, incidence (Table 1) and lung-water ratios (Table 2) were significantly larger at a pH of 6.5 than at 8.5 (P<0.05). In the atropinized rats, lung-water ratios obtained under light anesthesia were larger at a pH of 6.5 than at 8.5 (P<0.05), whereas under deep anesthesia, no such differences were observed. In the vagus-nerve intact rats, neither incidences of pulmonary edema nor lung-water ratios were different between the two pH values.

**Effects of interventions in the vagus nerve on development of pulmonary edema:** Incidence of pulmonary edema was larger in the vagotomized rats (12/16) than in the vagus-nerve intact rats (13/31) (P<0.05). At a pH of 6.5 under light anesthesia, lung-water ratios were significantly larger in the atropinized and vagotomized rats than in the vagus-nerve intact rats (P<0.01) (Table 2). At a pH of 8.5 under light anesthesia, lung-water ratios were larger in the vagotomized rats than in the atropinized rats (P<0.01).

Under deep anesthesia, interventions in the vagus nerves had little or no effect on the incidence of pulmonary edema and lung-water ratio (Tables 1 and 2).

**Latency in development of fibrin-induced pulmonary edema:** Figure 2 shows the recordings of blood pressure, heart rate and respiratory movement obtained in the vagotomized rats. Pulmonary edema occurred except in an experimental condition of pH 8.5 under deep anesthesia. Time of latency for the appearance of edema froth in the trachea

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**Table 1. Incidence of fibrin-induced pulmonary edema**

<table>
<thead>
<tr>
<th>Anesthesia</th>
<th>pH</th>
<th>Intervention in the vagus nerve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>intact</td>
<td>atropine</td>
</tr>
<tr>
<td>Deep</td>
<td>6.5</td>
<td>5/15</td>
<td>6/11</td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>0/6</td>
<td>1/4</td>
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<tr>
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<td>4/8</td>
<td>6/6</td>
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<td></td>
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<td>4/4*</td>
<td>2/4</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>13/31</td>
<td>15/25</td>
</tr>
</tbody>
</table>

Incidences were indicated as number of rats in which pulmonary edema of grades 2 and 3 was induced/total number of rats used in each group. * P<0.05 vs. pH of 8.5; ** P<0.05 vs. deep anesthesia.
was quite different with each condition. At a pH of 8.5 under light anesthesia, edema froth often flushed out from the tracheal tube at the first expiration after intracisternal injections. The time required for the edema froth to appear in the tracheal tube was 1.73±0.26 min after the injection of thrombin. At a pH of 6.5 under light anesthesia, the edema froth appeared in 4.7±1.0 min, and deep anesthesia prolonged the time (>4.5 min).

**Protein-ratio of edema froth to plasma:** In 9 experiments, edema froth could be collected in an amount sufficient to measure the protein-concentration. Protein concentration in

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### Table 2. Lung-water ratio

<table>
<thead>
<tr>
<th>Anesthesia</th>
<th>pH</th>
<th>Intervention in the vagus nerve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>intact</td>
</tr>
<tr>
<td>Deep</td>
<td>6.5</td>
<td>4.2±0.4</td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>3.2±0.4</td>
</tr>
<tr>
<td>Light</td>
<td>8.5</td>
<td>4.3±0.5</td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>5.3±0.4**</td>
</tr>
</tbody>
</table>

Values indicate the mean and S.E. * P<0.05 vs. pH of 8.5; ** and ***. P<0.05 and 0.01, respectively, vs. deep anesthesia; ††. P<0.01 vs. intact; ††. P<0.01 vs. atropine.

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**Fig. 2.** Tracings of experimental recordings. Rats were anesthetized with pentobarbital (PB), 25 or 50 mg/kg, i.p. The thick arrow indicates an intracisternal injection of fibrinogen and the thin arrow, that of thrombin. These solutions were adjusted with Tris buffer to a pH of 6.5 or 8.5. Before the intracisternal injection, midcervical bilateral vagotomy was performed. BP, blood pressure; HR, heart rate; Res, respiratory movement. Downward recording in the respiratory movement indicates the inspiration.
the edema froth was 70–80% of that in the plasma.

Discussion

Lung-water ratio corresponded to the severity of pulmonary edema estimated by recognizing the edema froth in the trachea or bronchi (Fig. 1). However, caution in interpreting the data is necessary when the occurrence of pulmonary edema is estimated using only the lung-water ratio or the findings of edema froth. First of all, edematous lungs may be mistaken for nonedema or nonedematous lungs mistaken for edema at a probability of 0.15, if the critical lung-water ratio is taken as 4.6. Secondly, lungs of grade 1 were not always nonedematous, and may even be a case of interstitial edema in which edema froth appears in the bronchi only after compression of excised lungs.

Generally, the results obtained in this study indicated that the incidences of pulmonary edema were larger in lightly anesthetized rats than in deeply anesthetized rats. As shown in our previous study (9), nerves which run to the heart and lungs participate in the genesis of neurogenic pulmonary edema. Furthermore, Dauber and Weil (10) reported that sympathetically mediated pulmonary venoconstriction increased the edema froth accumulation caused by oleic acid. As a possibility, deep anesthesia may inhibit such an augmented nerve activity, preventing the accumulation of edema fluid. However, it cannot be excluded that the effects of deep anesthesia on systemic vasculature may decrease a shift of blood volume from the systemic circulation into the pulmonary circulation (11), lowering pulmonary capillary pressure. Because of such nonspecific effects of deep anesthesia on nerves and/or the cardiovascular system, reasons why deep anesthesia hindered the development of pulmonary edema are still obscure.

Under light anesthesia, the lung-water ratios in the atropinized or vagotomized rats treated with fibrinogen and thrombin at a pH of 6.5 were greater than those in the vagus-nerve intact rats, a tendency not seen in rats anesthetized deeply (Table 2). These results indicate that efferent cholinergic fibers in the vagus nerves may play a role to lower the severity of pulmonary edema. Yet this was not the case in rats treated with fibrinogen and thrombin at a pH of 8.5. The fact that the lung-water ratio was larger in the vagotomized rats than in the atropinized rats treated at a pH of 8.5 under light anesthesia indicated rather that afferent nerves may inhibit the development of pulmonary edema. These controversial results seemed to be derived from a difference in pH at the time of intracisternal fibrin formation. Although the mechanism still must be investigated, the vagus nerve may exert its inhibitory actions on the development of pulmonary edema via either afferent or efferent nerves.

Contradictory results have been published, with some groups claiming that vagotomy or atropine is protective (12, 13), whereas others have found that eliminating vagal influence had no effect (14). The discrepancy between theirs and our results may be derived from differences in the methods used to induce the neurogenic pulmonary edema: 1) rapid increase in intracranial pressure (12, 13), 2) preoptic lesion (14) and 3) injection of fibrin (this study). More specific studies in which stimulated or inhibited sites in the central nervous system are ascertained will be required to unravel the influence of vagal tone on the development of neurogenic pulmonary edema.

Under deep anesthesia, both the incidence and lung-water ratios obtained in the vagotomized rats treated with fibrinogen and thrombin were greater at a pH of 6.5 than at 8.5, whereas under light anesthesia, no such a difference was observed. Okada and Blomback (6) reported that low pH, 6.5, favored the production of an infinite network of fibrin gels with large pore size, whereas high pH, 8.5, produced gels with a tight network and small pore size. It is probable that the difference in incidence of pulmonary edema which was distinguishably obtained under deep anesthesia may be related to such effects of pH on the fibrin formation.

Latency for development of pulmonary edema was the shortest in the vagotomized rats treated at a pH of 8.5 under light anesthesia (1.73 min after injection of thrombin). From such a fast reaction, an elevated vascular permeability as suggested from the
high protein concentration ratios of froth to plasma (>70%) was considered to be mediated by a so-called permeability nerve as proposed by Minnear and Malik (15).

Thus, three factors, i.e., anesthesia, pH of fibrinogen and thrombin and vagal activities (afferent and efferent) influenced the incidence and severity of fibrin-induced pulmonary edema. It should be noted that when rats are vagotomized and treated with fibrinogen and thrombin at a pH of 6.5, pulmonary edema occurs in all of the rats anesthetized either deeply or lightly, although lung-water ratios may be slightly modified by the depth of anesthesia. Such a definite experimental condition will be beneficial for investigating the mechanisms involved in the development of pulmonary edema and also to identify substances which can elevate the vascular permeability.

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References