INCIDENCE OF EPINEPHRINE-INDUCED PULMONARY EDEMA IN RABBITS AND ACCOMPANYING METABOLIC CHANGES

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Abstract—Searching for an experimental condition to produce pulmonary edema of uniform high grade severity in rabbits, five different doses of epinephrine (38, 60, 100, 160 and 260 µg/ml) were infused at a rate of 0.29 ml/min for 20 min into the femoral vein of fed and fasted animals. Body temperature was maintained at 38 to 40°C. The incidence of pulmonary edema was not dose-dependent, and was approx. 70% by the administration of 60 to 260 µg/ml of epinephrine solutions. In general, the edema was more severe in the fed group than in the fasted. The highest dose of epinephrine was often fatal in the fasted group. One hundred µg/ml (approx. 10 µg/kg/min), fed was regarded as a favourable condition for the experiment. The amounts of lung lipids increased in edema (+) cases of the fed groups. The plasma potassium level was elevated in proportion to the dose of epinephrine. The clotting time of blood was markedly prolonged in edema (+) rabbits. The significance of these observations was discussed in regard to the mechanism of epinephrine-induced pulmonary edema.

The mechanism of epinephrine-induced pulmonary edema has been discussed by many workers, but some critical factors which are responsible for the acute vascular permeability change still seem to remain unclarified (1-4). In the previous papers, one of the authors investigated the relationship between the hemodynamic changes and the occurrence of pulmonary edema induced by intravenous infusion of epinephrine in rats and rabbits (5, 6). In the experiments with rabbits, a considerable difficulty was experienced in consistently producing pulmonary edema of uniform high grade severity. The present study was undertaken to devise more adequate experimental conditions. Furthermore, to get useful information on the accompanying metabolic changes, we examined the levels of lipids in the lungs and some plasma components.

MATERIALS AND METHODS

Male rabbits, weighing 2.5 to 3.0 kg, were used. The experiments were carried out in two kinds of feeding states, and five different doses of epinephrine were given. Of the 75 rabbits used, 35 were fed and 40 were fasted overnight. Both were subdivided into 5 groups, and one of the five doses of epinephrine was administered to the animals in each

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group. Each group included 8 rabbits except in three groups (fed 38 µg/ml, 3; fasted 60 µg/ml, 11 and fasted 260 µg/ml, 5 cases).

The animals were anesthetized by an intravenous injection of sodium pentobarbital, 25 mg/kg. Body temperature was maintained at 38 to 40°C during the experiment by means of a vinyl bag containing warm water, on which the animals were fixed on their backs. Water of 41 to 42°C was circulated continuously through the bag. Systemic blood pressure was recorded from the femoral artery by means of polyethylene tubing connected to a high pressure transducer (Nihon Kohden, LPU-0.5). The heart rate was recorded simultaneously using tachograph (Nihon Kohden, RT-2). The respiratory movement was traced by a strain gauge transducer (Nihon Kohden, SB-1T) connected to the skin of the thoracic wall.

The concentrations of L-epinephrine (Bosmin, Daiichi-Seiyaku) solutions were 38, 60, 100, 160 and 260 µg/ml. One of these solutions was infused into the femoral vein at a rate of 0.29 ml/min for 20 min. After infusion, the lung was removed and the ratio of lung weight to body weight (L-B index) was examined. The degree of edema was divided into four grades as follows: grade 0, no change; grade 1, a group in which small amounts of edema froth were recognized in the bronchi with compression of the removed lung; grade 2, a group in which froth ran off spontaneously from the trachea upon thoracotomy; grade 3, a group in which froth ran off from the trachea during the infusion of epinephrine. In the following, cases which exhibited grade 2 and 3 are referred to as pulmonary edema (+), and the other cases are referred to as pulmonary edema (−).

The lipids were extracted from the lung and liver according to the method of Folch et al. (7). The extract was mixed thoroughly with 0.2 volume of 0.73% NaCl solution and the mixture was separated into two phases by centrifugation. The upper phase was removed and the lower one washed again with a mixture of chloroform, methanol and water (3:48:47). After the second washing, the lower phase was evaporated to dryness and the amounts of the total lipid were determined gravimetrically. The residue was then dissolved in the appropriate volume of chloroform and used as a source for the determination of triglyceride (8) and phospholipid (9).

The potassium ion level in the plasma was measured by flame spectrophotometry after the infusion of epinephrine. Proteins in the plasma before and after the infusion and in the edema froth were fractionated by cellulose acetate and polyacrylamide disc gel electrophoresis and then direct densitometry was carried out after staining. Protein concentrations were determined by the biuret method. The clotting time of blood was measured by Lee-White's method.

Statistical significance of differences of values was calculated using Student’s t test.

RESULTS

Hemodynamic changes

As shown in Fig. 1, epinephrine caused a sustained rise in the systemic arterial blood pressure, usually with initial bradycardia, in all groups. Many times, Cheyne-Stokes type respiration appeared during the early half of the infusion period. In the case of grade 3,
large fluctuations in blood pressure, accompanied by irregularities in the cardiac rhythm and respiratory movements, preceded the spontaneous runoff of edema froth from the tracheal cannula (bottom tracing in Fig. 1).

**Relationship between the dose of epinephrine and pulmonary edema**

The occurrence of pulmonary edema and the L-B indices are shown in Fig. 2. The numbers of animals belonging to grades 0 to 3 were 24, 6, 22 and 23, respectively. Taking grade 2 and 3 as edema (+) cases (○, ●, □ and ■ in the figure), the incidence was only 27% (3 out of 11) in the 38 μg/ml group. In the other four groups, the 60, 100, 160 and 260 μg/ml groups, the incidence of edema was 58% (10 out of 19), 63% (10 out of 16), 69% (11 out of 16) and 77% (10 out of 13), respectively. Thus, except in the 38 μg/ml group, no clear difference was observed among the results of the four groups. Definite differences between fed and fasted animals were not evident.

Regarding the L-B indices, those of edema (+) cases were clearly larger than...
TABLE 1. Levels of lipids in lungs and livers from the fed and fasted rabbits after epinephrine infusion

|                | Lung | Liver |
|----------------|------|-------|
|                | Total Lipid | Triglyceride | Phospholipid | Total Lipid | Triglyceride | Phospholipid |
|                | mg/kg BW | pmoles/kg BW | pmoles/kg BW | g/kg BW | pmoles/kg BW | pmoles/kg BW |
| Fed            |         |         |           |         |         |           |
| Grade 0 & 1 (14) | 115 ± 5.4 | 5.93 ± 0.30 | 112 ± 5.3 | 1.76 ± 0.11 | 311 ± 51 | 1.51 ± 0.08 |
| Grade 2 & 3 (21) | 152 ± 9.6* | 8.70 ± 1.10* | 145 ± 8.9* | 1.53 ± 0.05 | 257 ± 35 | 1.42 ± 0.05 |
| Fasted         |         |         |           |         |         |           |
| Grade 0 & 1 (14) | 145 ± 9.6 | 6.62 ± 0.93 | 117 ± 6.2 | 1.53 ± 0.08 | 246 ± 47 | 1.40 ± 0.06 |
| Grade 2 & 3 (21) | 128 ± 7.5 | 7.33 ± 0.71 | 101 ± 3.6* | 1.54 ± 0.06 | 372 ± 56 | 1.30 ± 0.04 |

The values are the means ± S.E. *: Significantly different from the corresponding value in Grade 0 & 1 (p<0.05). Figures in parentheses indicate numbers of rabbits.

Table 1 shows the levels of lipids in lungs and livers from the fed and fasted rabbits after epinephrine infusion. The results are summarized in Table 1. Lipid levels in the lungs of fed rabbits were increased in the edema (-) animals. No remarkable change was seen in fasted animals, except in phospholipid. In the liver, there was no marked difference between edema (-) and edema (+) animals.

**Lipid levels in the lungs**

The results of measurement of the lipid content in the lung and the liver are summarized in Table 1. Lipid levels in the lungs of fed rabbits were increased in the edema (+) animals. No remarkable change was seen in fasted animals, except in phospholipid. In the liver, there was no marked difference between edema (-) and edema (-) animals.

**Levels of plasma components**

As shown in Fig. 3, the potassium ion level in the plasma increased in proportion to the dose of epinephrine infused. This level in the fasted groups was higher than that of the fed. However, this result does not correlate directly with the dose of epinephrine infused. The highest dose of epinephrine was too toxic for the fasted animals. Eight rabbits died of ventricular fibrillation (one in each of the fed 100, the fasted 38, 60, 100, 160 μg/ml, and three in the fasted 260 μg/ml group). These cases were excluded from the results.

**Fig. 3. Potassium ion concentration in plasma after epinephrine infusion.**

Figures below doses of epinephrine represent numbers of rabbits used.

*: Significantly higher than the corresponding value in the fed animals (p <0.05).
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Protein concentration in the edema froth was measured and compared with that in the plasma. The latter was $87.9 \pm 2.5$ mg/ml (mean±S.E., $N=11$) in fed rabbits, and $89.2 \pm 1.6$ mg/ml ($N=27$) in fasted rabbits before administration of epinephrine. Protein concentration in the edema froth was 73% of that in the plasma of the same animals in 5 fed rabbits on the average, and 78% in 21 fasted ones. Furthermore, the major peaks in the electrophoretic pattern of the edema froth were nearly the same as those of the plasma.

The clotting time of the blood was determined only in the fasted groups. The time in 18 edema (+) rabbits was clearly prolonged compared to 14 edema (-) ones. Namely, in 15 cases of the edema (+) rabbits, it took more than 20 min—more than 30 min in 10 of them. On the other hand, in the edema (-) rabbits, the blood clotted within 20 min in 12 out of 14 cases.

DISCUSSION

Various parameters were examined herein in an attempt to find clues on the mechanism of epinephrine-induced pulmonary edema.

In consideration of the possibility that the feeding state of the animals might have a great influence on the variations of the metabolic changes induced by epinephrine, two sets of experiments were performed in the fed and the fasted rabbits. Although smaller variations had been expected in the latter, no marked differences were obtained between the two groups.

It is well known that lipids play a great role in maintenance of the structure and function of the lung (10-13). Harlan et al. reported that pulmonary edema induced by dextran in dogs was accompanied by significant decreases in the phospholipid level (14). Such being the case, the effect on lipids in the lungs during epinephrine infusion was deemed worthy of investigation. Our results showed that the amounts of total lipids, triglycerides and phospholipids increased unexpectedly in the fed, edema (-) rabbits. Although the significance of such findings is not clear, it is necessary to take into account the lipid contents of the plasma or blood contained in the edematous lung tissue.

Confirming the earlier observations (2), the high protein content in the edema froth indicates an alteration of vascular permeability in the lung.

In many of the animals exhibiting pulmonary edema, the blood clotting time was markedly prolonged. It is known that clotting of blood and production of some permeability factors, for instance bradykinin, are closely interrelated in their mechanisms. Thus, further analyses of the phenomenon may be worthwhile.

The potassium ion concentration increased in proportion to the dose of epinephrine infused (Fig. 3) and this hyperkalemia must have been toxic to the heart. However, the high plasma potassium ion concentration in itself cannot explain the occurrence of pulmonary edema, as the incidence of edema was almost the same in each dosage group.

Regarding the incidence and grade of edema, an outline was obtained in this study over the range of epinephrine dosage used. The incidence was not simply dose-dependent, and
was about 60 to 80% in the 60 to 260 μg/ml groups. From these results, it may be stated that one favourable condition for further experiment will be 100 μg/ml (approx. 10 μg/kg/min), fed, by which a relatively high incidence of severe edema will be secured without too great an increase in plasma potassium. High plasma potassium possibly makes the interpretation of results more complex. Furthermore, this dose of epinephrine will not cause a ventricular fibrillation frequently.

The mechanism of the pulmonary edema have been discussed by many authors. Recently, Robin et al. summarized the causes as follows: a) altered permeability, b) increased pulmonary capillary pressure, c) decreased oncotic pressure, d) lymphatic insufficiency, e) increased negative interstitial pressure, and f) mixed or unknown mechanisms (4). In our study, a) the number of grade 1 was few (only 6 out of 75, Fig. 2), b) the incidence was not dose-dependent and c) the L-B indices of the edema (+) cases were clearly larger than those of the edema (−) ones. These observations suggest that the epinephrine-induced pulmonary edema occurs in an all-or-none manner, rather than in a graded manner, and that, once the edema occurs, it quickly aggravates to grade 2 or 3. The occurrence may depend on some unknown factor which may be operative under the increased pulmonary vascular pressure, and which would discontinuously alter the permeability.

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