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Kinin-inactivating Enzyme from the Mushroom *Tricholoma conglobatum*. VII. Suppression of Epinephrine-induced Pulmonary Edema in Rats[1)]

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Shimeji kininase intravenously administered almost completely suppressed the pulmonary edema in rats induced by L-epinephrine. This observation suggested that kinin might be involved in the genesis of this edema because this enzyme maintained its kininase activity in the rat body for a fairly long time and was able to block the kinin action in the body. However, no inhibitory effect was observed when Shimeji kininase was intraperitoneally administered. The reason for this was probably that the amount of Shimeji kininase that entered into the vascular system was small, and the concentration of Shimeji kininase in the plasma was therefore inadequate.

On the other hand, attempts to determine whether the kallikrein–kinin system was really activated or not in this pathogenetic state were unsuccessful because L-epinephrine present in plasma interfered with the quantitative determination of components related to the kallikrein–kinin system in plasma. At present, the mechanism of the activation of the kallikrein–kinin system in this pathogenetic state is not clear, although the marked suppression of the pulmonary edema by Shimeji kininase strongly suggested the involvement of the kallikrein–kinin system in this pathogenetic state in rats.

Keywords——kinin-inactivating enzyme from mushroom; kinin; pulmonary edema; epinephrine; kallikrein–kinin system; kininogen

Pulmonary edema is an inflammation in the lung and its occurrence is considered to be based on vascular permeability changes leading to engorgement of the interstitial spaces between the vascular and alveolar walls.[2,3)] This edema is experimentally induced by the intravenous injection of large doses of L-epinephrine into experimental animals, and almost all the animals usually die. Many investigations to identify the substances that cause this vascular permeability change have been performed. For example, Di Mattei found bradykinin in high concentrations in the lung, liver and plasma of rabbits suffering from epinephrine-induced pulmonary edema,[4)] and the depletion of total kininogen in the plasma of rats given an intravenous injection of L-epinephrine was reported by Castania and Rothschild.[5)] On the other hand, Katori *et al.* reported that bromelain, which causes consumption of high molecular weight kininogen (HMW-K) in plasma, strongly suppressed the epinephrine-induced pulmonary edema whereas papain and ficin, which have no effect on plasma kininogen content, did not suppress this edema.[6)] These observations suggest that the kallikrein-kinin system might be involved in the epinephrine-induced edema.

The authors have been working on a potent kinin-inactivating enzyme, Shimeji kininase, from the mushroom *Tricholoma conglobatum* in the hope of blocking the kinin action in the body,[7)] because the roles of the kallikrein-kinin system in the body could be revealed by experiments in which the kinin action is specifically blocked. As already reported, this enzyme has extremely potent kinin-inactivating activity and is able to block kinin action in the body for a fairly long period.[7c)] Thus, this enzyme may be useful in research on the physiological and pathological significance of the kallikrein–kinin system in the body as a blocker of kinin action.

In the present investigation, the authors tested this enzyme in the case of epinephrine-induced pulmonary edema in rats, in order to investigate the possible involvement of the kallikrein-kinin system.

Materials and Methods

Materials——L-Epinephrine was purchased from Tokyo Kasei Kogyo Co. (Tokyo) and sodium pentobarbital was obtained from Pitman-Moore Inc. (Washington, N.J., U.S.A.). Bradykinin was a product of the Protein Research Foundation (Osaka). Shimeji kininase used was purified according to our previous paper.[7a] One kininase unit is the amount of enzyme degrading 1 μg of synthetic bradykinin in 1 min at 30°C, pH 7.4.

Pulmonary Edema——Wistar rats weighing 120—220 g were anesthetized by intravenous administration of sodium pentobarbital at a dose of 50 mg/kg of body weight and a polyethylene cannual was introduced into the left femoral vein. L-Epinephrine solution, 100 μg/ml, was infused into the femoral vein *via* this cannula at a uniform rate for 10 min at a dose of 250 μg/kg of body weight, and the mortality rate and changes in lung wet weight/body weight ratio were observed. Changes in lung wet weight/body weight ratio reflect the degree of pulmonary edema.[2,3]

Kinin Assay——Kinin assay was carried out by the Magnus method using isolated rat uterus.[7e]

Results

Inhibition of Pulmonary Edema by Shimeji Kininase

Table I shows the inhibitory effect of Shimeji kininase on epinephrine-induced pulmonary edema in rats. As shown in Table I, the ratio of lung wet weight/body weight of the normal rats expressed as percentage was 0.63±0.02. On the other hand, that of the rats administered L-epinephrine was 1.43±0.05, and all the rats died (treatment 2 in Table I). Petechiae and hyperemia were always observed in the pulmonary tissue of the dead rats. In contrast, the edema was markedly suppressed by the intravenous administration of Shimeji kininase 15 min before the infusion of L-epinephrine (treatments 4 and 5 in Table I). In particular, 500 kininase U of Shimeji kininase completely inhibited the edema; all the rats survived and the ratio of lung/body weight of the rats administered Shimeji kininase (0.66±0.01) was almost equal to that of the normal rats. Neither hyperemia nor petechiae was observed in the pulmonary tissue of the rats pretreated with 500 U of Shimeji kininase. However, no inhibitory effect was observed even when high doses of Shimeji kininase were intraperitoneally administered 30 min before the infusion of L-epinephrine (treatment 7 in Table I). It is possible that amount of Shimeji kininase that entered the vascular system was small, and the concentration of Shimeji kininase in the plasma was therefore insufficient. Shimeji kininase itself of course did not cause pulmonary edema (treatment 3 in Table I).

TABLE I. Inhibition of L-Epinephrine-induced Pulmonary Edema in Rats by Shimeji Kininase

Treatment	Edema (Lung weight/body weight) × 100 ± S.E.	Survival rate (%)
1. None (normal rats)	0.63±0.02 (1.0)	12/12 (100)
2. L-Epinephrine	1.43±0.05 (2.3)	0/9 (0)
3. Shimeji kininase 300 kininase U, *i.v.*	0.66±0.04 (1.0)	4/4 (100)
4. Shimeji kininase 200 kininase U, *i.v.*+L-epinephrine	1.13±0.12 (1.8)	3/9 (33)
5. Shimeji kininase 500 kininase U, *i.v.*+L-epinephrine	0.66±0.01 (1.0)	4/4 (100)
6. Shimeji kininase 400 kininase U, *i.p.*+L-epinephrine	1.50±0.33 (2.4)	0/3 (0)
7. Shimeji kininase 900 kininase U, *i.p.*+L-epinephrine	1.48±0.17 (2.3)	0/3 (0)

Shimeji kininase was intravenously (*i.v.*) or intraperitoneally (*i.p.*) administered to the rats 15 or 30 min before the infusion of L-epinephrine, respectively. The survival rates (number of surviving rats/number of rats employed in each group) were determined at 1 h after the infusion of L-epinephrine. The survival rates expressed as percentages are shown in parentheses. The ratios of lung weight/body weight were also expressed as percentages. The lungs of the dead rats were removed just after death and weighed. The surviving rats were killed 1 h after the infusion of L-epinephrine, their lung weights were measured. The ratios of edema formed are given in parenthesis relative to normal rats taken as 1.0.

Failure of Kinin Liberation in the Plasma of Rats treated with L-Epinephrine

Fig. 1 shows the failure of kinin liberation in the fresh plasma of rats treated with L-epinephrine. As shown in Fig. 1-C to -E, kinin activity was not observed in the incubation mixture of fresh rat plasma and L-epinephrine even after 25 min incubation.

Fig. 2 shows the kinin liberation in the above incubation mixture upon adding glass powder to it. It is reported that addition of glass powder to the plasma causes plasma prekallikrein activation, and the activated plasma kallikrein liberates bradykinin from HMW-K.[6] Thus, the kinin liberation shown in Fig. 2-B and -E means that HMW-K remained in the L-epinephrine-treated plasma. These findings suggested that rat plasma prekallikrein was not activated only by the treatment of plasma with L-epinephrine.

We also attempted to investigate the variations of kininogen content in plasma *in vivo* after the intravenous injection of L-epinephrine. However, this attempt was technically unsuccessful because L-epinephrine present in the plasma caused marked suppression of the contraction of rat uterus induced by bradykinin. Namely, as shown in Fig. 3-C and -F, contractile responses to bradykinin were strongly suppressed by adding 2 μg of L-epinephrine compared with the control responses (Fig. 3-A and -B). Thus, accurate determination of kininogen content in the plasma containing L-epinephrine could not be carried out. Washing of the uterus did not wholly remove this suppressive effect of L-epinephrine (Fig. 3-D) but it gradually disappeared during repeated washing of the uterus and addition of bradykinin

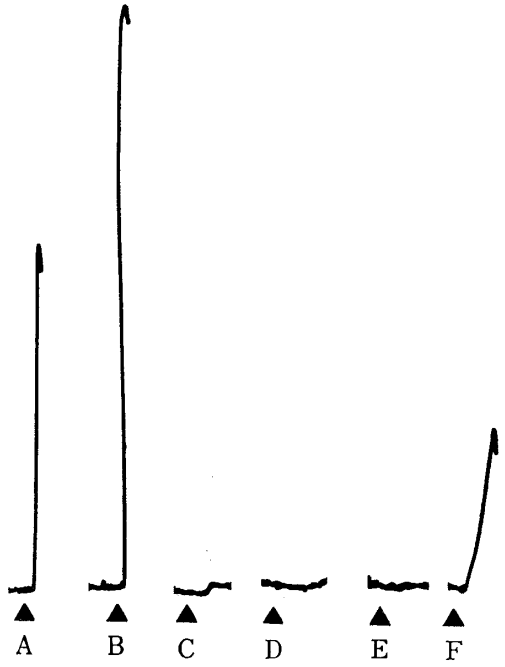

Fig. 1. Failure of Kinin Liberation in the Plasma of Rats treated with L-Epinephrine

A,B and F; bradykinin 4, 10 and 4 ng, respectively. C, D and E; Twenty μl of L-epinephrine solution (100 μg/ml) was added to the fresh rat plasma (500 μl) and the mixture was incubated at 30°C in the presence of 8-hydroxyquinoline (1 mg/ml). After 5 (C), 15 (D) and 25 min (E), 20 μl of this mixture was added to the organ bath. In order to remove the suppressive effect of L-epinephrine on the contraction of rat uterus induced by bradykinin, between the assay periods of C and D, and D and E, 4 ng of bradykinin was added to the bath and the preparation was washed (twice) (see Results).

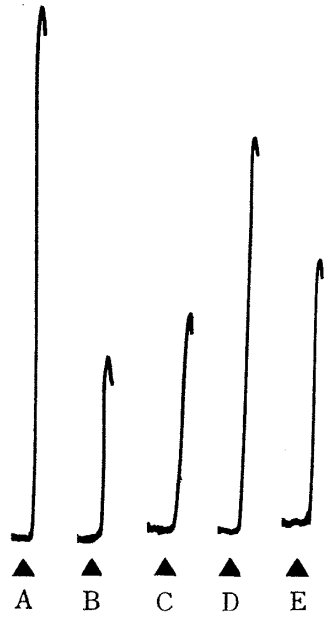

Fig. 2. Remaining High Molecular Weight Kininogen in the Plasma of L-Epinephrine-treated Rats

A, C and D; bradykinin 4 ng. B and E; Glass powder (0.5 g) was added to the rat plasma (500 μl) which had been incubated in advance with 20 μl of L-epinephrine solution (100 μg/ml) in the presence of 8-hydroxyquinoline (1 mg/ml) at 30°C for 30 min. After 5 (B) and 15 min (E), a 20 μl aliquot was assayed.

Fig. 3. Suppression of Kinin Activity by L-Epinephrine

A; bradykinin 10 ng. B, D and E; bradykinin 4 ng. C; bradykinin 4 ng and L-epinephrine 2 μg were added to the bath at the same time. F; bradykinin 10 ng and L-epinephrine 2 μg were added to the bath at the same time.

(Fig. 3-C to -E). The same result (suppression of kinin action by L-epinephrine) is also apparent in Fig. 1-F and Fig. 2-C and -D.

Meanwhile, the suppression of kinin action by L-epinephrine was not complete and the kinin activity could be qualitatively observed even in the presence of L-epinephrine (Figs. 1 to 3). Thus, the failure to detect kinin activity (Fig. 1-C to -E) is not due to the suppression of kinin liberated in the plasma of L-epinephrine-treated rats. Kinins would not be liberated by only the treatment to rat plasma with L-epinephrine.

Discussion

In the present investigation, the authors applied Shimeji kininase to a study of epinephrine-induced pulmonary edema in rats in order to investigate the involvement of the kallikrein-kinin system in this edema. This enzyme is able to block the kinin action in the body due to rapid destruction of kinins, but does not suppress the actions of acetylcholine, histamine and serotonin.[7c] As shown in Table I, Shimeji kininase intravenously administered almost completely suppressed the pulmonary edema in rats induced by L-epinephrine. This suggested that kinin might be involved in the genesis of this edema. However, as shown in Fig. 1, L-epinephrine could not activate the plasma prekallikrein when it was incubated with fresh rat plasma. Concerning this point, Shigei *et al.*[8] and Katori *et al.*[9] also observed that Trasylol, a kallikrein inhibitor, did not suppress the edema, and that no consumption of HMW-K or low molecular weight kininogen in plasma occurred after the intravenous injection of L-epinephrine. These observations seem to rule out the involvement of plasma kallikrein in this adema as a kinin releasing enzyme. Rothschild *et al.*, however, showed that plasma prekallikrein could be activated by the administration of L-epinephrine in the body.[10] Namely, they showed that when L-epinephrine was incubated only with plasma, plasma prekallikrein was not activated, but prekallikrein activation and an increase of BzArgOEt-esterase activity in plasma occurred when L-epinephrine was incubated with plasma containing peritoneal fluid cells such as mast cells. Mast cells are widely distributed in the body. Thus, it is possible that plasma prekallikrein was secondarily activated by the administration of L-epinephrine and that the liberated kinin contributed to the vascular permeability increase in the pulmonary tissue, though the exact role of mast cells in plasma prekallikrein activation was obscure.

On the other hand, Castania and Rothschild observed that the fibrinolytic activity of plasma following intravenous injection of L-epinephrine into rats showed a transitory increase.[5] This observation suggests that enzymes other than plasma kallikrein, such as plasmin, may play more important role(s) than plasma kallikrein; plasmin and some other enzymes, including glandular kallikrein, also have kininogenase activity although plasmin is not a good kininogenase. One of the ways to check whether plasma kallikrein or other enzymes are involved in this edema as a kinin-releasing enzyme might be to investigate the variations of plasma kininogen content in rats suffering from epinephrine-induced pulmonary edema, because plasma

kallikrein causes specific depletion of HMW-K. However, an attempt to do this failed for technical reasons (see Results). At present, we are not able to remove L-epinephrine from the sample without affecting substances related to the kallikrein-kinin system.

In conclusion, application of Shimeji kininase to studies on epinephrine-induced pulmonary edema strongly suggested that kinin(s) must be involved in this edema, but the enzyme(s) involved in kinin liberation in this pathogenic state have not yet been identified.

References and Notes

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