Plasma Extravasation by Chemical Mediators in Rat Skin and Trachea: A Role of Neurogenic Agents on Tracheal Edema Formation

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Abstract—The plasma extravasation inducing activities of several chemical mediators (allergic agents: histamine, leukotriene C₄ (LTC₄) and platelet activating factor (PAF); neurogenic agents: substance P, capsaicin and carbachol) have been investigated and characterized in rat skin and trachea. Substance P, histamine, LTC₄ and PAF induced dose-dependent plasma extravasation in rat skin. The activities of these mediators in inducing tracheal plasma extravasation were very different from those in the skin reactions. When these mediators were injected intravenously, substance P induced severe plasma extravasation, and the activities of histamine and PAF were weaker than that of substance P. When injected intratracheally, only substance P and capsaicin induced tracheal plasma extravasation, while none of the allergic mediators tested caused any plasma extravasation in the trachea. Carbachol did not induce any plasma extravasation in either skin or trachea. These results indicate that the stimulation of afferent substance P-containing nerve fibers has a more important role in the induction of tracheal plasma extravasation than that of allergic chemical mediators.

It is well-known that asthma is one of the allergic diseases. However, asthmatic attacks are induced not only by allergens but also by various nonspecific stimuli such as cold air, smoking, SO₂ gas and so on (1–9). Recently it has been suggested that the increase in airway reactivity to various stimuli, namely “hyperreactiveness”, plays an important role in the generation of asthma (7, 10). Of the many known causes of airway hyperreactiveness, inflammatory damage in the airway has recently received the most attention (10, 11). In a normal airway, irritant receptors, which are sensitive to various stimuli, are covered by airway mucosal membranes that prevent contact with these stimuli. On the other hand, in an asthmatic airway, inflammation causes damage to the mucosal membranes. Also, the exposed irritant receptors are easily activated by various nonspecific stimuli. The nervous system component which is activated through irritant receptors is considered to be afferent C-fibers containing tachykinins as neurotransmitters (12–16). In fact, the pungent agent of red peppers “capsaicin”, which stimulates C-fibers, leads to substance P release, inducing severe bronchoconstriction in the experimental animals (10, 17, 18). While many studies have been published about the mechanism of mediator-induced bronchoconstriction, still little is known about bronchial mucous edema.

Our results suggest that the excitation of afferent C-fibers and the release of substance P from their endinds is important in the generation of rat mucous edema in the airway.

Materials and Methods

Drugs: Substance P (Sigma), histamine (Nakarai), LTC₄ and carbachol (Nakarai) were dissolved in saline. Capsaicin (Sigma) and PAF were dissolved in ethanol and diluted with saline. LTC₄ and PAF were
synthesized in our laboratories according to the methods reported by Corey et al. (19) and Heymans et al. (20), respectively.

**Rat skin reactions**: Male Wistar rats (250–300 g) were injected intradermally with 0.1 ml of chemical mediator solution and immediately given intravenously 1 ml of saline containing 5 mg Evans blue (Merck). After one hour, the animals were decapitated, and the skin was exfoliated and the intensity of skin reaction was evaluated by expressing the size of the stained area as the mean of the longest and shortest diameter.

**Rat tracheal mucous edema reaction**: Male Wistar rats (250–300 g) anesthetized with urethane (3 g/kg, i.p.) were injected intravenously with Evans blue solution (20 mg/kg), followed by the injection of various chemical mediator solutions intravenously (2 ml/kg) or intratracheally. For the intratracheal injection, the trachea was exposed, and 50 ul of chemical mediator solution was injected into the trachea using a syringe. After 10 min, the animals were sacrificed by blood-letting, and the lungs were perfused with 50 ml of saline. Trachea and stem bronchi were dissected out, weighed, and then dissolved in 0.25 ml of 1 N KOH at 37°C for 6 hr. After the extraction with 2.25 ml of acetone-phosphate solution

![Fig. 1. Dose-response curve showing the rat skin reactions induced by substance P, capsaicin, PAF, histamine, LTC4 and carbachol. Each point indicates the mean±S.E.M. of 5 animals. (*P<0.05, **P<0.01).](image-url)
(0.6 N H₃PO₄ : acetone=5:13) the tissue Evans blue content was quantified colorimetrically at 620 nm.

**Data analysis:** The results were expressed as the mean±standard error (S.E.M). Student’s t-test was used to make a statistical comparison between the groups.

**Results**

In order to explore the mechanism and mediators of tracheal mucous edema formation, we examined rat tracheal plasma extravasation induced by allergic agents (histamine, LTC₄, PAF) and neurogenic agents (substance P, capsaicin, carbachol) and compared them with the skin reaction induced by the same agents.

As shown in Fig. 1, rat skin reactions were induced dose-dependently by histamine, LTC₄, PAF and substance P, whereas capsaicin and carbachol induced no significant reaction even at relatively high doses. Among the agents tested, substance P was the most potent inducer of the skin reaction. The ability of histamine to induce the skin reaction was very weak compared with those of substance P, PAF and LTC₄.

Results obtained from experiments on tracheal edema differed greatly from those of skin reactions. As shown in Fig. 2, when these mediators were injected intravenously, only substance P induced significant plasma extravasation.

![Fig. 2. Dose-response curve showing the rat tracheal plasma extravasation induced by intravenous injection of substance P, capsaicin, PAF, histamine, LTC₄ and carbachol. Each point indicates the mean±S.E.M. of 3-6 animals. (*)P<0.05, (***)P<0.01.](image-url)
extravasation in the trachea. The substance P response was maximum at the 32 nmol/kg dose. Histamine and PAF showed relatively weak activities in inducing the tracheal plasma extravasation. LTC₄, capsaicin and carbachol showed no activity. As shown in Fig. 3, when these mediators were injected intratracheally, only substance P and capsaicin induced plasma extravasation. Capsaicin induced a significant reaction at doses greater than 0.17 nmol/site and was stronger than substance P. The dose-response curve of capsaicin was typically bell-shaped and greatly differed from that of substance P. The reason for these differences is obscure. Carbachol did not induce any tracheal plasma extravasation or any skin reaction. Interestingly, none of the allergic mediators tested showed any significant reaction in the trachea when injected intratracheally.

We did not measure the extent of plasma extravasation in smaller bronchi due to technical reasons. However, by macroscopic observation, it was noticed that there were site differences in the edema formation by each agent. Following intravenous injection, plasma extravasation was induced in the entire airway by substance P, in the trachea by histamine, and in smaller-than-stem bronchi by PAF. When injected intratracheally, both substance P and capsaicin preferentially induced plasma extravasation in the smaller bronchi.

**Discussion**

Recently, in the studies of asthmatic
disease, much interest has been focused on the inflammatory reaction in the airway (10, 11). The typical clinical picture of asthma is bronchial constriction, mucosal edema and mucus hypersecretion. Thus, hyperreactiveness of the airway is considered to be a basic phenomenon of asthma. Since the inflammatory reaction in the airway may play an important role, not only in the induction of airway hyperreactiveness but also in the generation of the chronic disease, it will be very valuable to clarify the mechanism of airway inflammation. It is well-known that histamine, leukotrienes and PAF are released by allergic reactions. In addition to these mediators, recent studies suggest the important roles of neurogenic mediators such as substance P in the development of inflammation in the airway (5, 21, 22). We examined the plasma extravasation in the airway induced by these allergic and neurogenic mediators given intravenously or intratracheally in rats and compared them with the skin reaction induced by the same mediators.

In the rat skin, histamine, LTC$_4$, PAF and substance P induced plasma extravasation. Among these mediators, substance P was the most potent inducer of the skin reaction, and the order of potency in developing the reaction was substance P > PAF > LTC$_4$ > histamine. Carbachol and capsaicin did not show any reaction even in relatively high doses. Capsaicin is a pungent agent that stimulates afferent C-fibers of sensory neurons and releases substance P from their endings (10, 17, 18). However, the obtained results suggested that capsaicin could not release a sufficient amount of substance P to induce apparent plasma extravasation.

Next we examined the plasma extravasation in the trachea induced by intravenous injection of these mediators. Substance P induced plasma extravasation in the trachea in a dose-dependent manner. The reaction reached maximum at the 32 nmol/kg dose. Histamine and PAF also caused, significant increase in plasma extravasation in the trachea, but the intensity and potency were both weaker than that of substance P. LTC$_4$ which induced a severe reaction in the skin did not show any response, and carbachol and capsaicin were also inactive in the trachea. With regard to the effect of intravenous capsaicin on the rat trachea, Saria et al. (23) reported that 5 $\mu$mol/kg injection of capsaicin induced severe plasma extravasation in the trachea. Our experimental range was limited to 100 nmol/kg because of the acute toxicity induced by capsaicin injection. Animals receiving more than 1 $\mu$mol/kg capsaicin died from acute respiratory impairment. Saria et al. found it necessary to use artificial ventilation in order to avoid acute toxicity in their experiments. Although substance P induced severe plasma extravasation in the trachea, even at a high dose (100 nmol/kg), no sign of respiratory impairment was observed. Acute respiratory impairment by intravenous capsaicin is considered not to be the result of systemic release of substance P. Saria et al. also reported that the intravenous injection of compound 48/80 induced a substance P-dependent plasma extravasation in rat trachea (24).

Results obtained from the experiments using intratracheal injection of these mediators were very different from those using intravenous injection as described above. Substance P and capsaicin induced severe plasma extravasation in the trachea after local application. PAF, LTC$_4$ and carbachol did not induce any reaction. Histamine showed a slight tendency to cause plasma extravasation, but this was not significant. Although capsaicin did not induce any plasma extravasation in the skin or the trachea by intravenous injection, it induced significant reaction in the trachea by intratracheal injection. The potency of capsaicin to induce this reaction was stronger than that of substance P. This result suggests that rat airway is extremely sensitive to locally applied capsaicin. There may be a high density innervation of capsaicin sensitive C-fibers in the airway of rats. These results are in agreement with those of Lundberg and Saria (4, 25). These authors also showed that histamine induced significant plasma extravasation in the trachea by local treatment, but that its potency and intensity was less than that of substance P. In our experiments, histamine did not induce significant reaction, but the tendency to develop plasma extravasation was observed. Histamine, applied locally, has
a very weak activity in developing plasma extravasation in rat trachea. Effects of locally applied substance P and capsaicin in guinea pig trachea have been reported by other authors (26, 27). They showed that 0.1 nmol of capsaicin induced severe plasma extravasation in the trachea following local treatment, but this was not produced by 0.1 nmol of substance P. A slightly larger dose of substance P was needed to induce this reaction. These data suggest that guinea pigs, like rats, are very sensitive to intratracheal injection of capsaicin. In contrast, the effect of locally applied LTC₄ is different between the two species. In guinea pigs, severe plasma extravasation induced by intratracheal injection of LTC₄ has been found (27–30). Persson et al. (27) showed that 0.2 pmol of LTC₄ induced severe plasma extravasation in guinea pig trachea following local treatment. However, in our experiments, 8 nmol of LTC₄ did not induce any reaction in rat trachea. Other authors reported that guinea pig skin and rat skin have different sensitivities to LTC₄ (31). They showed that the sensitivity to LTC₄ in guinea pig skin was about 10 times more potent than that in rat skin. Then, in the trachea, the different sensitivity to LTC₄ between guinea pigs and rats might be bigger than in the skin. This suggests that in rat trachea, LTC₄ would be less important in the induction of tracheal edema than in guinea pig trachea.

From our present results, three important points arise. Firstly, histamine, LTC₄ and PAF, all of which are mediators released by allergic reactions, can only weakly induce plasma extravasation in the airway. Secondly, among the neurogenic mediators, substance P was very potent in causing tracheal plasma extravasation, while carbachol induced no plasma extravasation in the airway. Thirdly, capsaicin, which stimulates nerve endings following substance P release, induces a severe plasma extravasation in the airway after local application. These points strongly suggest that substance P is the most likely candidate to induce an inflammatory reaction in the airway, which is known to be well-innervated by capsaicin-sensitive substance P-containing neurons. Inflammation in the airway might be largely induced by the stimulation of nerve endings in the mucosal membrane through the endogenous release of substance P. In asthmatic disease, disruption of epithelial cells in the airway is a common feature. Furthermore, the exposure of nerve endings is considered to be a basic phenomenon leading to the acquirement of airway hyperreactiveness. Neurogenic inflammation in the airway, which is mediated by substance P release, must have an important role in the pathogenesis of asthma.

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