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Immediate inhibition by oral *l*-ephedrine of passive cutaneous anaphylaxis of rats: indirect inhibition of anaphylactic chemical mediator release from the mast cell

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Abstract. *Objective and Design:* We previously reported that oral *l*-ephedrine showed extraordinarily rapid inhibition of 48-h passive cutaneous anaphylaxis (PCA) in rats. In the present study, in vivo and in vitro experiments were performed to elucidate a possible mechanism for the inhibition.
Materials: Rat antiserum was prepared with dinitrophenylated *Ascaris suum* extract + *Bordetella pertussis*.
Treatment: Wistar rats were passively skin-sensitised, actively sensitised or non-sensitised. *l*-Ephedrine immediately before provocations was orally or intravenously administered in in vivo experiments. In in vitro experiments, the drug was added at various time and concentrations before the challenge.
Methods: The intensity of PCA was assessed by dye leakage method. Histamine and serotonin released in vitro or retained in the skin in vivo by anaphylaxis were assayed fluorometrically.
Results: Oral *l*-ephedrine rapidly inhibited the PCA by inhibiting the release of histamine and serotonin from the reaction site, whereas anaphylactic histamine and serotonin releases from skin fragments were not affected by the drug. Furthermore, the orally administered drug influenced neither the histamine- nor serotonin-induced cutaneous vascular permeability.
Conclusions: These results were strongly indicative that the prompt suppression of the PCA by oral *l*-ephedrine was not exerted following the drug was absorbed from the gastrointestinal tract. Thus, the result may be from an indirect inhibition of chemical mediator release, possibly through an unidentified stimulation of the nervous system, but not from the inhibition of chemical mediator release by the direct interaction of drug to mast cells and not from the decreased vascular permeability.

Key words: Ephedrine – Passive cutaneous anaphylaxis – Mast cell – Vascular permeability – Histamine

Introduction

We previously reported that *Mao-Bushi-Saishin-To*, a traditional Chinese medicinal formula that consists of three kinds of Chinese plants, *Ephedra* herb *(Mao*), *Aconiti* tuber *(Bushi*) and *Asiasarum* root *(Saishin*), inhibits 48-h passive cutaneous anaphylaxis (PCA) even when it is orally administered immediately before the antigen provocation in the rat. *l*-Ephedrine, which is contained in a large amount in the *Mao* component, is almost solely responsible for the inhibitory constituent of the formula [1]. In addition, we also showed that *l*-ephedrine does not possess the ability to inhibit the anaphylactic histamine release from isolated rat peritoneal crude mast cells in vitro [1]. Hence, the inhibitory effect on PCA exerted within an extremely short time following oral administration of the drug can result from one or more of the following possible mechanisms: 1) direct inhibition of chemical mediator (histamine and serotonin) release from skin mast cells but not peritoneal mast cells, 2) direct or indirect prevention of the increased vascular (mainly postcapillary venular) permeability caused by anaphylactically released chemical mediators and 3) a yet unidentified mechanism.

In this report, to clarify which of the above-mentioned mechanisms is involved in PCA inhibition, we examined whether *l*-ephedrine prevents the decreases of histamine and serotonin contents in the skin of an anaphylaxis-induced site in vivo, the histamine- or serotonin-induced cutaneous vascular permeability in vivo, and anaphylactic histamine or serotonin release from skin fragments in vitro in the rat.

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Materials and methods

Animals

Seven-week-old, male Wistar rats weighing 150–170 g were purchased from Japan SLC (Hamamatsu, Japan). Experimental procedures followed in this study adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals guideline.

Reagents

Reagents and their sources were Evans blue (Merck, Darmstadt, Germany), sodium pentobarbital (Abbot Lab., North Chicago, IL), and histamine dihydrochloride, serotonin creatinine sulphate and diethyl ether (Wako Pure Chem., Osaka, Japan). The other reagents used were the highest grade of commercial products available.

Drugs

The drug used was *l*-ephedrine, which was isolated from *Mao* extracts in the laboratory of Kotaro Pharm. Co. Ltd., Osaka, Japan [1]. The same lot of the drug was used through the experiments.

Antigen and antiserum

Dinitrophenylated *Ascaris suum* extracts (DNP-As) and the antiserum against them produced in rats were prepared as previously described [1].

PCA

Procedures for PCA and the estimation of Evans blue dye leaked into the skin as an index of the intensity of the reaction were performed as previously described [1]. In brief, one hundred µl/site of a 100-fold dilution of the anti-DNP-As serum was intradermally injected at the shaved back of the rat. After 48 h, PCA was evoked by intravenous injection of the antigen (0.3 mg DNP-As/5 mg Evans blue/ml/animal). Thirty minutes later, the skin was removed and the dye leaked into the skin was extracted for photometrical quantification at 610 nm.

l-Ephedrine and their vehicles (Aq. pur. for oral administration and physiologic saline for intravenous injection, for control groups) (2 ml/kg) were administered immediately (within 20 sec) before the antigen challenge.

Histamine and serotonin contents in the skin of anaphylaxis-induced site

In the experiments to estimate histamine and serotonin contents in anaphylaxis-induced skin, PCA was induced by injection of the antigen without Evans blue. Just before or at various times (or 1 h in the experiment on the effect of *l*-ephedrine) after the challenge, the animal was sacrificed and the skin was removed. The isolated skin of the anaphylaxis-induced site, to which physiologic saline (200 mg wet skin/5 ml) was added, was homogenised at 15,000 rpm for 60 sec (Physcotron; Niti-on, Chiba, Japan). The resultant skin suspension was heated in a boiling water bath for 15 min and then centrifuged at 1,700 × g for 15 min at 4 °C. The supernatant was isolated and stored at −20 °C until the histamine and serotonin assays.

Cutaneous vascular permeability induced by histamine and serotonin

Under diethyl ether anaesthesia, the dorsal hair of the rat was removed by an electrical trimmer. Forty eight hours later, following intravenous injection of 1 ml/animal of saline containing 0.5% Evans blue, 2 µg/50 µl/site of histamine or 0.04 µg/50 µl/site of serotonin was intradermally injected into the shaved back of the conscious rat. Thirty minutes later, the animal was sacrificed, its skin was removed and the dye leaked into the skin was colorimetrically assayed by the same procedures as used in the case of PCA [1].

l-Ephedrine and the vehicle (Aq. pur. for the control group) were orally administered in a volume of 2 ml/kg immediately before histamine and serotonin-intradermal injection.

Anaphylaxis of the skin fragments

Rats were sensitised with DNP-As according to Tada and Okumura [2]. Conditions for preparation and antigen challenge of the actively sensitised skin fragments were as described elsewhere [3]. In brief, on day 14 after the first immunisation, following sacrifice of the animal, the shaved dorsal skin was removed and cut into pieces of approximately 1 × 1 × 1 mm in size with a razor blade. The fragments were suspended in Tyrode's solution and then challenged with antigen (10^{-4} g/ml at final concentration) at 37 °C for 15 min. After centrifugation, the resultant supernatant was used for the histamine and serotonin assays. For the estimation of histamine and serotonin contents in skin fragments, the non-challenged specimen was treated as the same procedures as those in the skin described above.

Assays of histamine and serotonin

The supernatants obtained from the above respective experiments were divided into two portions, one for the histamine assay and the other for the serotonin assay. The histamine sample was deproteinised by adding perchloric acid to a final concentration of 3%, followed by centrifugation at 4 °C for 5 min. Then the histamine content was assayed fluorometrically following purification by a histamine analytical system using high performance liquid chromatography (HPLC, Toso, Tokyo, Japan) with a cation exchange column (TSK-gel SP-2SW, 4.6 ϕ × 50 mm, Toso). The conditions for HPLC were as follows: solvent, 0.28 M KH_2PO_4; flow rate, 0.6 ml/min, at room temperature [4, 5].

The serotonin sample was partially purified and then measured by HPLC [6]. Briefly, the specimen, to which NaCl and *n*-butanol had been added, was shaken and then centrifuged. The *n*-butanol layer was transferred into a tube and shaken following the addition of *n*-heptane and 0.3 M HCl. After centrifugation, the aqueous layer was isolated and its serotonin content was fluorometrically quantified on by HPLC [column (TSK-gel SP-2SW, 4.6 ϕ × 150 mm, Toso), solvent (0.5 M citrate-sodium citrate (pH 4.0) containing 20% methanol), flow rate (0.2 ml/min, at 60 °C), excitation and emission wavelengths (280 and 340 nm, respectively)]. Respective serial dilutions of histamine (30 pg to 100 ng/100 µl) and serotonin (30 pg to 50 ng/100 µl) were fluorometrically measured after the same purification procedures described above and served for standard curves. The concentrations of detection limit of histamine and serotonin were both approximately 30 pg/100 µl.

Statistical analysis

Statistical analyses were performed by one-way analysis of variance (ANOVA). If a significance was detected, the individual group difference was determined by Dunnett's test for in vivo experiments and the paired *t*-test for in vitro experiments. A probability value (P) of less than 0.05 was considered to be statistically significant.

Results

Effects of l-ephedrine on PCA

l-Ephedrine was administered orally or intravenously at the dose of 15 mg/kg immediately prior to the antigen challenge. The oral administration as well as intravenous injection of the drug significantly inhibited the PCA. The intravenous administration seems to be more effective than the oral one (Fig. 1).

Effects of l-ephedrine on cutaneous vascular permeability induced by histamine and serotonin

Figure 2 shows the effects of *l*-ephedrine, which was administered orally or intravenously at a dose of 15 mg/kg immediately before the induction, on histamine- and serotonin-induced cutaneous vascular permeability. *l*-Ephedrine by oral administration affected neither the histamine- nor the serotonin-induced reaction. On the other hand, the intravenous administration significantly prevented both reactions.

Time course changes of histamine and serotonin contents in the skin of the anaphylactic site following antigen challenge and effects of l-ephedrine on them

The time course changes of histamine and serotonin contents in the skin of the anaphylaxis-induced site by antigen challenge were investigated. Histamine and serotonin contents before the antigen challenge were on the average approx. 23 and 2.8 μg/g wet skin, respectively. Thirty minutes after the antigen challenge, their amounts were significantly decreased to approx. 17 and 2.0 μg/g wet skin, respectively, and at 1 h, they were further slightly decreased. At 2 and 3 h, the contents were similar to those at 1 h (Fig. 3).

When *l*-ephedrine was orally administered immediately before the antigen challenge, both histamine and serotonin in the skin of the anaphylactic site were almost completely preserved (Fig. 4).

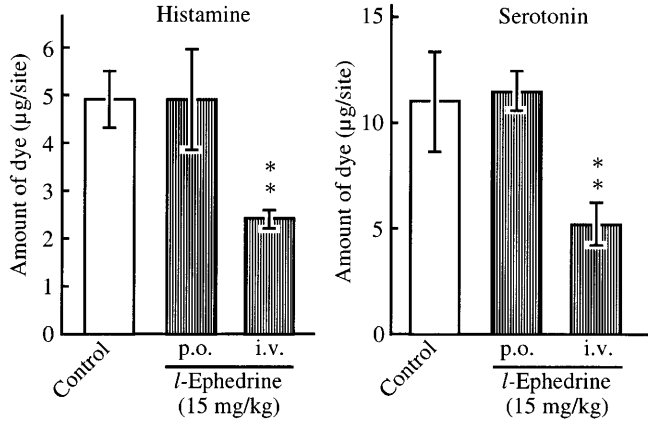

Fig. 2. Effects of *l*-ephedrine on the cutaneous vascular permeability induced by histamine and serotonin in the rat. *l*-Ephedrine was orally or intravenously administered just (within 20 sec) before the intradermal injection of histamine and serotonin. Each column represents the mean ± SE of 8 to 10 animals. **: significantly different from the control at $P<0.01$.

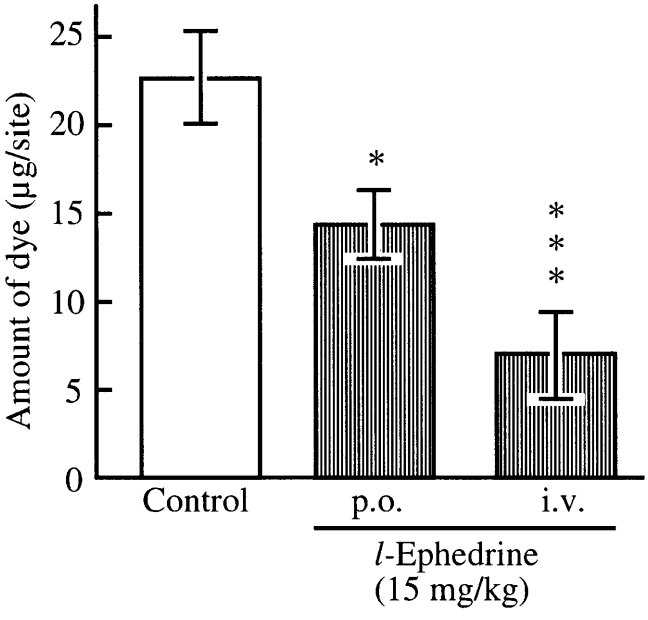

Fig. 1. Effects of *l*-ephedrine on 48-h homologous passive cutaneous anaphylaxis in the rat. *l*-Ephedrine was orally or intravenously administered just (within 20 sec) before antigen challenge. Each column represents the mean ± SE of 8 animals. * and ***: statistical significance from the control at $P<0.05$ and 0.001, respectively.

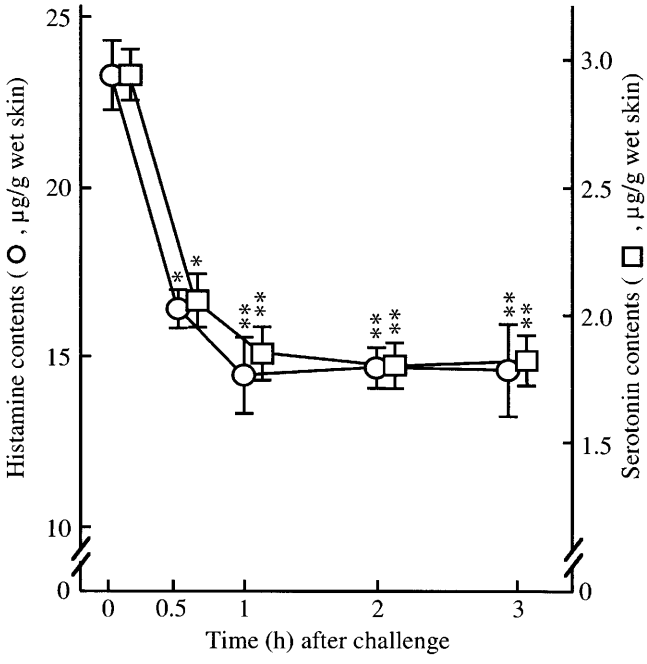

Fig. 3. Time-course changes of histamine and serotonin contents in the skin challenged by antigen in the rat. Each point represents the mean ± S.E. of 6 animals. * and **: significantly different from the levels before (0 min) the challenge at $P<0.05$ and 0.01, respectively.

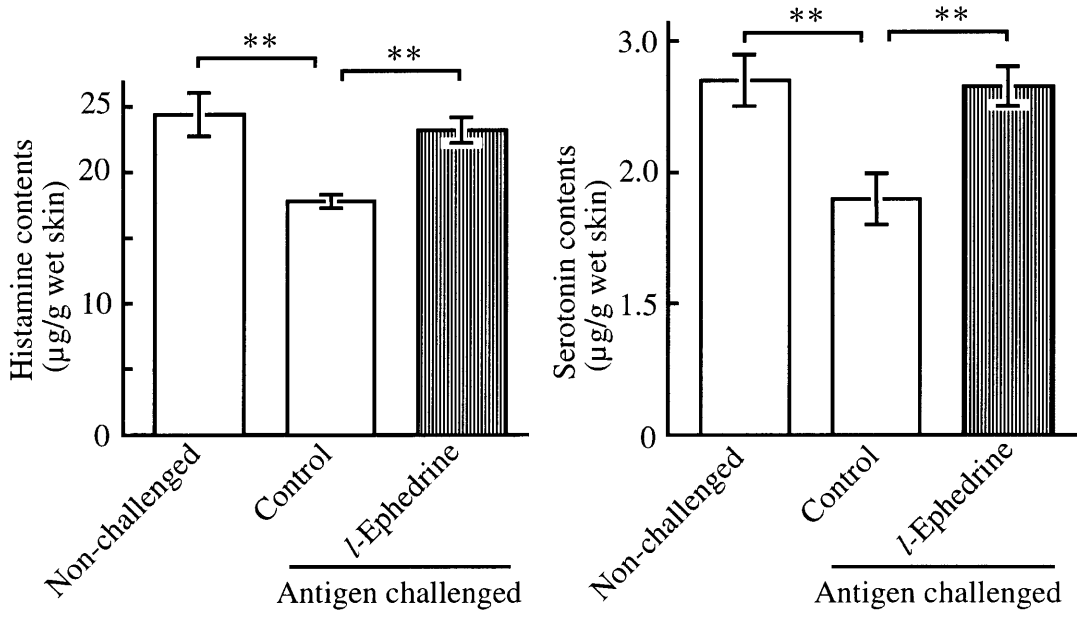

Fig. 4. Effects of *l*-ephedrine on the decreases of histamine and serotonin contents in the skin challenged by antigen in the rat. *l*-Ephedrine and Aq. pur (control group) were orally administered just (within 20 sec) before the antigen challenge. The skin of the anaphylactic site was removed 1 h after the challenge, and histamine and serotonin contents were measured by the procedures described in the text. Each column represents the mean ± SE of 6 animals. **: significant difference at $P < 0.01$.

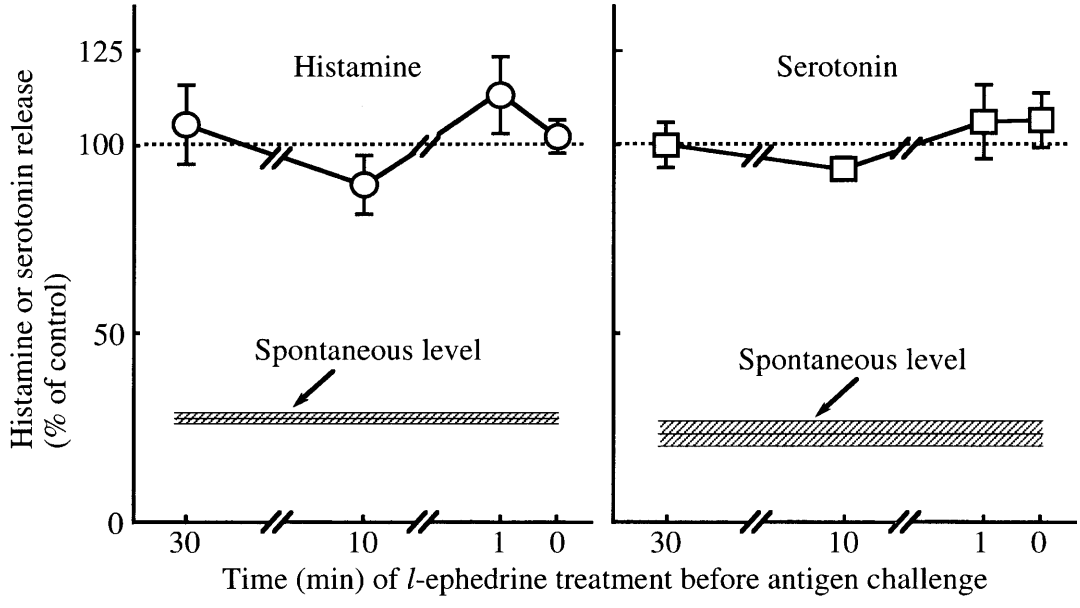

Fig. 5. Time-course effects of *l*-ephedrine on anaphylactic histamine and serotonin release from skin fragments in the rat. *l*-Ephedrine at the final concentration of 10^{-4} g/ml was added at the time indicated before the antigen challenge. Each point represents the mean ± SE of 3 experiments. Amounts of spontaneous and anaphylactic histamine release, and histamine content were 1.1 ± 0.06 and 4.0 ± 0.24, and 28.3 ± 3.25 μg/g wet skin, respectively. Amounts of spontaneous and anaphylactic serotonin release, and serotonin content were 74 ± 8.9 and 255 ± 17.8, and 2,650 ± 230 ng/g wet skin, respectively.

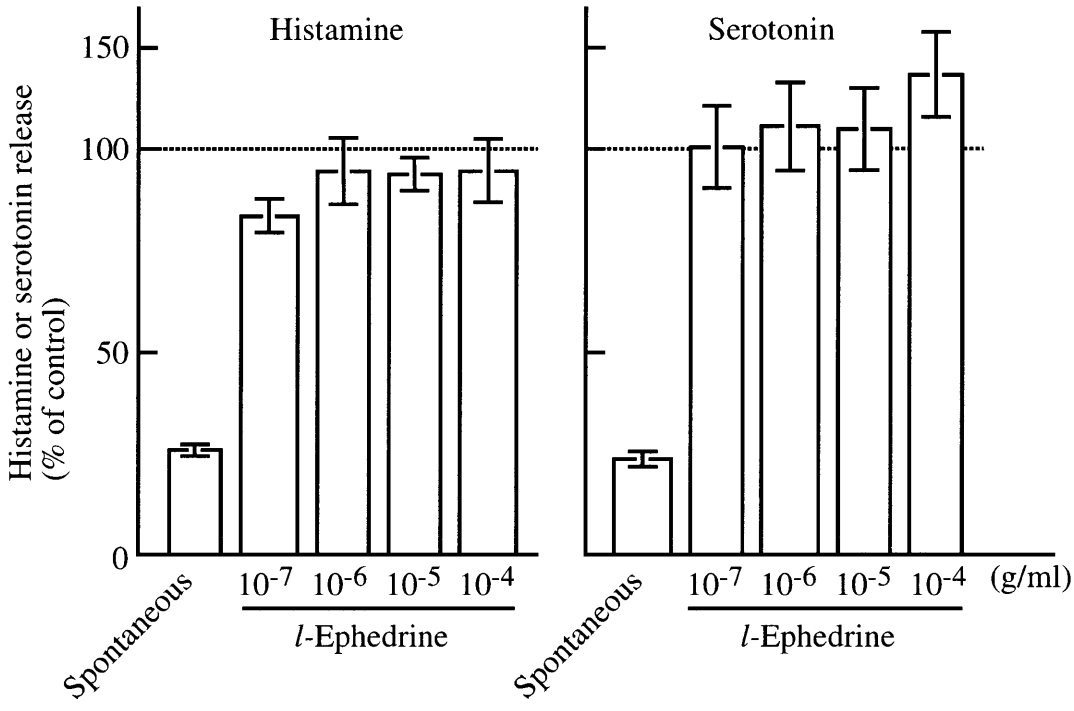

Fig. 6. Effects of *l*-ephedrine on anaphylactic histamine and serotonin release from skin fragments in the rat. *l*-Ephedrine at the final concentrations indicated was added 10 min before the antigen challenge. Each column represents the mean ± SE of 3 experiments. Amounts of spontaneous and anaphylactic histamine release, and histamine content were 0.9 ± 0.10 and 3.9 ± 0.30, and 26.0 ± 2.95 μg/g wet skin, respectively. Amounts of spontaneous and anaphylactic serotonin release, and serotonin content were 81 ± 9.7 and 283 ± 33.0, and 2,900 ± 330 ng/g wet skin, respectively.

Effects of l-ephedrine on the anaphylactic histamine and serotonin releases from skin fragments

Figure 5 shows the influence of *l*-ephedrine at 10^{-4} g/ml on the anaphylactic histamine and serotonin releases from skin fragments in vitro. Treatment with the drug for 0 to 30 min before the antigen challenge suppressed neither histamine nor serotonin release. Furthermore, the treatment with the drug at 10^{-7} to 10^{-4} g/ml for 10 min also did not affect their releases (Fig. 6).

Discussion

We previously reported that oral administration of *Mao-Bushi-Saishin-To*, one of the formulas of Chinese medicine, significantly inhibits the PCA in the rat [1, 7]. The potent inhibitory effect of the drug was observed rapidly (within 20 sec) after the administration of the drug and lasted for 240 min. Pharmacological analyses revealed that *l*-ephedrine, which is contained in *Mao*, one of Chinese plants in the formula, was found to be almost solely responsible for the prompt inhibitions of the PCA [1].

PCA results from the increased vascular permeability by anaphylactically released histamine and serotonin from mast cells [3]. Therefore, drugs suppressing any step from the antigen-antibody reaction to the vascular permeability results in the inhibition of the reaction. *l*-Ephedrine is well known to exert sympathomimetic action through the direct and indirect stimulation [8–11]. Intravenous administration of *l*-ephedrine significantly inhibited not only PCA, but also the cutaneous vascular permeability induced by histamine and serotonin; the results strongly suggested that the above mentioned pharmacological action of the drug can largely contribute to the inhibition of these reactions. In addition, it seems likely that the same mechanism of the action is involved in the late inhibition of the PCA by oral *l*-ephedrine, which is absorbed from the gastrointestinal tract. The cutaneous vascular permeability induced by histamine and serotonin may also be inhibited by oral *l*-ephedrine via the same mechanism when the drug is sufficiently absorbed from the gastrointestinal tract on the provocation. However, immediate oral administration of the drug did not influence the vascular permeability induced by them at all, while the drug significantly inhibited PCA. From this, it can be deduced that the rapid inhibition by oral *l*-ephedrine of PCA does not

result from the suppression of the vascular permeability that is the last step of the reaction. In other words, the drug must affect any event prior to the vascular permeability in the PCA.

As previously reported, the time courses of histamine- and serotonin-induced cutaneous vascular permeability, and PCA are almost identical [3]: they are completed within 10 min, suggesting that the time required from antigen-antibody reaction to histamine and serotonin release from the mast cell is quite short. In addition to this, no rapid inhibition of histamine- and serotonin-induced cutaneous vascular permeability by the oral administration of *l*-ephedrine in the present experiments further strongly suggested that the rapid inhibition of PCA by oral *l*-ephedrine is exerted through the mechanism that the drug affects a reaction prior to the absorption from the gastrointestinal tract.

In in vitro experiments, *l*-ephedrine did not suppress the anaphylactic release of either histamine or serotonin from the actively sensitised dorsal skin fragments. The results indicated that *l*-ephedrine did not possess the ability to inhibit the chemical mediator release by direct action to the mast cell. Therefore, it is not conceivable that the inhibition of PCA by *l*-ephedrine orally administered immediately before the challenge was attributed to the direct inhibition of release of chemical mediators even if the drug was rapidly absorbed.

Time-course experiments of histamine and serotonin contents in the PCA site revealed that their decreases were time-dependent at 0 to 1 h after the challenge. The reductions of these chemical mediators in the PCA site in non-drug-treated animals both reached approximately 30% 1 h after the provocation. Although released and retained histamine and serotonin in the PCA site could not be distinguishably measured, the decreased amounts of these chemical mediators in the PCA site are considered to be those released from the mast cell and then washed out by the blood stream. The consequence can be supported by the following reports. First, on the release, 15 to 40% or approx. 50% of histamine contents are released from rat mast cells by antigen-antibody [12, 13] or IgE-anti-IgE [14] reaction in isolated rat peritoneal mast cells, indicating that the mast cell has ability to release considerable amount of histamine in response to the reaction. Second, on the washout, plasma histamine level increases four fold, while the histamine contents in the skin are lowered when rats are exposed to water immersion stress [15]. In addition, Ind et al. observed that in atopic patients, local plasma elevation of histamine, which peaks at 5 to 15 min and lasts 30 to 60 min after the provocation, was induced with wheal and flare by intradermal injection of antigen at the forearm [16].

The amounts of anaphylactic histamine and serotonin release from the dorsal skin fragments in the present in vitro experiments were considerably lower than the in vivo PCA site. However, it has been reported that the cell-stored histamine in the pleural cavity was potently reduced to 1/7 when actively sensitised rats were intrapleurally challenged by antigen [17], considerably different from the weak releasability of rat isolated pleural mast cells [18]. Thus, the weak histamine and serotonin release from the skin fragments induced by antigen challenge in the present experiments, compared to the PCA site, appeared to manifest the circumstantial difference between in vitro and in vivo experiments including antigen penetrating ability to the tissue.

Taken together, these results strongly suggested that the rapid inhibition of PCA by oral administration of *l*-ephedrine was not due to the classical direct or indirect mechanism acting through the sympathetic system, but due to an unidentified mechanism(s).

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