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Role of bradykinin in the vascular permeability response induced by carrageenin in rats

[1] Seiichiro Kumakura, Izumi Kamo & Susumu Tsurufuji

Department of Biochemistry, Faculty of Pharmaceutical Sciences, Tohoku University, Aoba, Aramaki, Sendai 980, Japan

1 Bradykinin in carrageenin-induced inflammatory pouch fluid was measured by an enzyme immunoassay method.

2 The bradykinin showed a single peak in the 30–60 min period after the challenge and then decreased quickly, and there was a correlation between the bradykinin level and exudation of fluorescein-labelled bovine serum albumin in the first 60 min period.

3 Captopril (an inhibitor of kininase II) elevated both the bradykinin level in the inflammatory pouch fluid and vascular permeability, while DL-2-mercaptomethyl-3- guanidinoethylthiopropanoic acid (an inhibitor of kininase I) had no effect.

4 Soybean trypsin inhibitor (SBTI) inhibited the vascular permeability response in parallel with the decrease in the bradykinin level.

5 A bradykinin-degrading activity appeared in the pouch fluid within 1 h after the challenge and increased with time.

6 In the period of 3.5–4 h, bradykinin levels were suppressed below the sensitivity limit of the assay, i.e. 0.07 ng ml^{-1}, in spite of active generation. This was because degradation of bradykinin was very rapid in this late stage. Nevertheless, bradykinin still played a definite role in sustaining a high level of vascular permeability response in the late stage in conjunction with prostaglandins.

Introduction

In animal models of inflammation induced by carrageenin, bradykinin has been assumed to be a chemical mediator responsible for increased vascular permeability, especially in carrageenin-induced rat paw oedema (Van Arman *et al.*, 1965; Di Rosa *et al.*, 1971) and carrageenin-induced rat pleurisy (Katori *et al.*, 1978). However, quantitative measurements of bradykinin at the inflammatory site have not been successfully carried out because of various difficulties. In fact, bradykinin can be quickly degraded before extraction through the actions of arginine carboxypeptidase (EC.3.4.17.3, kininase I) and peptidyldipeptidase (EC.3.4.15.1, kininase II) (Yang & Erdös, 1967). In addition, there is a considerable risk of generation of bradykinin as an artifact in the process of sample collection (Girey, 1972). Therefore, a clear understanding of the role of bradykinin in inflammatory processes has not yet been obtained.

Recently, however, definite improvements in the measurement of bradykinin in biological materials have been made to overcome the above difficulties (Ueno *et al.*, 1981, Minami *et al.*, 1983). Based on these methodological improvements, we have carried out some experimental studies in order to gain insight into the role of bradykinin in the vascular permeability response in inflammation. We have employed experimental models of inflammation of the air pouch type in rats that have proved to be highly efficient for the determination of chemical mediators at the site of inflammation (Tsurufuji *et al.*, 1984; Ohuchi *et al.*, 1985; Hirasawa *et al.*, 1986). In the present paper we deal with the role of bradykinin in the vascular permeability response in carrageenin-induced air pouch inflammation which can be provoked subcutaneously on the back of rats.

Methods

Animals

Male rats of the Sprague-Dawley strain (specific pathogen free, 4 weeks old and weighing 60–90 g)

[1] Author for correspondence.

were purchased from Charles River Japan, Inc., Kanagawa, Japan, and maintained on laboratory food (available *ad libitum*) (CRF-1, Charles River, Japan) and chlorinated tap water in laminar flow racks for 2 weeks before use.

Carrageenin-induced inflammation of the air pouch type on the back of rats

Rats were injected under light ether anaesthesia with 7.0 ml of air subcutaneously on the back to make an air pouch in the shape of an ellipsoid or oval. After 24 h, 4 ml of 2% solution of carrageenin in 0.9% NaCl was injected into the air pouch to provoke an inflammatory reaction. The carrageenin solution was sterilized before injection by autoclaving at 110°C for 15 min and penicillin and streptomycin were added to 0.1 mg ml^{-1}.

Measurement of vascular permeability

Plasma exudation into the pouch tissues was measured as described elsewhere (Watanabe *et al.*, 1984) using fluoresceinisothiocyanate-conjugated bovine serum albumin (F-BSA) as a tracer. Briefly, at an appropriate time after the challenge with 2% carrageenin, 20 mg of the tracer, F-BSA, dissolved in 0.2 ml of 0.9% NaCl was injected into the tail vein of animals, and 10 or 30 min later the animals were killed by cutting the carotid artery under light ether anaesthesia. The entire fluid volume in the pouch was collected and the leakage of the tracer into the pouch fluid was determined. The volume of the entire pouch fluid was also measured. Fluorescence intensity at 521 nm of an aliquot of the pouch fluid from each rat was read in a spectrofluorophotometer (Shimazu RF-540) with excitation at 490 nm. Total fluorescence intensity for the entire pouch fluid was calculated and expressed in terms of the percentage of total fluorescence intensity injected. This served as an index of the plasma exudation into the pouch.

Histamine assay

Histamine in the pouch fluid were determined according to the fluorometric method described by Shore *et al.* (1959).

Extraction of bradykinin from the pouch fluid

Collection of pouch fluid for the extraction and determination of bradykinin was carried out according to the method of Minami *et al.* (1983). This approach was developed to prevent generation and degradation of bradykinin during and after collection of assay samples. Briefly, 1 ml of the fluid in the pouch was drawn into a plastic syringe holding 0.2 ml of an inhibitor solution consisting of PBS-ethanol (80 : 1) containing SBTI (62.5 μg), aprotinin (12.5 μg), polybrene (17.5 μg), EDTA-Na (75 μg) and *o*-phenanthrolin (42.5 μg). All of the procedures for the extraction and subsequent enzyme immunoassay of bradykinin were carried out with plastic equipment and siliconized glassware to protect samples from artificial activation of bradykinin-generating systems. Immediately after withdrawing pouch fluid, 2 ml of ethanol was added to the sample and mixed well. After standing at 0°C for 1 h, the mixture was centrifuged at 2,500 *g* for 15 min at 4°C. The pellet was removed and the supernatant was evaporated to dryness at 37°C in a rotating evaporator. The dried sample was dissolved in 200 μl of buffer A by sonication; 50 μl of 1,1,2-trichloro-1,2,2-trifluoroethane (TCTFE) was added and agitated to remove extractable lipids. After centrifugation at 2,500 *g* for 15 min, the aqueous layer (upper phase) was transferred quantitatively to another plastic tube by decantation. To the transferred aqueous portion, 40 μl of 20% (w/v) trichloroacetic acid aqueous solution was added and mixed. The mixture was centrifuged at 1,500 *g* for 10 min to remove precipitated protein. The supernatant was used as a sample solution to determine bradykinin by an enzyme immunoassay method described below.

Enzyme immunoassay for bradykinin

Bradykinin in the above sample solution was assayed according to the method of MARKIT-BK with modification. The composition of the various buffer solutions is given at the end of Methods. One hundred μl of a solution of β-D-galactosidase-labelled bradykinin, 100 μl of an unknown sample solution or a bradykinin standard solution, 100 μl of the anti-bradykinin anti-serum, 100 μl of the solution of insoluble anti-rabbit IgG and 100 μl each of buffer B and C were mixed. The mixture was incubated in an ice bath for 2 h. After the incubation, 4 ml of 0.9% NaCl was added. The tube was then centrifuged at 1,500 *g* for 10 min at 4°C. To the precipitate thus obtained, 500 μl of buffer E and 300 μl of buffer D containing 0.2 mM 4-methylumbelliferyl-β-D-galactoside as a substrate was added. After incubation for 40 min at 37°C with moderate shaking, 3 ml of 0.2 M sodium phosphate-NaOH buffer (pH 10.3) was added to terminate the reaction. After the centrifugation at 1,500 *g* for 10 min, the amount of 4-methylumbelliferone released in the supernatant was measured in a spectrofluorophotometer (Shimazu RF-540). Fluorescence intensity was read at 448 nm with excitation at 365 nm. The minimal reliable assay limit for bradykinin in the pouch fluid was 0.07 ng ml^{-1}.

Measurement of protein concentration of pouch fluid

Protein concentration of pouch fluids was measured according to the method of Lowry *et al.* (1951).

Statistical analysis

Results were expressed as the mean ± s.e. mean. The data were evaluated by Student's unpaired t test and a P value less than 0.05 were regarded as significant.

Chemicals

Carrageenin (Seakem No. 202) was purchased from Marine Colloid Inc., Springfield, NJ, U.S.A. Soybean trypsin inhibitor (SBTI), aprotinin and indomethacin were from Sigma Chemical Co., St. Louis, Mo., U.S.A., cyproheptadine was from Merck Ban-yu Co., Tokyo, Japan, and Polybrene was from Aldrich Chemicals, Milwaukee, Wisc., U.S.A. Captopril, an inhibitor of kininase II (Ondetti *et al.*, 1977), was a gift from Sankyo Co., Tokyo, Japan, and DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid (MGTPA), an inhibitor of kininase I (Plummer and Ryan, 1981), was purchased from Calbiochem., La Jolla, CA, U.S.A. MARKIT-A Bradykinin (Dainippon Pharmaceutical Co. Ltd.), a kit for bradykinin assay, consisting of β-D-galactosidase-labelled bradykinin, anti-bradykinin antiserum and secondary antibody, insoluble anti rabbit IgG, was a generous gift from Dainippon Pharmaceutical Co. Ltd., Osaka, Japan. 4-Methylumbelliferyl-β-D-galactoside was purchased from Nakarai Chemicals Ltd., Kyoto, Japan. Other reagents were all analytical grade and obtained from commercial sources.

Buffer solutions

Buffer A: 0.05 M Tris-acetate buffer (pH 8.5) containing gelatin at 0.1% (w/v), Tween 20 at 0.05% (w/v) and NaN_3 at 0.02% (w/v); buffer B: 0.04 M sodium phosphate buffer (pH 7.0) containing gelatin at 0.2% (w/v), NaCl at 0.9% (w/v), and NaN_3 at 0.1%; buffer C: 0.55 M Tris-HCl buffer (pH 8.0) containing gelatin at 0.2% (w/v), NaCl at 0.9% (w/v) and NaN_3 at 0.1% (w/v); buffer D: 0.02 M sodium phosphate buffer (pH 7.0) containing NaCl at 0.1 M, bovine serum albumin (BSA) at 0.1% (w/v), $MgCl_2$ at 0.2 mM and NaN_3 at 0.1% (w/v); buffer E: 0.04 M sodium phosphate buffer (pH 7.0) containing NaCl at 0.9% (w/v), bovine serum albumin (BSA) at 0.1% (w/v) and NaN_3 at 0.1%.

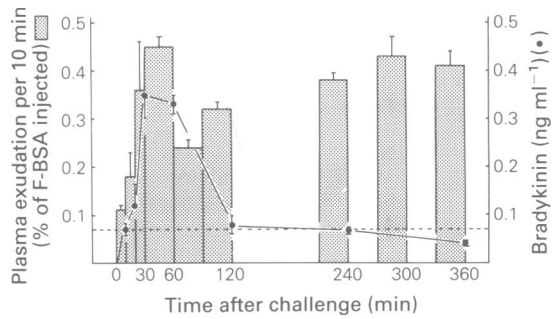

Figure 1 Time course of the vascular permeability (plasma exudation) and bradykinin levels during the first 6 h following challenge with carrageenin in the air pouch. Stippled columns and (●) indicate plasma exudation and bradykinin levels in pouch fluids, respectively, with the s.e. mean indicated by vertical bars. The column height indicates the leakage of F-BSA into the pouch fluid per 10 min.

Results

Vascular permeability and bradykinin level in the pouch fluid

Figure 1 shows the time courses of vascular permeability and amounts of bradykinin in air pouch fluids after challenge with carrageenin solution. Bradykinin in the pouch fluid showed a single peak with a maximum reached between 30 and 60 min after the challenge and then declined into the range lower than the assay limit of 0.07 ng ml^{-1}. During the first 60 min, vascular permeability increased with the passage of time in close parallel with the increase of bradykinin levels in the pouch fluid. After that, vascular permeability appeared to fall along with the decline of the bradykinin level. However, the time course of the vascular permeability did not follow the time course of bradykinin in the period after 120 min. High levels of vascular permeability were maintained up to 360 min in spite of the decline in the bradykinin level.

Analysis with the aid of enzyme inhibitors of a correlation between vascular permeability and bradykinin

In order to gain an insight into the role of bradykinin, the correlation between vascular permeability and the bradykinin level was examined with the aid of an inhibitor of the plasma kallikrein system as well as inhibitors of kininases I and II. SBTI (plasma kallikrein inhibitor), MGTPA (kininase I inhibitor) and captopril (kininase II inhibitor) were selected for this purpose. MGTPA at a dose of 0.2 mg per pouch

Table 1 Effects of kininase inhibitors and soybean trypsin inhibitor on the plasma exudation and bradykinin levels in pouch fluid

Treatment	*Plasma exudation* (% of F-BSA injected)	*Bradykinin* ($ng\,ml^{-1}$)
None	1.36 ± 0.22	0.37 ± 0.06
MGTPA + captopril (A)	4.66 ± 0.33***	17.83 ± 2.49***
SBTI (B)	0.61 ± 0.15**	<0.07***
A + B	0.83 ± 0.07**	<0.07***

Figures represent the mean ± s.e. mean from 7 animals.
DL-2-Mercaptomethyl-3-guanidoethylthiopropanoic acid (MGTPA, 0.2 mg per pouch), captopril (0.2 mg per pouch) and/or soybean trypsin inhibitor (SBTI, 8.4 mg per pouch) dissolved in 0.2 ml of 0.9% NaCl were injected into the pouch at 0.5 h after challenge with the carrageenin solution. At 1 h after the challenge, pouch fluids were withdrawn for bradykinin assay, then the rats were killed. Plasma exudation in 0.5–1 h period was determined.
Statistically significant differences from the corresponding control are shown: **$P < 0.01$; ***$P < 0.001$.

and captopril at a dose of 0.2 mg per pouch were injected at 30 min after the challenge. SBTI at a dose of 8.4 mg per pouch was injected at time 0 into the pouch. As shown in Table 1, in the stage from 30 to 60 min, SBTI caused a significant fall not only in the bradykinin level but also in the exudation of F-BSA. In the same stage, the combination of captopril and MGTPA caused significant rises both in bradykinin levels and exudation of F-BSA. In the presence of SBTI, the combination of the two kininase inhibitors was unable to exert any potentiating effect either on the bradykinin level or on the exudation of F-BSA.

Analysis of the role of histamine and 5-hydroxytryptamine (5-HT)

In order to investigate the role of histamine and 5-HT in the carrageenin-induced air-pouch inflammation, the effect of cyproheptadine, a histamine H_1-receptor and 5-HT antagonist, was examined in addition to the measurement of histamine levels in pouch fluids collected 30 min and 1 h after challenge. Cyproheptadine ($5\,mg\,kg^{-1}$) dissolved in distilled water was administered through a stomach tube 1 h before the challenge with carrageenin. The dose of cyproheptadine was selected referring to the results of preliminary experiments. These indicated that oral doses at 3 and $10\,mg\,kg^{-1}$ of cyproheptadine completely inhibited vascular permeability responses induced by the local application of histamine (40 μg in 4 ml of 2% CMC-Na solution) and 5-HT (4 μg in 4 ml of 2% CMC-Na solution) into the air pouch preformed on the back of rats. The results are summarized in Table 2. Histamine levels were in the range less than $22\,ng\,ml^{-1}$ and cyproheptadine was ineffective in inhibiting the vascular permeability.

Bradykinin-degrading activity in the pouch fluid

Three-fold dilutions of pouch fluids collected at various times after challenge were preincubated for 10 min at 37°C. Then 5 ng of bradykinin dissolved in 0.1 ml of 0.9% NaCl was added to 1.0 ml of the supernatant. The mixtures were then incubated for different periods at 37°C. Subsequently, bradykinin was extracted and measured by the enzyme immunoassay method. The results are summarized in Figure 2.

In the pouch fluid collected at 10 min after challenge, bradykinin-degrading activity was not

Table 2 Effect of cyproheptadine on the plasma exudation and histamine levels in the early stage (0–1.0 h) of the carrageenin-induced inflammation

Time (h)	*Treatment*	*Plasma exudation* (% of F-BSA injected)	*Histamine* ($ng\,ml^{-1}$)
0–0.5	None (5)	0.53 ± 0.07	13 ± 2
	Cyproheptadine (6)	0.63 ± 0.10	16 ± 3
0.5–1.0	None (5)	1.66 ± 0.16	22 ± 3
	Cyproheptadine (6)	1.61 ± 0.10	18 ± 3

Figures represent the mean ± s.e. mean. Figures in the parentheses show number of rats.
Rats were killed 0.5 or 1 h after challenge with the carrageenin solution and pouch fluids were collected to measure plasma exudation and histamine levels.
Plasma exudation for the first 30 min (0–0.5 h) and for the second 30 min (0.5–1 h) were determined.
Cyproheptadine ($5\,mg\,kg^{-1}$) was administered orally 1 h before the challenge.

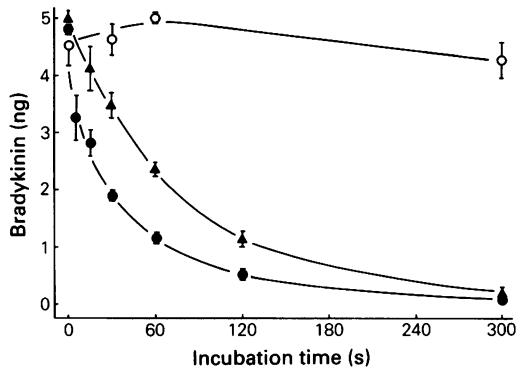

Figure 2 Bradykinin-degrading activity in pouch fluids collected at 10 min (○), 1 h (▲) and 4 h (●) after challenge with carregeenin. Each point represents the mean from 4 rats with s.e. mean shown by vertical bars.

detected, while a rapid degradation of bradykinin was observed in experiments with the pouch fluid collected at 1 h. Bradykinin was degraded much more quickly when incubated with the 4 h pouch fluid. Protein concentration of pouch fluids collected at 10 min, 1 h and 4 h were 2.5, 5.8 and 14.7 mg ml^{-1}, respectively.

Roles of kininases I and II in the degradation of bradykinin in the inflammatory pouch

MGTPA (0.2 mg per pouch) and captopril (0.2 mg per pouch) were injected into the pouch at 0.5 h after challenge with carrageenin and the entire volume of the pouch fluid was collected 30 min later. In the second series of experiments, the kininase inhibitors at the same doses were injected into the pouch at 3.5 h and the pouch fluids were collected 30 min later. F-BSA, an indicator for vascular permeability measurement, was injected intravenously 30 min before the collection of the pouch fluids. The results summarised in Figure 3 indicate that treatment with captopril caused considerable enhancement in both the bradykinin levels and vascular permeability in the earlier (0.5–1 h) and later (3.5–4 h) stages, while MGTPA was ineffective during both phases.

Effects of indomethacin and SBTI, alone and in combination, in the later stage

In the later stage (3.5–4 h), the effects of indomethacin and SBTI alone and in combination, on vascular permeability were examined. Indomethacin was applied locally in the pouch at time 0, dissolved in ethanol and then mixed in the carrageenin solution to a final concentration of 10 μg ml^{-1}. The final concentration of ethanol was adjusted to 0.1% in all of

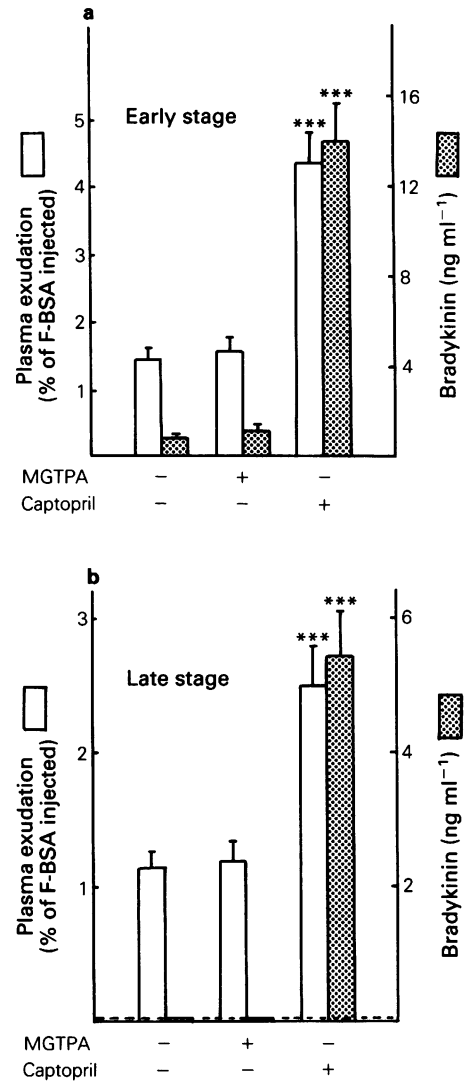

Figure 3 Effects of inhibitors of kininases I and II on the vascular permeability (plasma exudation) and bradykinin levels in the early (0.5–1.0 h) and late (3.5–4.0 h) stages. (a) DL-2-Mercaptomethyl-3-guanidoethylthiopropanoic acid (MGTPA, 0.2 mg per pouch) and captopril (0.2 mg per pouch) were injected into the pouch at 0.5 h. F-BSA was injected intravenously at 0.5 h. Rats were killed at 1 h. (b) MGTBA (0.2 mg per pouch) and captopril (0.2 mg per pouch) were injected into the pouch at 3.5 h. F-BSA was injected intravenously at 3.5 h. Rats were killed at 4 h. Open columns and stippled columns indicate the plasma exudation and bradykinin levels in the pouch fluid, respectively. Each column represents the mean from 5–6 animals with s.e. mean shown by vertical bars. Statistically significant differences from the corresponding control are shown by: ***$P < 0.001$.

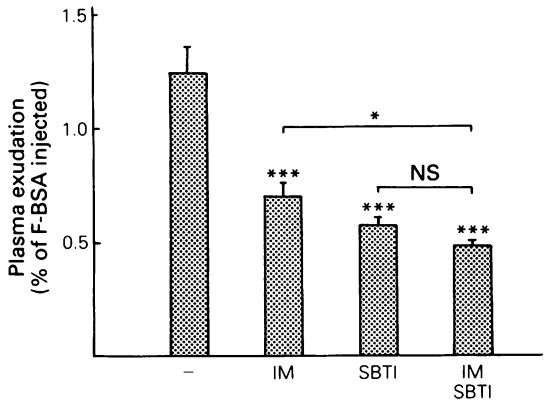

Figure 4 Effects of indomethacin and soybean trypsin inhibitor (SBTI) separately and together on plasma exudation in the period from 3.5 to 4 h (the late stage). Indomethacin (40 μg) was applied locally into the pouch at 0 h, SBTI (8.4 mg) was also locally applied at 3.5 h. Each column represents the mean from 5–6 animals with s.e. mean shown by vertical bars. Statistically significant differences from the corresponding control are shown: $*P < 0.05$, $***P < 0.001$.

the treated and control groups. SBTI (8.4 mg) dissolved in 0.2 ml of 0.9% NaCl or 0.2 ml of 0.9% NaCl was injected into the pouch at 3.5 h. The results are shown in Figure 4. Indomethacin and SBTI were both effective in inhibiting the increased vascular permeability. The combined treatment with SBTI and indomethacin did not cause any further decrease, in comparison with the decrease caused by SBTI alone, while the combination brought about a further significant fall compared with the single treatment with indomethacin.

Discussion

Carrageenin has been extensively used in experimental studies on the effect of nonsteroidal anti-inflammatory agents. In this connection, biochemical studies on the mechanisms responsible for inflammatory reactions provoked by carrageenin have been carried out in many laboratories (Van Arman *et al.*, 1965; Di Rosa *et al.*, 1971; Katori *et al.*, 1978), suggesting that bradykinin is an important chemical mediator responsible for increased vascular permeability, although no reliable measurements for bradykinin levels in the site of carrageenin-induced inflammation have been reported previously. The present study was undertaken with the aid of a sensitive enzyme immunoassay method for bradykinin (Ueno *et al.*, 1981) in combination with suitable procedures to preserve the generation and protect against the degradation of bradykinin. This revealed a definite role for bradykinin in plasma exudation in a carrageenin-induced experimental model of inflammation in rats.

In the early stage of the present experimental model, i.e. the first 60 min period after challenge with carrageenin, the vascular permeability increased with the passage of time in parallel with the rise of the bradykinin level in the pouch fluid (Figure 1). This fact seems to indicate a definite role for bradykinin in the vascular permeability response in the early stage. After reaching the maximum, the bradykinin level fell during the second 60 min to a level near to the assay limit of 70 pg ml^{-1}, and then maintained a low level until the end of the experiment at 360 min. On the other hand, the time course of vascular permeability changes was dissociated from that of the bradykinin level in the period after 120 min. In this late stage of the experiment the vascular permeability was maintained on a plateau in spite of the decline of bradykinin.

A quick decline in the bradykinin level during the period from 60 to 120 min suggests rapid appearance of some kininase activities in the inflammatory sites. Consequently, we examined pouch fluids for bradykinin-degrading activity and found a strong kininase activity in pouch fluids collected at 1 and 4 h after the challenge with carrageenin (Figure 2). In the 10 min pouch fluid, kininase activity was not found in spite of the presence of some proteins (2.5 mg ml^{-1}). The reason why kininase activity was not detected in 10 min pouch fluid was uncertain. Further experiments are required to discover the nature of the protein in the exudate at 10 min after challenge.

A combination of MGTPA, an inhibitor of kininase I, and captopril, an inhibitor of kininase II, when applied locally into the pouch, caused a significant elevation of the bradykinin level in the pouch fluid accompanied by an enhancement of the vascular permeability response. (Table 1). On the other hand, local application of SBTI, a strong inhibitor of plasma kallikrein (Garcia Leme *et al.*, 1978), resulted in a marked fall of vascular permeability (Table 1). The fall in vascular permeability was accompanied by a reduction of bradykinin in the pouch fluid. The parallel in the rise and fall between the bradykinin level and vascular permeability provides further evidence to support a concept that bradykinin is responsible for the vascular permeability response in the early stage within 1 h after challenge.

The complete blockade of the bradykinin generating system after treatment with SBTI was apparent, since kininase inhibitors were unable to cause any rise of plasma exudation when applied together with SBTI (Table 1), and in this case bradykinin was reduced to a level lower than the assay limit of 0.07 ng per ml even in the presence of the kininase

inhibitors. These results indicate that SBTI was extremely effective in inhibiting bradykinin generating system in the pouch.

The possibility that degranulation of mast cells might have a role in the vascular permeability response in the early stage was examined. However, histamine levels in the pouch fluid were very low (Table 2) compared with our previous data, i.e. 2–3 $\mu g\,ml^{-1}$, observed in the anaphylactic phase of allergic air pouch inflammation (Ohuchi *et al.*, 1985) and in experiments where the air pouch was treated with a histamine liberator, compound 48/80 (Konno & Tsurufuji, 1985). In addition, treatment with cyproheptadine did not exert any detectable influence on the vascular permeability and histamine level in response to carrageenin (Table 2).

As regards the specificity of the bradykinin-antibody used in the present study, the cross reactivity with Des-Arg^9-BK, the degradation product of bradykinin by kininase I, and Des-Phe^8-Arg^9-BK, the degradation product by kininase II, is reported to be 0.6% and less than 0.1%, respectively, as compared with its binding activity to bradykinin (Tanimoto *et al.*, 1986). Specificity of the inhibitory action of SBTI, MGTPA and captopril for the corresponding enzymes has been described in the literature (Ondetti *et al.*, 1977; Vogel, 1979; Plummer & Ryan, 1981). Moreover, we confirmed that SBTI did not exert any influence on histamine- or bradykinin-induced vascular permeability responses, and neither MGTPA nor captopril influenced histamine-induced vascular permeability responses (data not shown).

All of the above data indicate that bradykinin was mainly responsible for the vascular permeability response observed up to 1 h after challenge and that the appearance of some kininase activities in the pouch were responsible for the rapid fall in the bradykinin level and vascular permeability.

The significance of kinin-degrading enzymes in the early and late stages was examined with the aid of the respective inhibitors for kininases I and II. In both the early and late stages, kininase II seemed to be mainly responsible for the degradation of bradykinin, as shown in Figure 3. A kininase I inhibitor, MGTPA, brought about no significant change either in the bradykinin level or in vascular permeability. The concentration of MGTPA applied in the pouch was shown, in a separate experiment performed by the use of a synthetic peptide Bz-Gly-Arg as a substrate, to inhibit kininase I activity in the rat plasma by 88%. The reason why kininase I did not play any significant role will be reported in a separate paper.

At the late stage of about 4 h, the bradykinin level in the pouch fluid was lower than the sensitivity limit of the assay. However, it is likely that bradykinin was still actively generated because treatment with captopril caused enhancement of the vascular permeability response accompanied by a marked elevation of the bradykinin level in the pouch (Figure 3b). In order to examine the role of a small quantity of bradykinin in the late stage, the effects of SBTI, indomethacin and a combination of the two drugs were examined, since synergistic potentiation of plasma exudation by the collaboration of prostaglandin and bradykinin has been described by Williams & Morley (1973). Both SBTI and indomethacin caused significant falls in vascular permeability. The combined treatment with SBTI and indomethacin caused significant inhibition, but this inhibition was similar to that of SBTI alone (Figure 4). Thus, it is suggested that a very small amount of bradykinin escaping from the action of kininases still played a role in causing a vascular permeability response at the 4 h stage by collaborating with prostaglandins.

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