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Short communication

Involvement of bradykinin in endotoxin-induced vascular permeability increase in the skin of rats

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Abstract

The aim of the present study was to investigate the role of bradykinin as well as that of platelet-activating factor in the endotoxin-induced acute vascular permeability increase in the dorsal skin of rats by use of kininogen-deficient and normal Brown-Norway rats. In the kininogen-deficient rats, the dose-dependent dye exudation induced by endotoxin was about one half of that in the normal rats at any doses of endotoxin tested (0.1–1.0 mg per site), whereas the dose-response curves obtained by bradykinin (1–100 nmol per site), platelet-activating factor (0.1–1 nmol per site) or histamine (50–500 nmol per site) were the same in both rats. This effect induced by endotoxin in the kininogen-deficient rats was not changed by pretreatment with a bradykinin B_2 receptor antagonist, HOE140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin, 1 mg kg^{-1} i.v.), whereas the endotoxin-induced response in the normal rats was attenuated by the receptor antagonist. These responses in both kininogen-deficient and normal rats were significantly inhibited by a selective platelet-activating factor antagonist, TCV309 (3-bromo-5-[*N*-phenyl-*N*-[2-[[2-(1,2,3,4,-tetrahydro-2-isoquinolylcarbonyl-oxy)-ethyl]-carbamoyl]-ethyl]carbamoyl]-1-propyl-pyridinium nitrate, 0.1 mg kg^{-1} i.v.). These results suggest that bradykinin could be one of the major mediators in the endotoxin-induced vascular permeability increase in rat skin in addition to platelet-activating factor.

Keywords: Endotoxin; Vascular permeability; Kininogen-deficient rat; Bradykinin; PAF (platelet-activating factor); PAF receptor antagonist

1. Introduction

It is well known that intravenous injection of endotoxin into animals causes so-called septic shock, which is thought to be related to the increase in vascular permeability caused by endotoxin in many organ systems (Rubin et al., 1992; Fink et al., 1991). This universal increased vascular permeability is supposed to play a role in the development of septic shock and noncardiogenic pulmonary edema. In such a septic shock model, endotoxin can induce the production or release of many vasoactive mediators (Klosterhalfen et al., 1992), including platelet-activating factor (PAF; Terashita et al., 1985) and bradykinin (Gallimore et al., 1978). It is also well known that both bradykinin and PAF have potent activities in causing a vascular permeability increase. We reported very recently that both bradykinin and PAF might be involved in the increase in vascular permeability induced by endotoxin in Sprague-Dawley rats based on experiments using a bradykinin B_2 receptor antagonist and an antagonist against PAF (Ueno et al., 1995b). These data indicated the indirect involvement of bradykinin in the increase in vascular permeability induced by endotoxin. We also reported direct evidence for the involvement of bradykinin in endotoxin-induced septic shock by comparing the hypotensive responses to endotoxin in kininogen-deficient and normal rats (Ueno et al., 1995a).

In the present study, we examined the vascular permeability increase in the skin elicited by endotoxin treatment of kininogen-deficient Brown-Norway Katholiek rats (Damas and Adam, 1980) in comparison with that of normal Brown-Norway Kitasato rats in order to assess the role of bradykinin. And we also examined the effects of the antagonists, HOE140 (D-

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Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin), a bradykinin B_2 receptor antagonist, and TCV309 (3-bromo-5-[*N*-phenyl-*N*-[2-[[2-(1,2,3,4,-tetrahydro-2-isoquinolylcarbonyloxy)-ethyl]-carbamoyl]-ethyl]carbamoyl]-1-propylpyridinium nitrate), a novel selective PAF receptor antagonist, on the increase in vascular permeability caused by the endotoxin.

2. Material and methods

2.1. Vascular permeability

In order to investigate the involvement of kinin, male specific pathogen-free kininogen-deficient rats (Katholiek, 300–380 g), a mutant of Brown-Norway strain rats, were used, and the results obtained were compared with the same strain of specific pathogen-free kininogen-normal rats (Kitasato, 300–350 g), both of which were bred at Kitasato University (Oh-ishi et al., 1984). The experiments on vascular permeability were performed by the method previously reported (Ueno et al., 1981). Briefly, rats were anesthetized with pentobarbitone sodium (50 mg kg^{-1} i.p.). Five minutes after intravenous injection of pontamine sky blue (50 mg kg^{-1}), 0.1 ml of various concentrations of a given agent in filtered Tyrode's solution was injected intradermally into 8–10 sites of the shaved dorsal skin of a rat. The doses of agents were expressed as mol or mg per site. The rats were killed by exsanguination 40 min after the intradermal injection of the agents, because the response to endotoxin injected intradermally was found to have terminated by that time (Ueno et al., 1995b). The exuded dye in the skin at each site was extracted by the method of Katayama et al. (1978). In brief, the skin was incubated in 1 ml of 1 N KOH at 37°C overnight; then 2.5 ml of 0.6 N phosphoric acid was added to neutralize the base, and the exuded dye was extracted by addition of 7.5 ml of acetone. After centrifugation (1200 × *g* for 20 min), the optical density of each supernatant was measured at 620 nm with a spectrophotometer (UVIDEC-505, Japan Spectroscopic Co., Tokyo, Japan). The amount of exuded dye at each site was expressed as serum equivalents (μl serum eq.) in the same rat for uniformity.

2.2. Materials

Pentobarbitone sodium (Nembutal) was purchased from Abbot Lab. (North Chicago, IL, USA). Pontamine sky blue (Brilliant Blue 6B, Tokyo Kasei Co., Tokyo, Japan) was dissolved in sterile saline (50 mg ml^{-1}).

Bradykinin (Peptide Inst., Osaka, Japan) was taken from a frozen stock (10^{-4} M) and diluted in filtered Tyrode's just before use. PAF (Funakoshi Co., Tokyo, Japan) was dissolved in filtered Tyrode's containing 0.25% bovine serum albumin (fraction V, Sigma Chemical Co., St. Louis, MO, USA). Histamine hydrochloride (Wako Pure Chemicals Ind., Osaka, Japan) was also dissolved in the filtered Tyrode's. Endotoxin (lipopolysaccharide, from *Escherichia coli* 0111:B4, Sigma Chemical Co.) was dissolved in pyrogen-free physiological saline (Otsuka Pharmaceut. Co., Tokushima, Japan) just before use.

HOE140 (1 mg ml^{-1}, a gift from Hoechst, Frankfurt, Germany; Hock et al., 1991) and TCV309 (0.1 mg ml^{-1}, a gift from Takeda Chemical Ind., Osaka, Japan; Terashita et al., 1985) were freshly prepared in pyrogen-free physiological saline for each experiment and injected intravenously 30 min before the intradermal injection of agents.

2.3. Statistical analysis

All data were expressed as the means ± S.E.M. The effects of various antagonists were analyzed for statistical significance by Student's *t*-test by use of two-way analysis of variance. A *P* value of less than 0.05 was considered to be significant.

3. Results

3.1. Dose-responses to several agents and endotoxin in the kininogen-deficient and normal rats

In Fig. 1, the responses to the various agents in the kininogen-deficient Katholiek rats are shown together with those of the normal Kitasato rats. In both rats, bradykinin (1–100 nmol per site), PAF (0.1–1 nmol per

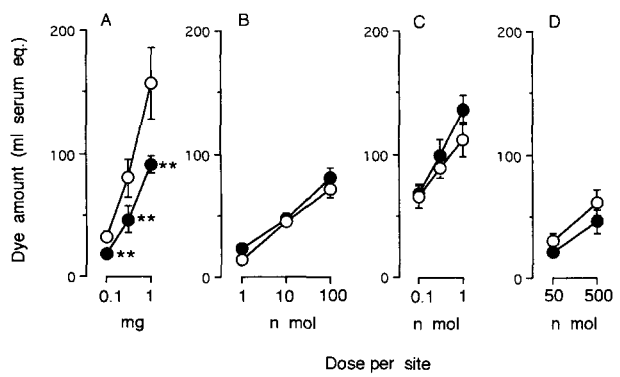

Fig. 1. Responses to various agents in kininogen-deficient Brown-Norway Katholiek (●) and normal Brown-Norway Kitasato (○) rats. Panel A shows the results for endotoxin (0.1–1 mg per site); panel B, those for bradykinin (1–100 nmol per site); panel C, those for PAF (0.1–1 nmol per site); and panel D, those for histamine (50–500 nmol per site). ** Significant difference ($P < 0.01$) between the response of kininogen-deficient rats and that of normal rats. Number of rats used for each datum point was 5–7.

Table 1
The effect of HOE140 and TCV309 on the increase in vascular permeability induced by lipopolysaccharide (LPS) in kininogen-deficient B/N Katholiek rats

Agent (dose)	Control	HOE140	TCV309
LPS (1 mg)	90.8± 7.0	83.8±12.3	39.7±5.1 [a]
BK (100 nmol)	80.4± 9.0	22.0± 2.0 [b]	69.1±5.6
PAF (1 nmol)	136.4±11.6	142.7±11.8	5.8±0.7 [c]

Numbers (mean±S.E.M., 5 rats) are the exuded dye amounts expressed in serum equivalents (μl) of the same rat. HOE140 (1 mg kg^{-1}) or TCV309 (0.1 mg kg^{-1}) was injected intravenously 30 min before the intradermal injections of the agents. The doses indicated in parentheses were in 0.1 ml of Tyrode. Significant differences are indicated: [a] $P < 0.05$ vs. control, [b] $P < 0.01$ vs. control, and [c] $P < 0.001$ vs. control.

site), and histamine (50–500 nmol per site) caused dose-dependent increases in vascular permeability. The potency of each agent in kininogen-deficient rats was not significantly different from that in kininogen-normal rats. Endotoxin (0.1–1.0 mg per site) also dose-dependently increased the vascular permeability in both kininogen-deficient and normal rats, but the response to endotoxin in the kininogen-deficient rats was significantly smaller than that in normal rats, that is, the response of kininogen-deficient rats was about one half of that of normal rats at any dose of endotoxin tested.

3.2. Effect of antagonists on the increased vascular permeability induced by endotoxin

As shown in Table 1, in the kininogen-deficient Katholiek rats, HOE140 (1 mg kg^{-1}) did not affect the response to PAF, but significantly inhibited that to bradykinin (BK) alone. TCV309 (0.1 mg kg^{-1}) also was specific in blocking the response to PAF, but not that to bradykinin. The increase in vascular permeability induced by endotoxin in the kininogen-deficient Katholiek rats was not affected by pretreatment with HOE140, but was inhibited significantly by TCV309.

On the other hand, in kininogen-normal Kitasato rats, both HOE140 and TCV309 inhibited significantly the response induced by endotoxin: 157.2 ± 29 μl serum eq. for control (5 rats), 43.5 ± 4.5 μl serum eq. for HOE140 treatment (5 rats) ($P < 0.05$ vs. control), and 72.9 ± 6.8 μl serum eq. for TCV309 treatment (5 rats) ($P < 0.01$ vs. control).

4. Discussion

In this study, we showed that the intradermal injection of endotoxin caused a dose-dependent increase in vascular permeability in the dorsal skin of Brown-Norway strain rats. Recently we have demonstrated that bradykinin might be involved in the increase in endotoxin-induced vascular permeability in Sprague-Dawley rats by showing the inhibition by HOE140 (Ueno et al., 1995b). However, it would be an indirect evidence for involvement of bradykinin, because only the inhibition by the antagonist was examined. The dose-response curve of endotoxin-induced vascular permeability in kininogen-normal Brown-Norway rats was almost the same as that in Sprague-Dawley rats (Ueno et al., 1995b), and there were no differences between the two strains in the increase in the vascular permeability induced by endotoxin. In this study, HOE140, at the dose that attenuated the increase by endotoxin in normal Brown-Norway rats, did not affect the increase by endotoxin in the kininogen-deficient Brown-Norway rats. This fact clearly suggests that bradykinin produced by activation of the kallikrein-kinin system would not be involved in the response of kininogen-deficient rats. From these results, we conclude that reduction of the endotoxin-induced vascular permeability increase in the skin of Katholiek rats is due to a genetical lack of bradykinin production because of the kininogen deficiency (Hayashi et al., 1992). Therefore, the kininogen-deficient Katholiek rats can be used as a model that excludes the involvement of the kallikrein-kinin system. Thus, in this study, the difference in the response to endotoxin between the kininogen-deficient and normal rats accounts for the involvement of bradykinin in the increase in vascular permeability induced by endotoxin in the normal rat; and its extent would be considerable, since the response to any doses of endotoxin in the kininogen-deficient rats was about one half of that in kininogen-normal rats.

Previously, we reported that the doses of antagonists used in this study, HOE140 and TCV309, were sufficient enough to inhibit the response induced by bradykinin or PAF, respectively, for a period of at least 150 min when the antagonists were injected intravenously (Ueno et al., 1995a). In this study, the effects of these antagonists were proved to be specific, because HOE140 in a dose that inhibited the action of bradykinin did not attenuate the response to PAF; and conversely, TCV309 inhibited the response induced by PAF but not that induced by bradykinin. The results obtained with these antagonists allow the following conclusions: PAF was not involved in the response to bradykinin itself and also the increase in vascular permeability induced by PAF was not mediated by bradykinin. Thus, the production of bradykinin and PAF induced by endotoxin would be independent of each other. Even when kininogen-deficient rats were pretreated with TCV309, the response induced by endotoxin was not perfectly inhibited, suggesting that other mediators aside from bradykinin and PAF would be involved in the increase in vascular permeability induced by endotoxin.

In this study, involvement of bradykinin in the in-

crease in vascular permeability induced by endotoxin was clarified by making a comparison of the responses between kininogen-deficient rats and normal rats.

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