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Life Sciences 70 (2002) 937–949

Life Sciences

The role of kinin B_1 in the plasma extravasation of carrageenin-induced pleurisy

Izumi Hayashi[a,c,*], Hideki Amano[b], Keiko Ishihara[c], Yuji Kumagai[a,c], Hirokuni Yoshimura[b], Masataka Majima[a,c]

[a]*Department of Pharmacology, Kitasato University School of Medicine, Sagamihara-shi, Kanagawa 228-8555, Japan*
[b]*Department of Thoracic and Cardiovascular Surgery, Kitasato University School of Medicine, Sagamihara-shi, Kanagawa 228-8555, Japan*
[c]*Department of Molecular Pharmacology, Kitasato University Graduate School of Medical Sciences, Sagamihara-shi, Kanagawa 228-8555, Japan*

Received 25 January 2001; accepted 4 July 2001

Abstract

The role of des-Arg[9]-bradykinin (des-Arg[9]-BK) and kinin B_1 receptor in the plasma extravasation of rat carrageenin-induced pleurisy was investigated employing B_1 receptor agonist and antagonists and kininogen-deficient rats. Expression of the B_1 receptor mRNA in pleura was induced from 3 to 5 h after the injection of carrageenin into the pleural cavity of Sprague-Dawley rats. Exogenous injection of des-Arg[9]-BK into the pleural cavity provoked a significant increase in plasma extravasation in 5 h carrageenin-induced pleurisy, but not in 20 min kaolin-induced pleurisy. The level of immunoreactive des-Arg[9]-BK in the exudate of 5 h carrageenin-induced pleurisy was higher than that of bradykinin (BK). Administration of the B_1 receptor antagonists, des-Arg[9]-[Leu[8]]-BK or des-Arg[9]-D-Arg-[Hyp[3], Thi[5],D-Tic[7],Oic[8]]-BK significantly reduced the exudation rate. However, intrapleural administration of des-Arg[9]-BK to plasma kininogen-deficient, Brown Norway-Katholiek rats did not result in a further increase in the plasma extravasation. In conclusion, endogenously generated des-Arg[9]-BK could contribute to the plasma extravasation in carrageenin-induced pleurisy via mediation of the inducible B_1 receptor.

Keywords: Des-Arg[9]-bradykinin; Kinin B_1 receptor; Pleurisy; Plasma extravasation; Brown Norway Katholiek rat

Introduction

The kinins, particularly bradykinin (BK), are important mediators involved in both the initiation and progression of the inflammatory response. They display several biological

* Corresponding author. Department of Pharmacology, Kitasato University School of Medicine, 1-15-1 Kitasato, Sagamihara-shi, Kanagawa 228-8555, Japan. Tel.: +81-42-778-9229; fax: +81-42-778-8441.
E-mail address: hayashii@med.kitasato-u.ac.jp (I. Hayashi)

0024-3205/02/$ – see front matter
PII: S0024-3205(01)01460-6

actions, such as pain, smooth muscle contraction, oedema formation, and cell proliferation [1]. In carrageenin-induced rat pleurisy, BK has an important role of increasing vascular permeability via the activation of the plasma kallikrein-kinin system [2, 3]. A non-peptide BK B_2 receptor antagonist, FR173657 [4] could effectively reduce the exudation rate and accumulation of pleural fluid in pleurisy [5]. Therefore it could be concluded that the B_2 receptor mediates the increase in vascular permeability by BK in pleurisy.

In 1980, one of degradative metabolites of BK, des-Arg^9-BK was demonstrated to evoke biological activity and the concept of a new and novel B_1 receptor was established [6]. Recently, molecular cloning of the B_1 receptor from human [7], rabbit [8], mouse [9] and rat [10] has been reported. The B_1 receptor is a heptahelical receptor distinct from the B_2 receptor in that it is highly inducible by inflammatory mediators such as bacterial lipopolysaccharide and interleukins [11]. This indicates that expression of the B_1 receptor is essential to enable des-Arg^9-BK to exert various biological functions. Thereafter, much information has accumulated suggesting that the B_1 receptor may participate in some pathophysiological conditions *in vivo.* Therefore in the present study, we revisited the involvement of des-Arg^9-BK and the B_1 receptor in plasma extravasation in rat carrageenin-induced pleurisy, which is widely used as an acute inflammatory model.

Methods

Induction of pleurisy in rats

Animals were housed in the Experimental Animal Center of the school for 1 week and acclimatized in the laboratory for 1 h before the commencement of each experiment. All of the following procedures were conducted in compliance with the Guideline Principles for the Care and Use of Laboratory Animals of the Animal Care Committee of Kitasato University. Male 7 or 8-week-old Sprague-Dawley (SD) rats were purchased from SLC Japan (Shizuoka, Japan). Inbred male Brown Norway Katholiek (plasma kininogens-deficient) and Brown Norway Kitasato rats (the normal strain), 9 or 10-week-old, were kept in the Experimental Animal Center, Kitasato University School of Medicine.

Rats were lightly anaesthetized with diethyl ether. Pleurisy was induced by injection of 0.1 ml of 2 % (w/v) carrageenin (lambda, type IV, Sigma-Aldrich Co., St. Louis, MO, U.S.A.) or 0.5 ml of 1 % kaolin (K-5, Fisher Scientific, Chicago, IL, U.S.A.) into the right pleural cavity as described previously [2]. Carrageenin was dissolved and kaolin was suspended in sterile physiological saline. The exudation rate of plasma for 20 min from circulation into pleural cavity was determined by the leakage of dye (pontamine sky blue, 50 mg/kg/ml saline, Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan). This dye was injected intravenously into the tail vein of rats under light ether anesthesia 20 min before exsanguination. After 5 h exposure to carrageenin-induced pleurisy or 20 min exposure to kaolin-induced, rats were lightly anaesthetized with ether. Blood was taken from a carotid artery through a polyethylene catheter to exsanguinate under anaethesia. Sufficient exsanguination was performed to minimize contamination of pleural fluid with blood. Serum was used for measurement of the concentration of the dye. The chest cavity was surgically opened and separated from the diaphragm in order to prevent injuring to blood vessels. Pleural fluid was collected using a plastic syringe with a

sonde for peroral administration. The sonde has a round edge to avoid damage to tissues in the pleural cavity. Such care was taken to avoid trauma to blood vessels in the chest wall to prevent contamination of the exudate with blood. Subsequently the pleural cavity was washed with 1 ml of saline and the residual dye collected. Concentrations of dye in the exudate and serum were determined spectrophotometrically at an absorbance of 630 nm. The exudation rate of plasma was expressed as the amount of dye exuded during the course of 20 min. Correction was made by reference to the concentration of dye in the serum, since the concentration in exudate differed among individual rats.

RNA extraction and reverse-transcription polymerase chain reaction (PCR)

Rats were killed at various time points after carrageenin injection to determine the time course of induction of the kinin B_1 receptor. Ten ml of denaturing solution (4 M guanidine thiocyanate, 25 mM trisodium citrate, 0.1 M 2-mercaptoethanol, 0.5 % (w/v) sodium N-lauroyl sarcosine, pH 7.0) was administered into the pleural cavity of rat after exsanguination. The solution was collected and passed through a needle (21G) with syringe 6 times to break deoxyribonucleic acid. The extracted solution was layered on 3 ml of 5.7 M cesium chloride in 13PA tube (Hitachi Koki Co. Ltd., Tokyo, Japan) and centrifuged at 40,000 rpm for 18 h at 20 °C in an automatic preparative ultracentrifuge Himac 70P-72 and RPS40T rotor (Hitachi Koki Co. Ltd.). Total RNA was collected as a pellet, which was washed with 70 % ethanol and dissolved in 10 mM Tris-HCl (pH 7.5) containing 1 mM EDTA, 5 % (w/v) sodium N-lauroyl sarcosine and 5 % (v/v) phenol. RNAs were further purified by extracting twice with phenol/chloroform [12]. Quantification of RNA was determined from the optical density at 260 nm.

Approximately 1 μg of total RNA was utilized as template for reverse transcription. First-stranded cDNA was synthesized with $p(dN)_6$ random primer and AMV reverse transcriptase (Roche Diagnostics GmbH). Fifty ng of cDNA was amplified by 2.5 units of Taq DNA polymerase (QIAGEN GmbH) in a 100 μl-reaction containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP, and 0.1 mM of the two primers, 5′-ccgaca tttatcatctccatctg-3′ (sense) and 5′-Ctggattcagggagctgttagtg-3′ (antisense) for BK B_1 receptor cDNA, or 5′-cccttcattgacctcaactacaatggt-3′ (sense) and 5′-gaggggccatccacagtcttctg-3′ (antisense) for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA [13]. The reaction was allowed to proceed for 25 cycles, each cycle consisting of 45 s at 94 °C, 60 s at 56 °C and 60 s at 72 °C, and finally 10 min extension at 72 °C. Amplified products in the reaction mixture were separated on a 2 % agarose gel and stained with ethidium bromide. The sequence of the amplified cDNA was verified using direct automated DNA sequencing using Dye Terminator Cycle Sequencing and Genetic Analyzer PRISM 310 (PE Applied Biosystems, CA, U.S.A.). Amplified cDNA by PCR was 807-base pairs for BK B_1 receptor and 470-base pairs for GAPDH. All substances other than those described above were of molecular biological grade and were purchased from commercial sources.

Effects of BK B_1 receptor agonist and antagonists on pleurisies

To evaluate the effects of a BK B_1 receptor agonist on pleurisy, 20 nmol of des-Arg^9-BK (Peptide Institute, Osaka, Japan) was injected into the pleural cavity of SD rat 20 min prior to the exsanguination and 5 h after the injection of carrageenin. For kaolin-induced pleurisy, the agonist was injected with kaolin solution simultaneously. One mg of soybean trypsin inhibi-

tor (SBTI, Sigma-Aldrich Co.) was simultaneously injected into the pleural cavity with des-Arg^9-BK. To examine effects of BK B_1 receptor antagonists, 3 nmol of des-Arg^9-[Leu^8]-BK (Peptide Institute) or 3 nmol of des-Arg^9-D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (des-Arg^{10}-HOE140, Peninsula Laboratories Inc., Belmont, CA, U.S.A.) was injected into the pleural cavity of SD rat 20 min prior to exsanguination and 5h after the injection of carrageenin. Des-Arg^9-BK, SBTI, des-[Arg^9, Leu^8]-BK, and des-Arg^{10}-HOE140 were dissolved in 0.1 ml of sterile physiological saline, respectively. Rats receiving the same volume of physiological saline were used as the vehicle group. At the end of experiment, serum and pleural exudate were collected as described above.

Determinations of an enzyme-linked immunosorbent assay (ELISA) for BK and des-Arg^9-BK in pleural exudate

Ice-cold absolute ethanol, the final concentration of which was 80 % (v/v), was added into pleural exudate. The mixture in a polypropylene tube was centrifuged at 3,000 × *g* for 20 min at 4 °C. The supernatant was evaporated to dryness in a vacuum centrifuge and dissolved in 1 ml of distilled water. The ethanol extract was washed 3 times with 2 ml of diethyl ether to remove lipid interference in the assay. The solution was acidified with 0.01 N HCl and was applied to a Sep-Pak C_{18} cartridge column (Waters, Milford, MA, U.S.A.). After being washed with 12 ml of distilled water and 4 ml of 0.1 M acetic acid, kinins were eluted with 6 ml of 80 % (v/v) acetonitrile containing 0.1 M acetic acid. The eluate was evaporated, and the remaining residue was dissolved in 500 μl of the assay buffer. The level of BK was determined with an ELISA kit (Markit M Bradykinin, Dainippon Pharmaceutical Corp.). For the quantification of des-Arg^9-BK, the antibody raised in rabbit and peroxidase-labeled des-Arg^9-BK was used [14].

Statistical analysis

Results are expressed as mean ± s.e.mean of the indicated number of observations. For comparison of data from two groups, Student's *t*-test was used to evaluate the significance of differences. For comparison of data from multiple groups, ANOVA followed by *post-hoc* Dunnett's test was used. A probability (*P*) value of less than 0.05 was taken to indicate statistical significance.

Results

Molecular expression of BK B_1 receptor mRNA in the pleura of rats during carrageenin-induced pleurisy

Kinin B_1 receptor mRNA was measured by RT-PCR of total RNA at 0, 1, 3, and 5 h after the injection of carrageenin. At 0 h, 0.1 ml of saline was injected into the pleural cavity of the rat. The saline-treated rat showed no detectable expression of B_1 receptor mRNA. In contrast, expression was increased at 3 to 5 h during the time course after the injection of carrageenin (Fig. 1). GAPDH mRNA, which is constitutively expressed in numerous tissues and housekeeping gene control, was consistently detected in all the samples. The resulting PCR product using specific primers for the B_1 receptor was subjected to DNA sequencing. The nucleotide sequence of the amplified cDNA with 807-bp was identical to that of rat kinin B_1 receptor reported previously (15).

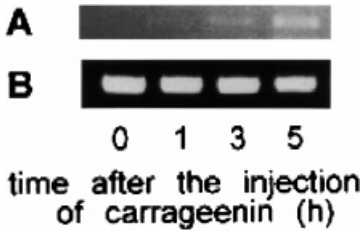

Fig. 1. Expression of BK B_1 receptor in the pleura during rat carrageenin-induced pleurisy. Pleurisy was induced in SD rats by intrapleural injection of 2 % (w/v) carrageenin (0.1 ml/cavity). At indicated time, total RNA in the pleura was prepared by the method of acid guanidinium thiocyanate-phenol-chloroform extraction followed by ultracentrifugation with cesium chloride. The RNA was utilized as template for reverse transcription. BK B_1 receptor (*A*, 807 bp) and GAPDH (*B*, 470 bp) products were amplified from cDNA by PCR. The resulting products were separated on a 2% agarose gel and stained with ethidium bromide. The rat at 0 h received 0.1 ml of sterile physiological saline intrapleuraly.

Effect of des-Arg^9-BK on carrageenin-induced pleurisy at 5 h

Effect of a B_1 receptor agonist on carrageenin-induced pleurisy was examined. Exogenous administration of des-Arg^9-BK (20 nmol/cavity) to rats with 5 h carrageenin-induced pleurisy doubled the rate of plasma exudation (Fig. 2B). Intrapleural injection of plasma kallikrein inhibitor, SBTI (1 mg/cavity) alone decreased the exudation rate. Simultaneous injection of des-Arg^9-BK with SBTI also resulted in a statistically significant increase ($P<0.01$, Fig. 2C). The administration of des-Arg^9-BK to rats that had not been treated with carrageenin failed to increase the exudation rate (Fig. 2A).

Determination of BK and des-Arg^9-BK in the exudate of carrageenin-induced pleurisy

Amounts of immunoreactive BK and des-Arg^9-BK in pleural fluid were quantified by ELISA. The pleural cavity was washed with 1 ml of physiological saline in saline-treated rats. The saline was collected for the measurements. The levels of both BK and des-Arg^9-BK were under the detection limit ($<$65 pg/rat and $<$936 pg/rat, respectively) in saline-treated rats. At 5 h after the injection of carrageenin, the intrapleural levels of BK and des-Arg^9-BK were dramatically increased to 0.59 $\pm$ 0.18 ng/rat (n=16) and 9.75 $\pm$ 2.57 ng/rat (n=8), respectively. A statistically significant difference of the level was observed between BK and des-Arg^9-BK ($P<0.01$).

Effects of BK B_1 receptor antagonists on carrageenin- and kaolin-induced pleurisies

Effects of BK B_1 receptor antagonists on plasma exudation rate were evaluated. The antagonists were injected intrapleuraly 20 min before sacrifice of rats that had been exposed to 20 min or 5 h of carrageenin-induced pleurisy, or to 20 min of kaolin-induced pleurisy. Administration of des-Arg^9-[Leu^8]-BK (3 nmol/cavity) or des-Arg^{10}-HOE140 (3 nmol/cavity) significantly reduced the plasma exudation rate in 5 h carrageenin-induced pleurisy (Fig. 4A, $P<0.01$ and Fig. 4B, $P<0.05$). In contrast, des-Arg^9-[Leu^8]-BK failed to attenuate the exudation rate in 20 min carrageenin- or kaolin-induced pleurisy (Fig. 4C, D).

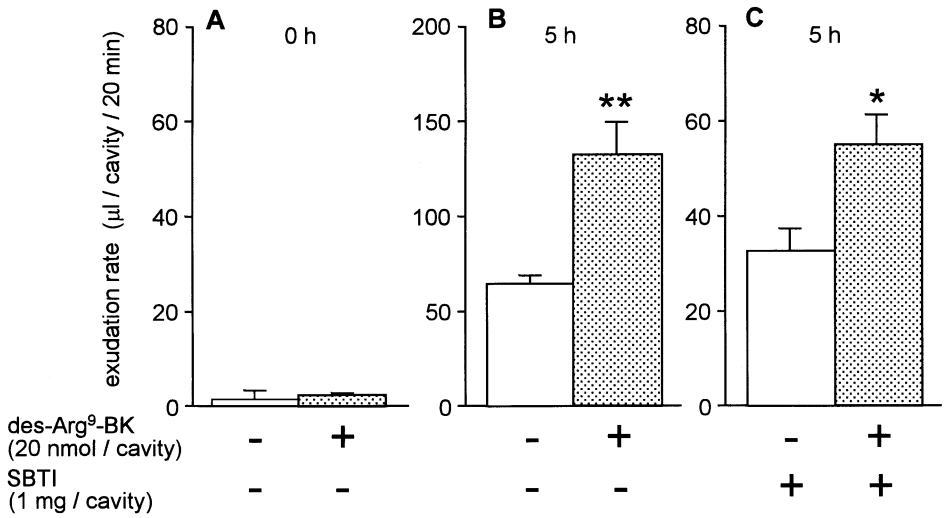

Fig. 2. Effects of des-Arg^9-BK and saline on the exudation rate of carrageenin-induced pleurisy at 0 and 5 h. Seven-week-old SD rats were used. (*A*) Sterile physiological saline (−; 0.1 ml/cavity) or des-Arg^9-BK (+; 20 nmol/cavity) was intrapleuraly injected into non-inflamed rats. (*B*) Twenty min before 5 h after the intrapleural injection of 2 % (w/v) carrageenin (0.1 ml/cavity), sterile physiological saline (−; 0.1 ml/cavity) or des-Arg^9-BK (+; 20 nmol/cavity) was intrapleuraly injected. (*C*) Twenty min before 5 h-period of the carrageenin-induced pleurisy, sterile physiological saline (−; 0.2 ml/cavity) or des-Arg^9-BK (+; 20 nmol/cavity) was intrapleuraly injected with 1 mg of SBTI (+) simultaneously. The exudation rate was determined by the leakage of pontamine sky blue (50 mg/kg/ml saline), which was administered intravenously 20 min prior to exsanguination. Results show mean ± s.e.mean, $n=4$. Student's *t*-test was used to evaluate the significance of differences: $^*P<0.05$, $^{**}P<0.01$.

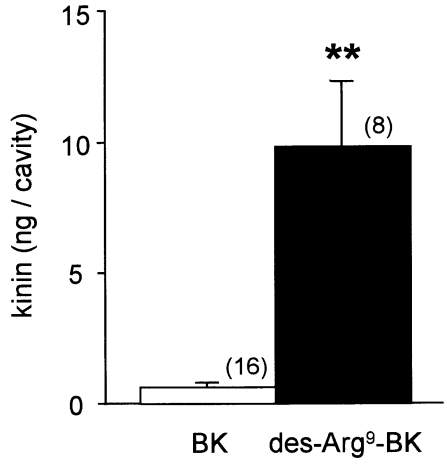

Fig. 3. The levels of BK and des-Arg^9-BK in the pleural exudate at 5 h in carrageenin-induced pleurisy. Pleurisy was induced in 7-week-old SD rats. At 5 h after the injection of carrageenin, rats were sacrificed and the exudate in pleural cavity was collected followed by extraction of kinins. Amounts of BK and des-Arg^9-BK were determined by ELISA, respectively. Results show amounts of immunoreactive kinin (ng/cavity) and are mean ± s.e.mean with the number of observations indicated in parentheses. Student's *t*-test was used to evaluate the significance of differences: $^{**}P<0.01$.

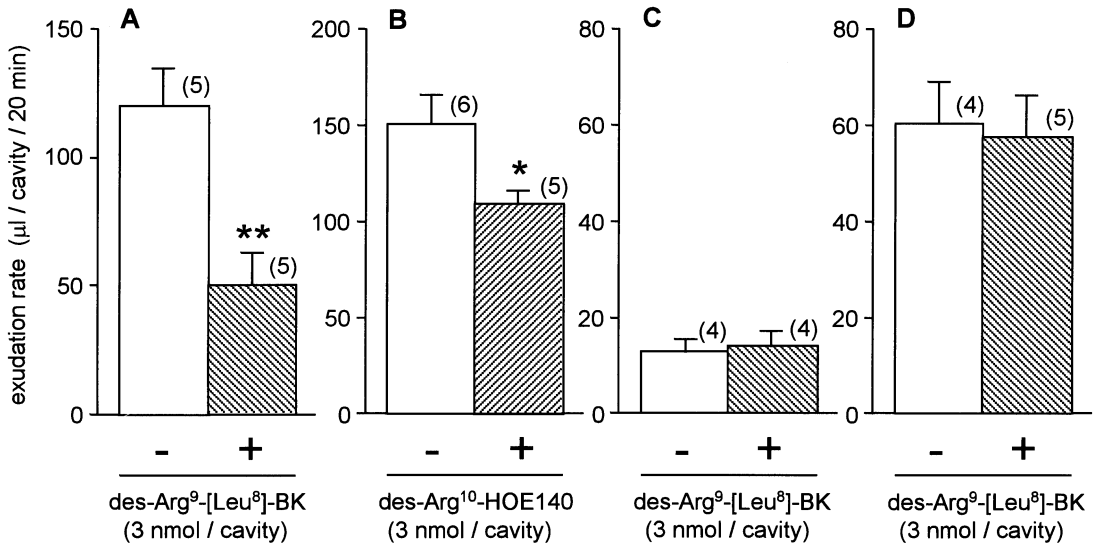

Fig. 4. Effects of BK B_1 receptor antagonists on carrageenin- (*A, B, C*) and kaolin-induced pleurisies (*D*). (*A*) Twenty min before 5 h after the intrapleural injection of 2 % (w/v) carrageenin (0.1 ml/cavity), sterile physiological saline (−; 0.1 ml/cavity) or des-Arg^9-[Leu^8]-BK (+; 3 nmol/cavity) was intrapleuraly injected into 7-week-old SD rats. (*B*) Twenty min before 5 h-period of the carrageenin-induced pleurisy in 8-week-old SD rats, sterile physiological saline (−; 0.1 ml/cavity) or des-Arg^{10}-HOE140 (+; 3 nmol/cavity) was intrapleuraly injected. (*C*) Carrageenin (2 % (w/v), 0.1 ml/cavity) was intrapleuraly injected with sterile physiological saline (−; 0.1 ml/cavity) or des-Arg^9-[Leu^8]-BK (+; 3 nmol/cavity) into 7-week-old SD rats. Rats were sacrificed at 20 min after the injection. (*D*) Pleurisy was induced by the injection of 0.5 ml of 1 % kaolin without (−) or with (+) des-Arg^9-[Leu^8]-BK (3 nmol/cavity) in 8-week-old SD rats. Rats were sacrificed 5 h (*A, B*) or 20 min (*C*) after the stimulations. The exudation rate was estimated during the course of 20 min before sacrifice. Results show mean ± s.e.mean with the number of observations indicated in parentheses. Student's *t*-test was used to evaluate the significance of differences: * $P<0.05$; ** $P<0.01$.

Effects of des-Arg^9-BK on carrageenin-induced pleurisy in the kininogens-deficient and the normal strain of rats

The responses to the administration of des-Arg^9-BK were compared between plasma kininogen-deficient, B/N-Katholiek and the normal strain, B/N-Kitasato rats. At 5 h after the injection of carrageenin, the exudation rate in B/N-Katholiek rats was significantly less than that in B/N-Kitasato rats ($P<0.01$). Intrapleural administration of des-Arg^9-BK (20 nmol/cavity) induced significant plasma exudation in B/N- Kitasato rats ($P<0.01$), but not in B/N-Katholiek rats (Fig. 5). In this experiment, B/N-Kitasato rats were used as the control strain for B/N-Katholiek rats. A significant difference of the exudation rate between B/N-Kitasato and SD rats was not observed (Fig. 2B).

Discussion

In the acute inflammatory response, exudation of plasma protein provides kininogen and kallikrein to generate kinins locally. This assures the early availability of these mediators at

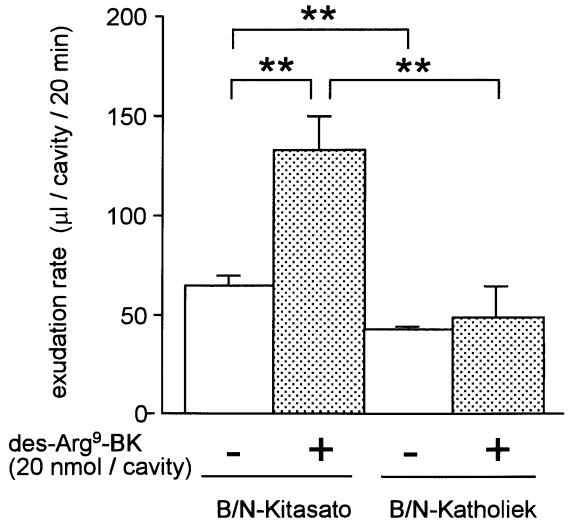

Fig. 5. Comparable effects of exogenously administered des-Arg^9-BK on the exudation rate of carrageenin-induced pleurisy in the kininogens-deficient and the normal strain of rats. The pleurisy was induced in the kininogen-deficient (*B/N-Katholiek*) and the normal strain (*B/N-Kitasato*) of rats. Twenty min before 5 h-period of the pleurisy, sterile physiological saline (−; 0.1 ml/cavity) or des-Arg^9-BK (+; 20 nmol/cavity) was intrapleuraly administered. Rats were sacrificed 5 h after the injection of carrageenin. The exudation rate was estimated during the course of 20 min before sacrifice. Results show mean ± s.e.mean, $*P<0.01$, ANOVA plus Dunnett's test, c.f. saline-injected animals.

an inflammatory site and highlights their potential involvement in the manifestation of the complex process of inflammation. Kinins act on two receptor types, B_1 and B_2, classified according to the relative potency of their endogenous agonists [1, 6]. While the B_2 receptor is typically activated by BK and lysyl BK, the B_1 receptor is selectively sensitive to kinin metabolites lacking a carboxyl-terminal arginine residue, e.g. des-Arg^9-BK and Lys-des-Arg^9-BK. The B_2 receptor is constitutively expressed and mediates many kinin-dependent inflammatory responses including increase in vascular permeability, contraction of smooth muscle, accumulation of inflammatory cells, and pain [1, 6]. In contrast, the B_1 receptor is usually not expressed under normal physiological conditions but can be induced during inflammation. Knowledge of the contribution of the B_1 receptor to the regulation of inflammatory processes is of importance, since this receptor could replace the B_2 receptor in its significance in these processes. The role of des-Arg^9-BK in the development and progression of inflammation is just becoming acknowledged with the renaissance of interest in the B_1 receptor.

Here, we have presented four pieces of evidence to indicate the involvement of endogenous kinin B_1 in rat carrageenin-induced pleurisy. 1) Expression of the B_1 receptor was induced after the intrapleural injection of carrageenin. This expression is essential for des-Arg^9-BK to exhibit its biological activities; 2) exogenous administration of the B_1 agonist, des-Arg^9-BK enhanced plasma extravasation in 5 h carrageenin-induced pleurisy, but not in 20 min carrageenin- or kaolin-induced pleurisies. Detectable expression of the B_1 receptor was not observed in the latter case (data not shown). Therefore the failure to increase extrava-

sation by des-Arg^9-BK in those pleurisies at 20 min might be due to less expression of the B_1 receptor; 3) a considerable amount of immunoreactive des-Arg^9-BK was measurable in the pleural fluid of 5 h carrageenin-induced pleurisy. This indicates endogenous production of des-Arg^9-BK at that time. Kininase I (carboxypeptidase N) and kininase II (angiotensin-converting enzyme) are predominantly kinin-degrading enzymes [16, 17]. Indeed, incubation of authentic BK with rat plasma produced des-Arg^9-BK as well as des-Phe^8-Arg^9-BK and Arg^1-Pro^2-Pro^3-Gly^4-Phe^5. An addition of D,L-2-mercaptomethyl-3-guanidinoethyl-thiopropanoic acid, which is a potent inhibitor of kininase I, into the incubation mixture inhibited the degradation of BK [14]. These three results indicate that des-Arg^9-BK is produced and the B_1 receptor is expressed. Furthermore, authentic des-Arg^9-BK has a potency of plasma extravasation in 5 h carrageenin-induced pleurisy. These are all essential and sufficient to exert actions of kinin B_1; 4) effects of the B_1 receptor antagonists on pleurisy were evaluated to clarify the involvement of kinin B_1. Two antagonists, des-Arg^9-[Leu^8]-BK [18] and des-Arg^{10}-HOE140 [19], which are well characterized to be potent and highly specific for the B_1 receptor [20], were used. Both antagonists showed a significant suppression of the exudation rate in the pleurisy. This provides crucial evidence for an important role of endogenously metabolized des-Arg^9-BK in the vascular permeability increase *in vivo*.

It was reported that the inflammatory response induced by intrathoracic injection of des-Arg^9-BK was mediated by stimulation of the constitutive B_1 receptor in a model of mouse pleurisy [21]. The B_1 agonist elicited a significant increase in vascular permeability, which appeared immediately after injection of the peptide. A definitive piece of evidence concerning the presence of constitutive B_1 receptor in the mouse was reported [22, 23]. The B_1 agonist failed to induce plasma extravasation in non-inflamed rats in the present study. Therefore, unlike the mouse, the B_1 receptor does not seem to function constitutively in the rat. Des-Arg^9-BK and the B_1 receptor are also gaining much attention in other experimental inflammatory models, such as angiogenesis [24], oedema [25], hyperalgesia [26–28], arthritis [29], heat stress [30], fever [31], allergen-induced bronchial hyperresponsiveness [32], coronary vasodilatation associated with endotoxemia [33] and myocardial infarction [34]. Since des-Arg^9-BK can be produced in human plasma [35], the actions of BK associated with both acute and chronic inflammatory insults could be partly mediated by the B_1 receptor in the human. In addition, B_1 receptor antagonists may eventually have important therapeutic relevance in the management of such inflammatory processes [36].

Kinin B_2 has preventative effects on salt-sensitive hypertension [44] and myocardial infarction [45]. In such pathophysiological conditions, use of B_2 receptor antagonists may have disadvantages. Therefore the B_1 receptor antagonist would have potential benefit when its receptor is highly expressed and mediates actions such as inflammatory exudation as was shown here and in hyperalgesia [36, 37].

In the present study, prepared RNA was derived from vascular endothelial cells, fibroblasts, epithelial cells, and other tissue or neural cells in pleura, and inflammatory leukocytes in the exudates. Cells expressing B_1 receptor mRNA could not be identified. Kinin B_1 showed an increase in vascular permeability in 5 h carrageenin-induced pleurisy. Thus it is possible that vascular endothelial cells may in part be involved in its expression. Kinin B_1 receptor mRNA was expressed at 3 to 5 h after the induction of pleurisy. This interval is much shorter than that in the previous observation [37], in which the B_1 receptor mediated

hyperalgesia 48 h after the injection of Freund's adjuvant. The difference in the time interval to induce the receptor may involve an activation of pre-existing, non-functional B_1 receptors in the present model. On the other hand, the B_1 receptor mRNA could be inducible within a few hours by the stimulation with a proinflammatory cytokine [38]. Upregulation of B_1 receptor expression is achieved by both transcriptional activation and post-transcriptional mRNA stabilization without depending on protein synthesis. This report suggests that it is likely to induce B_1 receptor mRNA within a few hours after inflammatory stimuli.

We observed that intrapleural injection of des-Arg^9-BK did not potentiate plasma extravasation in kininogen-deficient, B/N-Katholiek rats. Both high-molecular weight and low-molecular weight kininogens are deficient in the plasma of this strain, so that almost no kinin release occurs [39, 40]. The exudation rate in B/N-Katholiek rats was approximately 60 % of that in the normal strain, B/N-Kitasato rats, that had been exposed to 5 h carrageenin-induced pleurisy. Moreover, the number of leukocytes infiltrated into the pleural cavity was almost the same in both strains of rats [3]. These results indicate that a weak inflammatory response occurs in B/N-Katholiek rats. Nevertheless, the plasma extravasation was not potentiated by des-Arg^9-BK. One possible explanation for this observation is that the inflammatory reaction in B/N-Katholiek rats was not sufficient to induce the expression of the B_1 receptor. Otherwise, signaling from the B_2 receptor may mediate induction of the B_1 receptor. Activation of BK B_2 receptor resulted in Ca^{2+} influx followed by time-dependent tyrosine phosphorylation of mitogen-activated protein kinase in ras-transformed NIH/3T3 fibroblast [41]. On the other hand, p38 mitogen-activated protein kinase is recruited to trigger the *de novo* synthesis of the B_1 receptor [42]. These findings suggest a possible induction of the B_1 receptor by the B_2 receptor via the mitogen-activated protein kinase cascades. Autoregulation of BK receptors was reported [43] in which incubation of human lung fibroblasts with BK or des-Arg^{10}-kallidin led to an increase in the B_1 receptor. This indicates a crucial role of kinin production and metabolism in shifting the repertoire of kinin receptor subtypes in favor of the B_1 receptor during inflammation. Our data shown here may also be interpreted in terms of the autoregulation of BK *in vivo*.

We conclude from this study that, in rat carrageenin-induced pleurisy, des-Arg^9-BK partly mediates plasma extravasation by direct stimulation of the inducible B_1 receptor, which is likely to be expressed in the pleura. Concomitant with consequence of kinin B_1 under pathophysiological conditions, the present study would also propose the B_1 receptor as a novel target for the development of anti-inflammatory drugs with a potentially inhibitory action on plasma extravasation.

Acknowledgments

Part of this work was supported by Academic Frontier Project from the Japanese Ministry of Education, Science, Sports and Culture (to M.M.), grants from Integrative Research Program of the Graduate School of Medical Sciences, Kitasato University (to I.H. and M.M.), and Parent's Association grant of Kitasato University School of Medicine (to I.H.).

First author would like to thank to Ms Yuko Toyo-oka, Laboratory of Virology and DNA Research, Department of Clinical Laboratory, Kitasato University Hospital for kind help with DNA sequencing, Dr Noriyuki Sunahara, Dainippon Pharmaceutical Corp. for help with ELISA

for BK and des-Arg^9-BK, and Ms Misako Uemura and Ms Chieko Nakamigawa, the Experimental Animal Center for taking care of experimental animals.

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