Presence of T-kininogen and kinins in sponge-induced exudates in rats

¹J. Damas, ²A. Adam, V. Bourdon & G. Remacle-Volon

Physiologie humaine, Université de Liège, 17 Place Delcour, B-4020 Liège, and Laboratoire de Biologie Clinique, CHU de Sainte-Ode, Baconfoy, Belgium

1 The content of kinins and T-kininogen (the third kininogen) in exudates induced by the subcutaneous implantation of saline-soaked sponges have been measured by radioimmunoassay in normal Wistar rats and in Brown Norway rats from a strain which is deficient in high and low molecular weight kininogens.

2 In both strains, sponge implantation induced a rise of T-kininogen in plasma with subsequent accumulation in the sponge exudate. This accumulation correlated with the extravasation of plasma proteins during the first 6 h. Bioassays showed that the T-kinin moiety was retained in T-kininogen.

3 In Wistar rats, a large release of immunoreactive kinins up to a mean value of 6.4 ng ml^{-1} was observed during the first 6 h and on the second day after the implantation. In Brown Norway rats, the kinin level in the exudates did not exceed 0.53 ng ml^{-1} .

4 Of the kinins present during the first 6 h in the exudates withdrawn from Wistar rats, 60% were identified by high performance liquid chromatography as bradykinin.

5 The volume of the exudate induced by the implantation of dry sponges was smaller in Brown Norway rats than in Wistar rats.

6 We conclude that the role of T-kininogen in this kind of exudate was mainly the inhibition of thiol proteinases and not the release of T-kinin. In Wistar rats, bradykinin acts as a pro-inflammatory factor during the first hours and may play a role during the healing process.

Introduction

Kinins are pro-inflammatory peptides released from plasma kininogens. In most mammalian species, two kinds of kininogens, low and high molecular weight kininogens, have been identified. Tissue kallikreins release bradykinin from both rat kininogens (Alhenc-Gelas *et al.*, 1981), while plasma kallikrein releases bradykinin only from the high molecular weight kininogen. The participation of kinins in some inflammatory reactions has been studied for several years (Review in Lewis, 1970; Garcia Leme, 1978). However, in rats, the interest in this topic was renewed by two recent findings. First, we discovered that the plasma of rats from a particular Brown Norway strain is devoid of both kininogens and poor in plasma prekallikrein (Damas & Adam, 1980; 1985). These animals have been used as a negative

index of the participation of kinins in some inflammatory reactions. Oedemas and pleurisies induced by carrageenan, kaolin or urate crystals were greatly reduced in these rats indicating the role of the kinins in these models (Damas *et al.*, 1984; 1986; Oh-ishi *et al.*, 1987). Secondly, Okamoto & Greenbaum (1983a, b) discovered a second low molecular weight kininogen in rat plasma. This kininogen is resistant to the kininogenase activity of kallikreins but releases Ile-Ser-bradykinin or T-kinin after hydrolysis by high concentrations of trypsin. It was thus named T-kininogen. T-kininogen is similar if not identical to the α_1 -cysteine proteinase inhibitor, the major acute phase plasma protein of the rat (Enjyoji *et al.*, 1988). Inflammation induced by adjuvant or turpentine increases the plasma level of T-kininogen from 0.25 mg to 3 to 8 mg ml^{-1} (Barlas *et al.*, 1985a; Bouhnik *et al.*, 1987). During inflammatory processes, T-kininogen could thus represent a large plasma pool of a kinin-releasing substrate (Greenbaum, 1984). Indeed, Barlas *et al.* (1985b)

¹ Author for correspondence.

² Present address: Faculté de Pharmacie, Université de Montréal, 2900 Bd. Monpetit, Montréal, H3W 1R4 Québec, Canada.

have observed the accumulation of large amounts of T-kininogen and the release of T-kinin in carrageenan-induced oedema in an air-pouch model. Brown Norway rat plasma devoid of high and low molecular weight kininogens has been proved to contain T-kininogen (Hayashi *et al.*, 1984; Damas & Adam, 1985). As paw oedema and pleurisy induced by carrageenan in these rats were small, the role of T-kininogen as a kinin-forming substrate was considered as insignificant (Damas *et al.*, 1986; Damas, 1987; Oh-ishi *et al.*, 1987).

However, in the rat, the above reactions are short-lasting as compared to the granulomatous reaction studied by Barlas *et al.* (1985b). Moreover, in the granulomatous tissues, Sakomoto *et al.* (1987) reported the presence of an acid proteinase able to release T-kinin from T-kininogen. A release of T-kinin could thus take place during long-lasting reactions. As we have developed specific radioimmunoassays to quantify T-kininogen and bradykinin (Adam *et al.*, 1989), we measured, during the first three days, the accumulation of T-kininogen and the release of kinins in inflammatory exudates induced by sponge implantation in normal rats and in kininogen-deficient Brown Norway rats. Our observations could shed some light on the role of T-kininogen in long-lasting inflammatory processes as either a kinin-releasing substrate or a cysteine proteinase inhibitor.

Methods

We used male Wistar rats as normal control rats. The deficient Brown Norway rats were bred by the farm of the Catholic University of Leuven (Heverlee, Belgium). The mean weight of the animals was 220 g for Brown Norway rats and 260 g for Wistar rats. Polyvinyl sponges (diameter 15 mm, thickness 4.5 mm) weighing 17–17.5 mg, were first sterilized in ethanol, rinsed in twice distilled water and oven-dried. They were used as such (dry sponges) or soaked in physiological saline. The rats were anaesthetized with ether. After sterilisation of the skin of the back with ethanol, a 15 mm dorsal incision was made and the dermis separated from the muscular layer. One sponge was implanted in each side. Three to seventy-two hours later, the rats were anaesthetized with sodium pentobarbitone (30 mg kg⁻¹); 10 min later, blood was withdrawn by cardiac puncture and mixed with 0.1 vol. 3.8% citrate. Plasma was separated by centrifugation at 2500 *g* for 10 min and frozen at -25°C. The dorsal incision was opened and the sponges removed carefully to avoid local bleeding.

After removal, the sponges were directly immersed in polyethylene tubes containing either 2 ml physio-

logical saline or 0.5 mg ml⁻¹ trypsin in 2 ml physiological saline or 80% cold ethanol in water. The tubes containing trypsin were incubated at 37°C for 30 min. Then the sponges were gently squeezed with a syringe plunger and the total cell count was performed by phase contrast microscopy using a Thoma's plate. The other sponges were gently squeezed three times and removed. The exudates diluted in saline were frozen at -25°C until the assay of T-kininogen. The exudates mixed with ethanol were centrifuged at 2500 *g* for 10 min. The supernatant was evaporated and the dried residue was dissolved in phosphate buffer for measurement of the kinin content.

T-kininogen levels in plasma or in exudates were measured by a specific radioimmunoassay previously described (Adam *et al.*, 1989): 100 µl of samples or increasing quantities of unlabelled T-kininogen and antiserum (final dilution 10⁻⁴) were incubated with ¹²⁵I-labelled T-kininogen (20,000 c.p.m.) at 4°C in a total volume of 0.4 ml of incubation buffer KH₂PO₄/Na₂HPO₄ · 2H₂O 200 mM, pH 7.5 containing 5 g bovine albumin, 0.1 g NaN₃ and 0.37 g EDTA per litre. After 4 h, the reaction was stopped by adding 2 ml of isotonic saline containing sheep anti-rabbit gamma globulin antiserum (final dilution 100 fold), polyethylene glycol 6000 (40 g l⁻¹) and microcrystalline cellulose (500 mg l⁻¹). After incubation at room temperature for 30 min and centrifugation at 1000 *g* for 15 min, the supernatant was decanted, and the radioactivity of the pellet counted. Our antibodies raised in rabbits against pure rat T-kininogen did not cross-react with T-kinin, bradykinin or rat high molecular weight kininogen.

In order to measure the kinin-releasing activity of T-kininogen in exudates, 0.4 ml of diluted exudate was incubated with 0.1 ml N-tosyl-L-phenylalanine chloromethyl ketone (TPCK)-treated trypsin (final concentration 1 mg ml⁻¹) in the presence of 0.1 ml EDTA (1 mg ml⁻¹ final concentration) and 0.2 ml Tris HCl 0.15 M, pH 7.8, for 30 min at 37°C. The reaction was stopped with 0.2 ml soya bean trypsin inhibitor (SBTI, final concentration: 2.5 mg ml⁻¹) and the released kinins were measured by bioassay using rat stomach strip, rat uterus and rat duodenum superfused with Tyrode solution (2.5 ml min⁻¹) at 37°C and aerated with 95% O₂ and 5% CO₂. The threshold level varied between assays from 0.5 to 2 ng ml⁻¹. The standard bradykinin was prepared in 0.15 M Tris HCl pH 7.8 containing EDTA (1 mg ml⁻¹).

Kinin levels in exudates were measured by a specific radioimmunoassay (Adam *et al.*, 1989). This radioimmunoassay was performed under similar conditions to the one described for T-kininogen, except that albumin was substituted by lysozyme (1 g l⁻¹) in the incubation buffer. The antiserum was

obtained in rabbits against bradykinin coupled to swine thyroglobulin and used at a final dilution 10^{-5} . The radioimmunoassay was standardised using bradykinin but Ile-Ser-bradykinin, kallidin and des-Arg⁹-bradykinin showed a complete cross-immunoreactivity on an equimolar basis.

Kinins in exudates were also separated by a reverse phase high performance liquid chromatography (h.p.l.c.); the dried residue of exudates mixed with ethanol was dissolved in 200 μ l phosphate buffer; 50 μ l were injected and the column (0.46 $\times$ 25 cm) of TSK-gel ODS-120 T, 5 μ m (LKB) was isocratically eluted with 19% acetonitrile in 0.05 M phosphate buffer pH 2.5 at a flow rate of 1 ml min⁻¹. Fractions of 0.33 ml were analysed by radioimmunoassay for the presence of kinins.

Total proteins in plasma or in exudates were quantified by the Folin-Ciocalteu reagent as described by Lowry *et al.* (1951).

Bradykinin and kallidin were purchased from UCB (Belgium), T-kinin, trypsin, N-tosyl-L-phenylalanine chloromethyl ketone (TPCK) and soya bean trypsin inhibitor (SBTI) from Sigma.

Results are given as the mean $\pm$ s.e.mean of *n* determinations. Comparisons between groups were made with two-tailed Student's *t* test for unpaired data.

Results

Plasma level of T-kininogen

After the implantation of saline-soaked sponges, a progressive and similar increase in the plasma level of T-kininogen in Wistar rats and in Brown Norway rats was observed. This increase was statistically significant from 24 h onwards. The peak increase: 811 μ g ml⁻¹ was reached, in Wistar rats, at 48 h (Figure 1).

T-kininogen in sponge exudates

Simultaneously, in both strains, an accumulation of T-kininogen occurred in the sponges. The maximum level of T-kininogen, measured by radioimmunoassay, was observed at 72 h (Figure 2). We checked that T-kininogen in the exudates was still able to release a kinin activity. Trypsin (1 mg ml⁻¹) in presence of a kininase inhibitor EDTA (1 mg ml⁻¹) released $12.46 \pm 1.26 \mu$ g bradykinin equivalent per ml of the exudates withdrawn at 48 h from Wistar rats (*n* = 6). This amount corresponded to about 700 μ g ml⁻¹ of T-kininogen, a value slightly higher than the level of $553 \pm 71 \mu$ g ml⁻¹ measured by radioimmunoassay. In fact, bioassays measured not

Figure 1 Plasma level (μ g ml⁻¹) of T-kininogen after sponge implantation (continuous line) in Wistar rats and (broken line) in Brown Norway rats. Mean (*n*) determinations; vertical bars show s.e.mean.

only T-kininogen but total kininogens. This could explain the difference between the two results.

The extent of the inflammatory reaction induced by the saline-soaked sponges was determined by the measurement of protein and leucocyte levels in the exudates. The accumulation of proteins in the exudates was rapid in Wistar rats and reached a maximum, about 62 to 76% of the plasma level, from 24 h to 72 h after the implantation (Figure 3). The extravasation of proteins in the exudates was delayed in Brown Norway rats: the protein level was significantly lower at 3 h. Thereafter, it reached 67 to 69% of the plasma level (Figure 3). Simultaneously, leucocytes were found in the inflammatory lesion. Their level, similar in both strains, was maximum at 24 h and then decreased. The diminution was however slower in Brown Norway rats (Figure 3).

Figure 2 Accumulation of T-kininogen (μ g ml⁻¹) in sponge exudates (continuous line) from Wistar rats and (broken line) from Brown Norway rats. Mean (*n*) determinations; vertical bars show s.e.mean. The levels in Wistar rats were not significantly different from those in Brown Norway rats.

Figure 3 Evolution of the level of total proteins (mg ml^{-1}) (a) and total leucocytes (b) in sponge-induced exudates withdrawn from Wistar rats (continuous line) or from Brown Norway rats (broken line). The figure shows the mean of 10–12 determinations for leucocytes and of (n) determinations for proteins; vertical bars show s.e.mean. *** $P < 0.005$.

In Wistar rats, during the first 6 h, the level of T-kininogen in exudates correlated with the extravasation of proteins in the exudates (Figure 4). Thereafter, the T-kininogen content of the exudates expressed in $\mu\text{g mg}^{-1}$ of proteins did not correlate with the plasma level (Figure 4).

Kinin level in exudates

Figure 5 shows the evolution of kinin levels as measured by radioimmunoassay in sponge-exudates from both rat strains. In Wistar rats, the level of immunoreactive kinins was elevated as soon as the inflammatory reaction started. Thereafter, this level decreased up to 24 h and increased again during the second day up to a maximum level of $6.4 \pm 0.93 \text{ ng ml}^{-1}$ ($n = 11$). The ranges of the levels observed were from 1.57 to 17.9 ng ml^{-1} at 3 h and from 1.3 to 9.34 ng ml^{-1} at 48 h. We tested for the presence of kinins in the exudates by bioassays. The exudates withdrawn 3 h after the implantation contained: $4.62 \pm 2.98 \text{ ng ml}^{-1}$ ($n = 12$) and $9.8 \pm 1.9 \text{ ng ml}^{-1}$ ($n = 5$) after 48 h. On the other hand, in Brown Norway rats, the content of immunoreactive kinins was low, between 0.08 to

0.53 ng ml^{-1} , during the whole inflammatory process (Figure 5).

We tried to determine the nature of the immunoreactive peptides present in the exudates, using separation by a reverse phase h.p.l.c. Successful measurements were only obtained for the exudates withdrawn from Wistar rats, 3 h after the sponge implantation. The kinins present in these exudates were detected partly as bradykinin and partly as unidentified peptides which were eluted between kallidin and bradykinin (Figure 6). Bradykinin accounted for about 60% of the immunoreactive peptides separated by h.p.l.c. ($n = 6$).

Comparison between the inflammatory reaction in Wistar and kininogen-deficient Brown Norway rats

To compare the importance of the inflammatory reactions induced in both rat strains, we implanted dry sponges and weighed them in order to measure the volume of the exudates. The sponges removed from Wistar rats, 3 h after implantation, were heavier than those removed from Brown Norway rats: $201 \pm 12 \text{ mg}$ ($n = 12$) and $142 \pm 14 \text{ mg}$ ($n = 12$; $P < 0.005$) respectively. The level of proteins in the exudates was similar: $38.2 \pm 3.9 \text{ mg ml}^{-1}$ in Wistar rats ($n = 12$) and $33.5 \pm 3.3 \text{ mg ml}^{-1}$ in Brown Norway rats ($n = 12$).

Discussion

The inflammatory reaction induced by sponge implantation increased the plasma level of T-kininogen. This increase was relatively small in comparison with the levels observed after turpentine (Bouhnik *et al.*, 1987; Adam *et al.*, 1989) or carrageenan injections (Barlas *et al.*, 1985b). This difference would indicate that the inflammatory reaction following the implantation of saline-soaked sponges is relatively weak. The two other kininogens are not acute phase plasma proteins and their level in non-injured rats accounts for 35% of total kininogens (Barlas *et al.*, 1985a; Bouhnik *et al.*, 1987; Adam *et al.*, 1989).

Confirming previous results (Barlas *et al.*, 1985b; Sakamoto & Yoshikawa, 1986), we observed the presence of T-kininogen in the exudate. During the first 6 h of the inflammatory reaction, the accumulation of this substance was proportional to the extravasation of plasma proteins. After 24 h, the concentration of T-kininogen in the exudate exceeded the protein level. This suggests that the kallikrein-resistant kininogen was accumulating in the exudate without releasing kinins during the whole inflammatory process. Indeed, the T-kinin moiety was shown to be present in T-kininogen withdrawn from the exudate, as previously observed by Sakamoto &

Figure 4 Correlation between the plasma level and the sponge level of T-kininogen in Wistar rats. The concentration of T-kininogen is expressed in $\mu\text{g mg}^{-1}$ of proteins. In (a) values from sponges at 3 and 6 h; in (b) values from sponges at 24, 48 and 72 h. In (a) $y = 1.09x + 0.07$; $r = 0.75$; $n = 29$; in (b) $y = 0.33x + 11.56$; $r = 0.18$; $n = 52$.

Yoshikawa (1986) for T-kininogen recovered from the exudate filling the granulomatous inflammation induced by carrageenin. Whatever the accumulation of T-kininogen, only a significant release of kinins

was observed in Wistar rats. In these rats, the two other kallikrein-sensitive kininogens are present. Furthermore, we did not find T-kinin in the exudates but bradykinin and another immunoreactive peptide

Figure 5 Occurrence of kinins ng ml^{-1} in sponge exudates (a) from Wistar rats; (b) from Brown Norway rats. Mean of (n) determinations; vertical bars show s.e.mean. The results obtained in Brown Norway rats were significantly different from those recorded in Wistar rats at 3 h and 24 h ($P < 0.02$) and at 48 h ($P < 0.001$).

Figure 6 Identification by h.p.l.c. of the kinins present in the exudates obtained 3 h after sponge implantation in Wistar rats. The exudates were directly mixed with ethanol in order to precipitate kininases and kinin-forming enzymes. After centrifugation; the supernatant was lyophilised. Kinins were dissolved in phosphate buffer, separated by reverse phase h.p.l.c. and measured by radioimmunoassay.

which could be a bradykinin metabolite. These results indicate that T-kininogen was not a significant kinin-releasing substrate during the whole inflammatory process induced by sponge implantation.

Its main function might be the inhibition of thiol proteinases like cathepsins released during inflammatory reactions. Indeed, Nakagawa & Hirata (1986) purified two species of cysteine proteinase inhibitors identical with T-kininogen, from the fluid studied by Barlas *et al.* (1985b).

At first sight, our results contrast with the observations of Barlas *et al.* (1985b) who detected the presence of T-kininogen, T-kinin and bradykinin in the pouch fluid withdrawn 24 h after the injection of carrageenin. The level of T-kinin in their study was 0.34 ng ml^{-1} . This level is close to the values of immunoreactive kinins measured in the exudates of our kininogen-deficient Brown Norway rats. We did not identify the nature of these kinins. But these peptides were released from T-kininogen, the only kinin-forming substrate present in these rats. They could thus be T-kinin. In Wistar rats, plasma contains the three kininogens and the content of the exudates in kinins was higher. According to Kumakura *et al.* (1988), in carrageenin-induced inflammation, a release of bradykinin was detected mainly during the first hour. After 2 h, the level of bradykinin was lower than the assay limits. This could explain the low level of bradykinin found by Barlas *et al.* (1985b) at 24 h. In sponge exudates, the profile of kinin release during the first 24 h was rather similar: the level was high during the first 3 h and decline up to 24 h. Thus at this time, the concentrations of T-kinin in sponge-exudates from Brown Norway rats, and of total kinins measured in Wistar rats were close as in the results of Barlas *et al.* (1988). In both inflammatory reactions, a large release of bradykinin occurred during the first hours, while the level of T-kinin was

very small in comparison with the T-kininogen content of the exudates. During the first hours, the difference between the ranges of our values of kinins and the ones reported by Kumakura *et al.* (1988) could be explained in the following way. First, we measured immunoreactive kinins. The volume of the exudate in sponge is very small preventing a dilution of plasma proteins and of kinins. Second, sponge exudate has a lower degree of protein clearance than carrageenin-induced inflammation in air pouches (Hambleton *et al.*, 1988).

On the other hand, our observations suggest that the other kininogens were implicated in the development of the inflammatory reaction induced by sponge implantation. The protein content of the exudate was reduced in Brown Norway rats and the volume of the exudate was diminished in dry sponges withdrawn from these animals, at the beginning of the reaction.

We also observed a significant kinin release in normal rats during the second day after implantation when the healing process is taking place. We have no idea about the mechanism of this release which did not depend on an infectious process as demonstrated by the leucocyte count in the sponges, and did not originate from T-kininogen as shown by the low values recorded in Brown Norway rats. But this release would indicate that the kinins did not only participate in the vascular changes mediating the inflammatory reaction but also could influence the cellular response leading to the formation of the granulomatous tissue. Indeed, some effects of bradykinin on the fibroblasts have been already described (Hong & Levine, 1976; Newcombe *et al.*, 1977; Vincenzi & Villereal, 1984).

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References

- ADAM, A., DAMAS, J., CALAY, G., RENARD, Cl., REMACLE-VOLON, G. & BOURDON, V. (1989). Quantification of rat-T-kininogen using immunological methods. Application to inflammatory processes. *Biochem. Pharmacol.*, **38**, 1569–1575.
- ALHENC-GELAS, F., MARCHETTI, J., ALLEGRI, J., CORVOL, P. & MENARD, J. (1981). Measurement of urinary kallikrein activity. Species difference in kinin production. *Biochim. Biophys. Acta*, **677**, 477–488.
- BARLAS, A., OKAMOTO, H. & GREENBAUM, L.M. (1985a). T-kininogen — The major plasma kininogen in rat adjuvant arthritis. *Biochem. Biophys. Res. Commun.*, **129**, 280–286.
- BARLAS, A., SUGIO, K. & GREENBAUM, L.M. (1985b). Release of T-kinin and bradykinin in carrageenin induced inflammation in the rat. *FEBS Lett.*, **190**, 268–270.
- BOUHNICK, J., BAUSSANT, T., SAVOIE, F., ALHENC-GELAS, F., GAUTHIER, F. & ESNARD, F. (1987). Direct radioimmunoassay for rat T-kininogen. *Biochem. Biophys. Res. Commun.*, **144**, 1090–1097.
- DAMAS, J. (1987). The novel rat kininogen, T-kininogen. A pro-inflammatory factor or a thiostatin? *Arch. Int. Physiol. Biochim.*, **95**, 67–73.
- DAMAS, J. & ADAM, A. (1980). Congenital deficiency in plasma kallikrein and kininogen in the Brown Norway rat. *Experientia*, **36**, 586–587.
- DAMAS, J. & ADAM, A. (1985). The relationship between kininogens and kallikreins in deficient Brown Norway rat plasma. *Mol. Physiol.*, **8**, 307–316.

- DAMAS, J., REMACLE-VOLON, G. & ADAM, A. (1984). Inflammation in the rat paw due to urate crystals. Involvement of the kinin system. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **325**, 76–79.
- DAMAS, J., REMACLE-VOLON, G. & DEFLANDRE, E. (1986). Further studies of the mechanism of counter-irritation by turpentine. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **332**, 196–200.
- ENJOJI, K.I., KATO, H., HAYASHI, I., OH-ISHI, S. & IWANAGA, S. (1988). Purification and characterization of rat T-kininogen isolated from plasma of adjuvant-treated rats. *J. Biol. Chem.*, **263**, 973–979.
- GARCIA LEME, J. (1978). Bradykinin system. In *Inflammation*, ed. Vane, J.R., Ferreira, S.H. Handbook of Experimental Pharmacology, Vol. 50, I, pp. 464–522. Berlin, Heidelberg: Springer-Verlag.
- GREENBAUM, L.M. (1984). T-kinin and T-kininogen – Children of new technology. *Biochem. Pharmacol.*, **33**, 2943–2944.
- HAMBLETON, P., CHANDLER, J. & MILLER, P. (1988). Comparison of exudation, vascular permeability changes and clearance properties in two models of acute inflammation in rats. *Br. J. Pharmacol.*, **93**, 216P.
- HAYASHI, I., INO, T., KATO, H., IWANAGA, S., NAKANO, T. & OH-ISHI, S. (1984). Demonstration of the third kininogen in high and low molecular weight kininogens-deficient Brown Norway katoliek rat. *Thrombosis Res.*, **36**, 509–516.
- HONG, S.C.H. & LEVINE, L. (1976). Stimulation of prostaglandin synthesis by bradykinin and thrombin and their mechanisms of action on MC5-5 fibroblasts. *J. Biol. Chem.*, **251**, 5814–5816.
- KUMAKURA, S., KAMO, I. & TSURUFUJI, S. (1988). Role of bradykinin in the vascular permeability response induced by carrageenin in rats. *Br. J. Pharmacol.*, **93**, 739–746.
- LEWIS, G.P. (1970). Kinins in inflammation and tissue injury. In *Bradykinin, Kallidin and Kallikrein*. ed. Erdös, E.G., Handbook of Experimental Pharmacology, Vol. 25, pp. 516–530. Berlin, Heidelberg: Springer-Verlag.
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
- NAKAGAWA, H. & HIRATA, K. (1986). Purification of two species of exudate cysteine-proteinase inhibitors that are acute-phase reactants in the carrageenin-induced inflammation in rats. *FEBS Lett.*, **207**, 58–62.
- NEWCOMBE, D.B., FAHEY, J.V. & ISHIKAWA, Y. (1977). Hydrocortisone inhibition of the bradykinin activation of human synovial fibroblasts. *Prostaglandins*, **13**, 235–244.
- OH-ISHI, S., HAYASHI, I., UTSUNOMIYA, I., HAYASHI, M., YAMAKI, K., YAMASU, A. & NAKANO, T. (1987). Roles of kallikrein-kinin system in acute inflammation: studies on high- and low-molecular weight kininogens-deficient rats (B/N-Katholiek strain). *Agents & Actions*, **21**, 384–386.
- OKAMOTO, H. & GREENBAUM, L.M. (1983a). Kininogen substrates for trypsin and cathepsin D in human, rabbit and rat plasmas. *Life Sci.*, **32**, 2007–2013.
- OKAMOTO, H. & GREENBAUM, L.M. (1983b). Isolation and structure of T-kinin. *Biochem. Biophys. Res. Commun.*, **112**, 701–708.
- SAKAMOTO, W. & YOSHIKAWA, K. (1986). T-kininogen in rats with carrageenin-induced inflammation. *Biochem. Pharmacol.*, **35**, 4283–4290.
- SAKAMOTO, W., SATOH, F., GOTOH, K. & UEHARA, S. (1987). Ile-Ser-bradykinin (T-kinin) and Met-Ile-Ser-bradykinin (Met-T-kinin) are released from T-kininogen by an acid proteinase of granulomatous tissues in rats. *FEBS Lett.*, **219**, 437–440.
- VICENTINI, L.M. & VILLERREAL, M.L. (1984). Serum, bradykinin and vasopressin stimulate release of inositol phosphates from human fibroblasts. *Biochem. Biophys. Res. Commun.*, **123**, 663–670.

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