Release of T-kinin and bradykinin in carrageenin induced inflammation in the rat

Aydin Barlas, Kazuo Sugio and Lowell M. Greenbaum

Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912, USA

Received 12 August 1985

Plasma and inflammatory fluid kininogen levels, and blood and inflammatory fluid free kinin levels were determined in rats 24 h after the injection of carrageenin into an air pouch. Plasma T-kininogen levels increased 7-fold. In the inflammatory fluid levels reached 8 μg/ml. Blood levels of free kinin showed a 5-fold increase. The kinins were identified on HPLC as T-kinin (Ile-Ser-bradykinin) and bradykinin, 63 and 37%, respectively. These results indicate for the first time that free T-kinin as well as bradykinin is released during an inflammatory response in rat and confirms our previous finding that T-kininogen may be a major acute-phase protein in inflammation.

T-kinin *T-kininogen* *Bradykinin* *Inflammation* *Acute-phase protein* *Carrageenin*

1. INTRODUCTION

T-kininogen, which contains the pharmacologically active T-kinin molecule (Ile-Ser-bradykinin), has been shown by our laboratory to be highly elevated in the plasma of rats with adjuvant arthritis [1]. This is most significant since T-kininogen has now been identified as a major acute inflammatory protein [2]. In this investigation we studied the major question whether T-kinin itself is released in an inflammatory condition. We also determined whether bradykinin itself is released. Carrageenin was used to induce an inflammation in an air pouch in the rat. The subsequent fluid accumulation allowed for the study of the various kininogens and free kinins in the fluid as well as in plasma and blood of normal and animals responding to carrageenin.

2. MATERIALS AND METHODS

2.1. *Carrageenin air-pouch*

6-week-old male Sprague-Dawley rats (160–180 g) were used. The method of Fukuhara and Tsurufuji [3] was used to induce a carrageenin air-pouch inflammation. Rats were injected subcutaneously at the dorsum with 8 ml air to make an air pouch. After 24 h, 4 ml of 2% carrageenin solution (sterilized) containing penicillin G (0.1 mg/ml) and streptomycin sulfate (0.1 mg/ml) was injected into the pouch (day 0).

2.2. *Collection of blood, plasma and inflammatory fluid*

24 h after the carrageenin solution injection, blood samples were collected from 5 rats under light ether anesthesia. Two samples were drawn from the abdominal aorta of each rat into two separate polyethylene syringes connected to a 3-way stopcock. The first sample was collected in a 1 ml syringe containing 0.1 ml of 3.8% sodium citrate and was used for kininogen determinations. The second sample was collected in a 10 ml syringe containing 5 ml of 30% trifluoroacetic acid (TFA) and was used to measure the concentration of free kinins. Inflammatory fluid from the pouch was collected in a plastic container. 0.5 ml of this fluid was transferred to a polyethylene tube containing 0.05 ml of 3.8% sodium citrate (for kininogen determination). The remaining fluid was im-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

mediately (within 2–3 s) mixed with an equal volume of 30% TFA (for free kinin determination).

2.3. *Assay of kininogen and free kinin levels*

Plasma and inflammatory fluid T-kininogen were determined as described [1]. The procedure involves pretreatment of the plasma and/or inflammatory fluid with HCl and conversion of the T-kininogen to T-kinin by an excess amount of trypsin, followed by radioimmunoassay of the released kinin [4]. For high-M_r kininogen determinations, the plasma and the inflammatory fluids were shaken with glass powder to activate kallikrein which releases bradykinin from high-M_r kininogen. The released bradykinin was determined by radioimmunoassay. The level of low-M_r kininogen was calculated by subtracting the concentrations of high-M_r kininogen and T-kininogen from the concentration of total kininogen (total amount of kinin released by excess amount of trypsin). Free kinins in the TFA-treated blood and inflammatory fluid were determined following centrifugation at 5000 × *g* for 10 min of the TFA precipitate. The supernatants were then applied to a C_{18}-extraction column (1 × 2 cm), previously primed with 5 ml methanol and 5 ml of 1% TFA. After washing the column with 5 ml of 1% TFA, kinins were eluted with 3 ml of 50% acetonitrile in 1% TFA. The eluate was evaporated and redissolved in 200 μl distilled water. Kinins extracted from the blood and the fluid were separated by a reverse-phase HPLC as in [5]. The fractions were collected and evaporated. The residue was redissolved in the buffer and radioimmunoassayed. The results were corrected in accordance with the recovery percentage (70% through all steps).

2.4. *Materials*

The sources were: carrageenin (Seakem no.202, Marine Colloid); trypsin (type X11, TPCK-treated from bovine pancreas, Sigma); bradykinin, [Tyr1]-kallidin (Peninsula Corp.); $Na^{125}I$ (carrier-free, New England Nuclear); octadecyl-extraction column (J.T. Baker). T-kinin (Ile-Ser-bradykinin) was synthesized and supplied by courtesy of Dr J.M. Stewart (Department of Biochemistry, University of Colorado Health Science Center).

3. RESULTS

Table 1 illustrates the levels of kinins and kininogens in plasma, blood and inflammatory fluid of normal and experimental animals (carrageenin-treated). It may be seen that free T-kinin as well as bradykinin appear to be elevated in all fluids 24 h after the carrageenin injection (about 4–5-fold). Similarly, free T-kinin and bradykinin appear in the inflammatory fluid 24 h after the injection.

The level of T-kininogen was increased some 7-fold in plasma after 24 h following the carrageenin injection; high-M_r kininogen on the other

Table 1

Kininogen and kinin levels of rats treated with carrageenin

Kininogen and kinin levels	Day after carrageenin injection	
	Day 0	Day 1
Plasma		
T-kininogen (μg BK equiv./ml)	5.04 ± 1.56	36.99 ± 5.34
High-M_r kininogen (μg BK equiv./ml)	0.82 ± 0.06	1.04 ± 0.04
Low-M_r kininogen (μg BK equiv./ml)	0.96 ± 0.09	0.91 ± 0.10
Blood		
T-kinin (ng/ml)	0.053 ± 0.031	0.265 ± 0.067
Bradykinin (ng/ml)	0.037 ± 0.010	0.153 ± 0.021
Inflammatory fluid		
Fluid volume (ml)	–	6.75 ± 0.34
T-kininogen (μg BK equiv./ml)	–	8.48 ± 1.11
High-M_r kininogen (μg BK equiv./ml)	–	0.05 ± 0.01
Low-M_r kininogen (μg BK equiv./ml)	–	0.78 ± 0.17
T-kinin (ng/ml)	–	0.340 ± 0.072
Bradykinin (ng/ml)	–	0.134 ± 0.040

Plasma and inflammatory fluid kininogen levels, and blood and inflammatory fluid free kinin levels were measured 24 h after the injection of carrageenin. The results were calculated after HPLC analysis followed by radioimmunoassay. Each value indicates a mean value of 5 rats ± SE

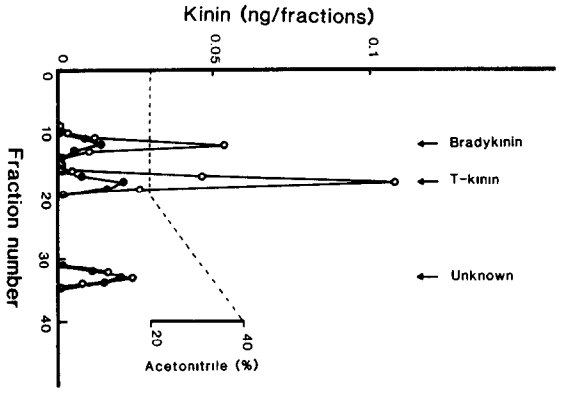

Fig.1. Reverse-phase HPLC of immunoreactive free kinins from the plasma of normal (●—●) and carrageenin-treated (○—○) rats. Kinin in each fraction was assayed by radioimmunoassay using bradykinin as a standard.

hand showed little change. The inflammatory fluid itself contained as its major kininogen, T-kininogen.

Fig.1 confirms by HPLC procedures the presence of free T-kinin and bradykinin in normal rat blood and their elevation 24 h after carrageenin injection.

4. DISCUSSION

Recently Okamoto and Greenbaum [6–8] discovered in rat plasma a kininogen which contained the novel sequence Ile-Ser-bradykinin and which they called T-kininogen. Barlas et al. [1] showed that plasma T-kininogen is the major plasma kininogen of the rat and increases 15-fold in rats with adjuvant arthritis. Other kininogens, high-M_r kininogen and low-M_r kininogen, showed little or no change under these conditions. Here, we have confirmed that T-kininogen is of major importance in the inflammatory response; plasma levels increased 7-fold, 24 h after the carrageenin injection while no detectable change occurred in high-M_r and/or low-M_r kininogens. It is of significant interest that Cole et al. [2] have described the major acute-phase α-protein of the rat as a kininogen. We note that this kininogen contains the sequence of T-kinin and therefore is T-kininogen.

Heretofore, bradykinin has generally been thought to be the only kinin mediator released in the inflammatory response. Our results demonstrate for the first time that T-kinin appears in free form in blood and fluids of rats following an inflammatory challenge. The appearance of bradykinin is also observed. Whether bradykinin is released directly or is a consequence of T-kinin metabolism is unknown at this time. Since kallikreins do not release T-kinin from T-kininogen, the appearance of T-kinin indicates that an enzyme system other than kallikreins is present in rat blood or tissues and is activated to release T-kinin during inflammation. Whether this enzyme is cathepsin D or an enzyme yet to be described is under study. The T-kinin-T-kininogen system is obviously of major importance in the inflammatory challenge since it releases a potent vasoactive mediator (T-kinin) and acts as a major acute-phase protein.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs J. Jones and Mr S. Cooper for their technical assistance. This work was supported by NIH grant HL-32183.

REFERENCES

[1] Barlas, A., Okamoto, H. and Greenbaum, L.M. (1985) Biochem. Biophys. Res. Commun. 129, 280–286.
[2] Cole, T., Inglis, A.S., Roxburgh, C.M., Howlett, G.J. and Schreiber, G. (1985) FEBS Lett. 182, 57–61.
[3] Fukuhara, M. and Tsurufuji, S. (1969) Biochem. Pharmacol. 18, 475–484
[4] Odya, C.E., Goodfriend, T.L., Stewart, J.M. and Pena, C. (1978) J. Immunol. Methods 19, 243–257.
[5] Narayanan, T.K. and Greenbaum, L.M. (1984) J. Chromatogr. 306, 109–116.
[6] Okamoto, H. and Greenbaum, L.M. (1983) Biochem. Biophys. Res. Commun. 112, 701–708.
[7] Okamoto, H. and Greenbaum, L.M. (1983) Biochem. Pharmacol. 32, 2637–2638.
[8] Okamoto, H. and Greenbaum, L.M. (1983) Life Sci. 32, 2007–2013.