Endotoxin-Induced Kinin Production in Man[1] (36302)

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Kinins are a group of polypeptides possessing potent biological properties including vasodilatation and hypotension, smooth muscle contraction, changes in vascular permeability, and excitation of pain (1). These vasoactive peptides arise from the conversion of precursor kininogen, an α_2 globulin, by the sequential activation of factor XII (Hageman factor) and a plasma proteolytic enzyme, kallikrein (1). Kinins are rapidly inactivated by kininases present in both plasma and granulocytes (2). The possible role of kinins in bacterial or endotoxin shock has been of interest to many investigators, but because of differences in animal species used in experimental shock studies and the difficulty and diversity of methods for measuring kinins, their importance in this pathologic state is unsettled. In this report, we have demonstrated for the first time elevated blood bradykinin levels in normal human subjects receiving purified bacterial endotoxin.

Materials and Methods. All subjects in this study were normal, healthy volunteers aged 18–21 of both sexes. Informed consent was obtained for all studies performed. Purified *S. abortus equi* endotoxin (Lipexal, Dorsey Company, Lincoln, Nebraska) or 1 ml of saline was administered intravenously in doses of 3.0 and 5.0 ng/kg. Blood pressure, pulse, respirations, and rectal temperatures were recorded at 30-min intervals for 8 hr. Blood was collected at 0, 30, 60 min and 2, 4, and 7 hr after injection. Etiocholanolone (Sigma Chemical Company, St. Louis, MO), a synthetic steroid pyrogen, or propylene glycol (vehicle) was administered intramuscularly and vital signs recorded for a 24-hr period. Blood was collected at 0, 9, 12, and 17 hr after injection. All subjects were at bed rest during the study period; foods and fluids were allowed as desired.

For bradykinin determinations, 10 ml of blood were drawn into a plastic syringe containing 20 ml of 1.2% perchloric acid and gently mixed. After centrifugation at 8000 rpm for 20 min at 4°, the supernatant fluid was decanted, pH adjusted to 7.5 with 2 *N* KOH, and stored at −20° for further preparation. Blood bradykinin levels were measured first by modification (3) of the rat uterus bioassay of Webster and Gilmore (4), which employs IRC-50 exchange resin chromatography for separation of kinins from other peptides and vasoactive amines. The specificity of the assay for bradykinin was verified by appropriate studies with blocking agents in the tissue bath and by incubation of the peptide sample with trypsin and chymotrypsin. Later, aliquots of each IRC-50 eluate were measured using minor modifications of an immunoassay method specific for bradykinin (5). After a 2-hr incubation period of the antigen–antibody, 1 ml of pH 7.4 veronal acetate buffer was added to each tube. Instead of the charcoal step, the entire volume was filtered through a Millipore filter (No. HAW PO-2500), pore size of 0.45 μm, diameter of 25 mm). The filters were rinsed twice with 5-ml aliquots of veronal acetate buffer both before and after application of the sample. The filter discs were then transferred directly to a counting vial and 10 ml of aquasol added for scintillation counting.

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TABLE I. Pyrogens and Blood Kinin Levels in Man.

	Saline 1 ml	Endotoxin 3.0 ng/kg	Endotoxin 5.0 ng/kg	Propylene glycol 1 ml	Etiocholanolone 3.0 mg/kg
Number of subjects	4	2	2	2	2
Temperature (°)					
Maximum[a]	37.5 (37.2–37.8)[b]	38.6 (38.5–38.8)	38.0 (37.9–38.0)	37.7 (37.6–37.8)	38.0 (37.8–38.2)
Systolic blood pressure (mm Hg)					
Maximum increase	16 (3–28)	17 (12–22)	15 (13–16)	13 (8–18)	17 (14–20)
Maximum decrease	9 (5–12)	3 (0–6)	3 (2–4)	4 (2–6)	5 (4–6)
Pulse (beats/min)					
Maximum increase	11 (4–18)	19 (12–25)	23 (20–26)	6 (3–8)	18 (15–20)
Maximum decrease	5 (2–10)	2 (0–4)	0	9 (8–10)	0
Granulocytes (cells/mm^3)					
Baseline	2,900	2,950	2,600	4,100	3,500
Maximum[a]	3,550	12,505	10,650	5,050	7,800
Change (% of baseline)	122	480	410	123	218
Minimum[a]	2,450	1,650	900	3,600	5,850
Change (% of baseline)	84	56	35	86	0
Peak bradykinin levels (ng/ml)	<1.0 (<1.0–1.2)[b]	3.65[c] (2.8–4.5)	5.4[c] (4.8–6.0)	<1.0	<1.0

[a] During the study period.
[b] Mean and range in parentheses.
[c] Difference from saline controls significant ($p < 0.01$, t test).

Differences between the two assays were less than 10% and only the immunoassay results are reported. Plasma histamine was measured by the method of Miller *et al.* (6). All samples were coded and assays were performed in a blind fashion.

Results. The responses of the subjects to endotoxin and ethiocholanolone are summarized in Table I. At the doses employed in these studies, blood pressure did not change significantly from control (saline) values during any phase of endotoxemia. Small but consistent increases were noted in the pulse rate and in general paralleled the temperature response. Temperature began to rise 90–120 min after endotoxin and fever persisted for approximately 4 hr. The greater fibrile response with the lower dose of endotoxin may be attributed to the well-known variability of febrile responses among different individuals. Fever with etiocholanolone began approximately 12 hr after injection and persisted for an additional 8 hr.

Transient granulocytopenia was observed to some degree in all subjects receiving endotoxin (Table I). The lowest granulocyte levels were observed 60–90 min after injection and by 2 hr all had entered the leukocytosis phase characteristic of endotoxin. The degree of granulocytopenia ranged from an absolute low of 600/mm^3 (19% of zero time baseline) to 2000/mm^3 (60% of baseline) and was dose related. Leukopenia was not observed after etiocholanolone and granulocytosis paralleled the temperature pattern.

Elevations of blood bradykinin levels were found in all subjects during endotoxemia and ranged from a low of 2.8 ng/ml to a high of 6.0 ng/ml (Table I). Higher levels were observed with the larger doses of endotoxin. Levels were apparent as early as 30 min after injection (two subjects) but reached a peak at 60 min in three of the four subjects studied. In one volunteer, the peak response was observed 2 hr after injection. Bradykinin levels were minimally elevated in one volunteer at 4 hr and were undetectable 7 hr after endotoxin. Control studies were performed in

every subject and of the 24 determinations all were < 1.0 ng/ml (upper limits of normal range) except two values which were 1.2 ng/ml. In these studies, the degree of granulocytopenia was closely associated with the value of the peak bradykinin levels ($r_s = 1.0$, $p < .05$, Spearman rank correlation test). No elevation of bradykinin levels were observed after etiocholanolone or propylene glycol. The temporal relationship of temperature, granulocytopenia, and kinin levels after endotoxin are shown in Fig. 1. Plasma histamine levels were performed on all samples, but only 2 of 54 showed minimal elevations (3.8 and 4.5 ng/ml; normal 1–3.4 ng/ml).

Discussion. The present studies clearly demonstrate elevations of bradykinin in blood during noninfectious, nonhypotensive endotoxemia in man. Bradykinin levels follow a smooth pattern appearing as early as 30 min after injection, reach a peak at approximately 1 hr, and return to normal by 4 hr. The *in vivo* formation of kinins induced by endotoxin occurs, therefore, as an early event in endotoxemia and prior to the well-known findings of fever and leukocytosis. The steroid pyrogen etiocholanolone regularly promotes fever, myalgias, leukocytosis, inflammation, and pain at the site of injection, but unlike endotoxin does not produce hypotension or leukopenia even in very large doses (7). The failure of this agent to evoke increases in blood bradykinin levels excludes the possibility that kinin formation by endotoxin is the result of nonspecific "stress" and further attests to the fundamental differences between these pyrogens.

The hypothesis that the kinin system may contribute to the clinical syndrome of endotoxin or bacteremic shock has received support recently by the finding in several septicemic and hypotensive patients of increased spontaneous plasma kallikrein activity (measured as plasma arginine esterase activity), coupled with reductions in substrate kallikreinogen and kallikrein inhibitor (8, 9). Blood kinin levels were not measured directly, but were presumed to be elevated as a consequence of the increased arginine esterolytic activity. Such presumptions may not always hold (10). A group of normotensive

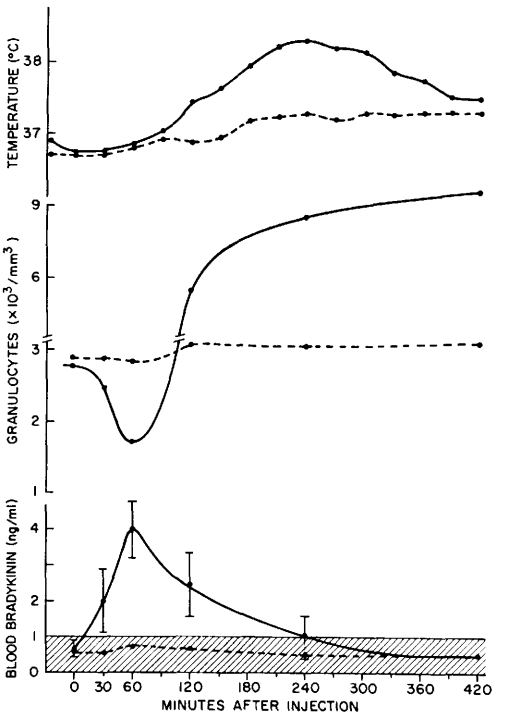

FIG. 1. Mean temperature, granulocyte and bradykinin responses (± standard error) for four subjects administered intravenous endotoxin (two received 3 mg/kg and two received 5 mg/kg, solid line) or control saline (broken line). Shaded areas for bradykinin levels represents the range for normal values.

patients with bacteremia showed no increase in plasma esterase activity although mild reductions in substrate and inhibitor were noted (9). In another study, endotoxin (Pyrexal) was noted to cause a decrease in plasma substrate kininogen in man (11). Our results offer direct evidence that bacterial endotoxin will promote the release of vasoactive bradykinin in man, and, moreover, this need not occur in a setting of uncompensated hypotension or major disturbance of the cardiovascular system. Our data suggest that in man histamine does not precede kinin generation and, therefore, it seems unlikely that this amine can be considered a significant trigger vasodilator.

In vivo kinin production by endotoxin in animals appears to be dependent on the species used for study. In the dog, a decrease in plasma substrate kininogen has been reported

by some investigators (12–14) but not by others (15–17). In the rabbit, significant reductions in glass but not pancreatic kallikrein-activatable kininogen were observed 5 min after endotoxin infusion (18) but in another study no decrease in kininogen was reported either in the rabbit or the cat (19). Elevations of spontaneous arginine esterolytic activity in the plasma of mice and rats given endotoxin have been described (9). In the monkey, both elevation of blood kinins and reduction in plasma kininogen substrate have been demonstrated after infusion of endotoxin (3). Similar to the present study in man, blood kinins appeared early, were maximal by 1 hr and then declined. Granulocytopenia was likewise found to parallel kinin release. The similarity of responses in the subhuman primate and man confirms the usefulness of this animal model for the study of kinin–endotoxin relationships.

Kinin release by endotoxin is probably mediated by several different mechanisms, although a common feature appears to be a requirement for factor XII (Hageman factor). Thus, endotoxins may cause activation of factor XII directly (10, 20), by promoting endothelial damage, which, in turn, activates factor XII (9), or by releasing an activating substance in granulocytes (2). Once activated, factor XII causes conversion of kallikreinogen to kallikrein leading to kinin formation from substrate kininogen. It is also possible that granulocytes may contain a kallikrein-like substance capable of acting directly on substrate kininogen (21). Of note in the present studies, the magnitude and temperal characteristics of granulocytopenia after endotoxin, possibly a consequence of sequestration and some degree of destruction in the microcirculation, closely paralleled the appearance of bradykinin in blood. While a causal relationship for these two events cannot be established from these studies, the data do suggest that the leukocyte may play a contributing role in kinin release during endotoxemia.

Summary. Elevations of bradykinin levels in blood were regularly induced in normal subjects by intravenously administered bacterial endotoxin. Bradykinin was detected as early as 30 min after injection, was maximal by 1 hr and thereafter declined. The magnitude and timing of kinin formation coincided with granulocytopenia and preceded fever and granulocytosis. No increase in blood bradykinin levels were observed after the administration of a steroid pyrogen, etiocholanolone. The data are consistent with the contention that kinin may contribute to the biologic events accompanying endotoxemia in man.

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1. Kallermeyer, R. W., and Graham, R. C., N. Engl. J. Med. **279**, 754, 802, 859 (1968).

2. Melmon, K. L., and Cline, M. J., Biochem. Pharmacol. Suppl. 271 (1968).

3. Nies, A. S., Forsyth, R. P., Williams, H. E., and Melmon, K. L., Circ. Res. **22**, 155 (1968).

4. Webster, M. E., and Gilmore, J. P., Biochem. Pharmacol. **14**, 1161 (1965).

5. Talamo, R. C., Haber, E., and Austin, K. F., J. Lab. Clin. Med. **74**, 816 (1969).

6. Miller, R. L., McCord, C., Sanda, M., Bourne, H. R., and Melmon, K. L., J. Pharmacol. Exp. Ther. **175**, 228 (1970).

7. Kimball, H. R., Vogel, J. M., Perry, S., and Wolff, S. M., J. Lab. Clin. Med. **69**, 415 (1967).

8. Colman, R. W., Mason, J. W., and Sherry, S., Ann. Intern. Med. **71**, 763 (1969).

9. Mason, J. W., Kleeberg, V., Dolan, P., and Colman, R. W., Ann. Intern. Med. **73**, 545 (1970).

10. Nies, A. S., and Melmon, K. L., Biochem. Pharmacol. **20**, 29 (1971).

11. Sicuteri, F., and Periti, P., *in* "Bradykinin and its Precursor" (F. Sicuteri, ed.), p. 53 Publ. C.E.P.I., Rome, 1963a.

12. Greeff, K., Luhr, R., and Strobach, H., Arch. Exp. Pathol. Pharmakol. **253**, 235 (1966).

13. Meyer, A., *in* "Neue Aspekte der Trasylol-Therapie" (R. Gross and G. Kroneberg, eds.), p. 142. Schattauer, Stuttgart (1966).

14. Diniz, C. R., Reis, M. L., and Corrado, A. P., *in* "Bradykinin and Related Kinins" (M. Rocha e Silva and H. Rothschild, eds.), p. 15. Plenum, New York (1967).

15. Webster, M. E., and Clark, W. R., Amer. J. Physiol. **197**, 406 (1959).

16. Urvanitz, D., Sailer, R., and Habermann, E., Advanc. Exp. Med. **8** (1970).

17. Carretero, O. A., Nasjletti, A., and Fasciolo, J. C., Experientia **26**, 63 (1970).

18. Erdos, E. G., and Miwa, I., Fed. Proc. **27**, 92

(1968).

19. Habermann, E., Sailer, F., and Müller, J., Schockssymposium Freiberg, (1969). Doútsche Gesellschaft for Fortscheritte Auf Dem Gebiet Der Inneren Medizen.

20. Pettinger, W. A., and Young, R., Life Sci. **9**, 313 (1970).

21. Melmon, K. L., and Cline, M. J., Nature (London) **213**, 90 (1967).

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