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Evidence for the involvement of a plasma kallikrein-kinin system in the immediate hypotension produced by endotoxin in anaesthetized rats

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1 *In vitro* incubation of normal rat plasma with endotoxin from *E. coli* (3–10 mg ml^{-1} in the incubation mixture) caused a dose-dependent increase in levels of free kinin and plasma kallikrein in the presence of *o*-phenanthroline, together with a mirror-image, dose-dependent decrease in the residual levels of the precursors, plasma prekallikrein and high-molecular-weight kininogen. Low-molecular-weight kininogen levels were not modified.

2 Intravenous injection of endotoxin (3–30 mg kg^{-1}) into the femoral vein of anaesthetized rats resulted in dose-dependent hypotension. In blood collected up to 15 min after injection, the levels of prekallikrein and high-molecular-weight kininogen in plasma were decreased while levels of the active forms, plasma kallikrein and free kinin, showed a transient increase in the blood 1 min after administration of endotoxin.

3 A degradation product of bradykinin, des-Phe^8-Arg^9-bradykinin, as measured by a newly developed enzyme immunoassay, was detectable up to 5 min after administration of endotoxin.

4 Intravenous infusion of soybean trypsin inhibitor inhibited both the formation of bradykinin and des-Phe^8-Arg^9-bradykinin and the initial hypotension.

5 It can be concluded from our results that plasma prekallikrein is activated in the blood immediately after administration of endotoxin to rats and that bradykinin is a major cause of the immediate hypotension.

Introduction

Circulating bacterial endotoxins are known to induce shock (Lillehei *et al.*, 1967; Cardis *et al.*, 1972). Because of the seriousness of the clinical symptoms associated with gram-negative septicaemia, several studies have been conducted to clarify the mechanisms responsible for the development of metabolic and haemodynamic abnormalities observed during endotoxin shock (Baue, 1968; Neuhof *et al.*, 1970; Sardesai & Walt, 1971). Among the vasoactive substances released by endotoxin, such as histamine (Hinshaw *et al.*, 1961; Vick *et al.*, 1971), 5-hydroxytryptamine, catecholamines (Rosenberg *et al.*, 1961) and kinins, bradykinin has been suspected of playing a major role in the widespread vascular disturbances seen during endotoxaemia. The involvement of the plasma kallikrein-kinin system in endotoxin shock has been examined and predicted by many authors: Robinson *et al.* (1975) measured kallikrein activity in blood of patients in endotoxin shock, using TAME (*p*-tosyl-arginine methyl ester hydrochloride), a non-specific synthetic substrate of esterase. Sardesai & Rosenberg (1974) also measured the increase in the TAME esterase activity, with an increase in levels of free bradykinin and a decrease in levels of total bradykininogen after injection of rats and dogs with endotoxin. They did not identify the types of kininogen involved. Gillimore *et al.* (1978) reported a reduction in the levels of prekallikrein and of high-molecular-weight kininogen in plasma during endotoxin shock, but free kinin was not detected. Similar results were obtained by Nies *et al.* (1968), who showed only an increase in levels of free kinin and a reduction in levels of total kininogen during endotoxin shock in monkeys. These workers presented only partial evidence of activation of plasma kallikrein-kinin

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systems. Conclusive evidence for the activation of plasma prekallikrein and in particular, the time course of the activation still remain to be demonstrated.

We have established, in our laboratory, systems for the measurement of the levels of plasma prekallikrein (Oh-ishi & Katori, 1979), high- and low-molecular-weight kininogens (Uchida & Katori, 1979; Uchida *et al.*, 1986) and bradykinin itself (Ueno *et al.*, 1981). A novel assay system for measurement of levels of a degradation product of bradykinin, des-Phe^8-Arg^9-bradykinin, was recently developed and has been used as a marker of the release of kinin at sites of inflammation (Katori *et al.*, 1988; Majima *et al.*, 1988a). Using these assays, we have attempted to obtain the conclusive evidence for the involvement of the plasma kallikrein-kinin system in the immediate phase of endotoxin shock.

Methods

Activation of the plasma kallikrein-kinin system in rat plasma by endotoxin in vitro

Blood was collected under light anaesthesia with ether from the carotid artery of rats (Sprague-Dawley, male rats, specific pathogen free, 8–10 weeks old, from the Shizuoka Laboratory Animal Center, Hamamatsu) into tubes that contained 1/10 volume of 3.8% sodium citrate and the mixture was centrifuged at 1500 *g*. The citrated rat plasma was mixed with an equal volume of a solution of endotoxin (6–20 mg ml^{-1} saline). After a 30 min incubation at 37°C, the residual levels of prekallikrein and kininogens in the incubation mixture were determined. Plasma kallikrein activity in the incubation mixtures was also assayed using a selective, synthetic substrate, Z-Phe-Arg-MCA for plasma kallikrein, according to the method for the measurement of levels of prekallikrein described below. The amount of free kinin released in the incubation mixture was measured in the presence of *o*-phenanthroline (0.2 mg ml^{-1} plasma), an inhibitor of kininase I and II, by use of an enzyme immunoassay (Markit-A, Dainippon Pharmaceutical Co., Osaka).

Assay of prekallikrein

The residual level of prekallikrein was measured by the method described by Oh-ishi & Katori (1979). Briefly, plasma prekallikrein was activated by kaolin in the presence of acetone, and plasma kallikrein activity was assayed using a peptidyl fluorogenic substrate, Z-Phe-Arg-MCA, whch is a selective, synthetic substrate for plasma kallikrein (Morita *et al.*, 1977). One unit was arbitrarily defined as the amount of prekallikrein that released 1×10^{-7} M of AMC during a 10 min period under our assay conditions. The difference between the amidolytic activities in the presence of soybean trypsin inhibitor and limabean trypsin inhibitor was considered to represent the plasma kallikrein activity, since the synthetic substrate is also hydrolyzed by other serine proteases, such as plasmin. The residual levels of plasma prekallikrein were expressed as arbitrary units per mg protein after activation. Levels of plasma proteins were determined by the method of Lowry *et al.*, (1951).

Assay of high-molecular-weight and low-molecular-weight kininogens

The residual levels of kininogens were determined by the method reported previously (Uchida & Katori, 1979; Uchida *et al.*, 1986). For high-molecular-weight kininogen, plasma was incubated with glass powder (Ballotini, Jencons, England) in the presence of *o*-phenanthroline. Bradykinin released from high-molecular-weight kininogen was measured by the enzyme immunoassay (Markit-A, Dainippon Pharmaceutical Co, Osaka). For low-molecular-weight kininogen, plasma was incubated with glass powder in the absence of *o*-phenanthroline. The plasma, in which the high-molecular-weight kininogen was consumed, was acidified at pH 2.0 and was incubated with trypsin after neutralization. The bradykinin released was measured by the enzyme immunoassay. The levels of high-molecular-weight and low-molecular-weight kininogens were expressed as the amount, in ng, of bradykinin released per mg of protein.

Endotoxin shock model

Male Sprague-Dawley rats (specific pathogen-free, weighing 270–390 g, obtained from Shizuoka Laboratory Animal Center Hamamatsu), were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.) before experimental procedures were started. Anaesthesia was maintained by occasional intraperitoneal administration of sodium pentobarbitone (25 mg kg^{-1}). The right femoral vein was cannulated with a polyethylene cannula (PE-50, Clay Adams, NJ, U.S.A.). Injection of endotoxin and infusion of soybean trypsin inhibitor were given through this cannula. The mean systemic blood pressure was measured by a pressure transducer (MPU-0.5, Nihon Kohden, Tokyo, Japan) attached to a cannula inserted into the right femoral artery and this parameter was recorded on a polygraph (RM-85, Nihon Kohden, Tokyo, Japan). Respiratory movements were recorded by a force-displacement transducer (SB-1T, Nihon Kohden, Tokyo, Japan). The

heart rate was measured from the ECG using a tachometer (RT-5A, Nihon Kohden, Tokyo, Japan). The blood samples were collected from a polyethylene cannula (PE-60, Clay Adams, New Jersey, U.S.A.) inserted into the left carotid artery. Blood (5 ml) was collected only once from each rat.

After completion of the experimental setup, endotoxin (3–30 mg ml^{-1} saline kg^{-1}), was injected as a single bolus into the right femoral vein. For control studies, only saline (1 ml kg^{-1}) was injected. For inhibition of release of kinins, soybean trypsin inhibitor (5 mg 0.5 ml^{-1} saline 10 min^{-1}) was infused for 20 min into the right femoral vein. Endotoxin was injected 15 min after the start of the infusion of soybean trypsin inhibitor.

Haematocrits of the samples of blood from the carotid artery were determined by a micro-method (Chien *et al.*, 1965).

Measurements of plasma kallikrein activity in blood

One ml of blood from the carotid artery was collected directly into a plastic tube (Falcon 2029) that contained 2.0 ml of the solution of MCA synthetic substrate (Z-Phe-Arg-MCA, 1.0×10^{-4} M in 150 mM phosphate buffer, pH 7.4) and incubated at 37°C for 15 min in the presence of soybean trypsin inhibitor or limabean trypsin inhibitor (Oh-ishi & Katori, 1979). The reaction was terminated by addition of 750 μl of a 20% solution of trichloracetic acid (TCA). After centrifugation (1500 *g*, for 15 min, at 4°C), the supernatant was diluted 10 fold with a 17% solution of acetic acid in order to prevent quenching. The amounts of AMC released were measured fluorometrically (excitation, 380 nm; emission, 436 nm). The difference between the amidolytic activities in the presence of soybean trypsin inhibitor and in the presence of limabean trypsin inhibitor was calculated and taken as the plasma kallikrein activity.

Detection of bradykinin and des-Phe^8-Arg^9-bradykinin in blood by enzyme immunoassay

Five ml of blood from the carotid artery was collected directly into the plastic tubes (Falcon 2099) which contained absolute ethanol in which 10 μM *o*-phenanthroline was dissolved. The mixture was heated at 70°C for 10 min. After centrifugation (1500 *g*, at 4°C, for 15 min), the supernatant was evaporated under reduced pressure. The residue was washed three times with 10 ml of diethylether in order to remove lipids, and it was then dissolved in the assay buffer for the enzyme immunoassay of bradykinin or des-Phe^8-Arg^9-bradykinin. The amounts of bradykinin were determined by a previously developed enzyme immunoassay (Markit-A, Dainippon Pharmaceutical Co., Osaka, Japan) (Uchida *et al.*, 1986), whereas amounts of des-Phe^8-Arg^9-bradykinin were measured by a recently established enzyme immunoassay (Katori *et al.*, 1988; Majima *et al.*, 1988a). Limits of detection for either peptide were 80 pg ml^{-1} blood. The recovery rate was greater than 90% for each peptide, as evaluated from measurements of authentic bradykinin or des-Phe^8-Arg^9-bradykinin added to the blood of normal, healthy rats.

Drugs

Drugs used were: *o*-phenanthroline (Wako Pure Chemical Co., Osaka, Japan); peptidyl fluorogenic substrate, Z-Phe-Arg-MCA (Peptide Institute Inc., Osaka, Japan); soybean trypsin inhibitor and limabean trypsin inhibitor (Worthington Biochemical Co., Freehold, NJ, U.S.A.); trypsin (twice-crystallized, salt-free, from bovine pancreas, Nutritional Biochemical Co., Cleveland, OH, U.S.A.); sodium pentobarbitone (Nembutal, Abbot Labs., North Chicago, IL, U.S.A.); endotoxin (lipopolysaccharide W, from *E. coli*, 0127 : B5, Difco Laboratories, Detroit, Michigan, U.S.A.). Other reagents were all of analytical grade and were obtained from commercial sources.

Statistical analysis

When variances were not heterogeneous, Student's *t*-test was used to evaluate the significance of differences. When variances were heterogeneous, statistical analysis was performed by the Aspin-Welch method or the Wilcoxon's rank sum test.

Results

Activation in vitro *of the plasma kallikrein-kinin system in rat plasma by endotoxin*

As shown in Figure 1b, the incubation of rat plasma with a solution of endotoxin for 30 min generated plasma kallikrein activity in a dose-dependent manner (to levels 2, 15 and 27 times the control value for 3, 5 and 10 mg ml^{-1} endotoxin, respectively). Concomitantly, the residual level of plasma prekallikrein was reduced in a dose-dependent manner (by 23%, 32% and 38% for 3, 5 and 10 mg ml^{-1} endotoxin, respectively) (Figure 1a).

Levels of free bradykinin in the incubation mixture also increased in a dose-dependent manner (to 16 and 23 times the control value for 3 and 10 mg ml^{-1} endotoxin, respectively) in the presence of *o*-phenanthroline (Figure 1d). Concomitantly, the residual level of high-molecular-weight kininogen

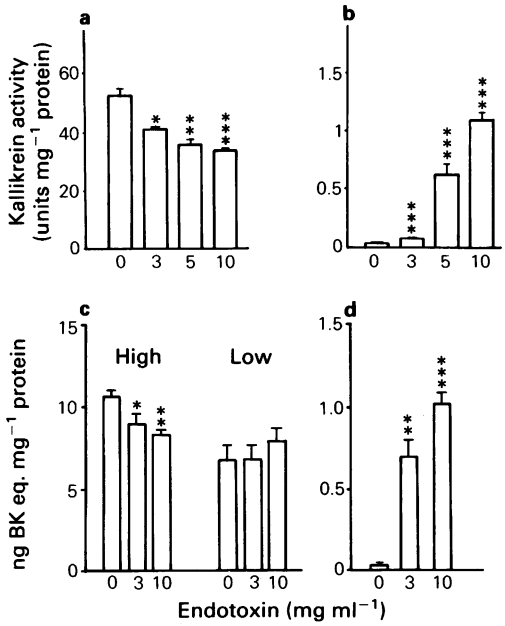

Figure 1 Changes of the levels of the components of the plasma kallikrein-kinin system after incubation of rat plasma with endotoxin *in vitro*. This figure illustrates residual levels of plasma prekallikrein (a), high-molecular-weight (High) and low-molecular-weight (Low) kininogens (c), plasma kallikrein activity (b), and free bradykinin (d). The ordinates in (a) and (b) are arbitrary units mg^{-1} of plasma protein and those in (c) and (d) are ng of bradykinin (BK) mg^{-1} of plasma protein. Levels of endotoxin are final concentrations in the incubation mixture. Each column represents the mean of results of four experiments (vertical bars show s.e. mean) and is compared with an appropriate saline control (no endotoxin). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

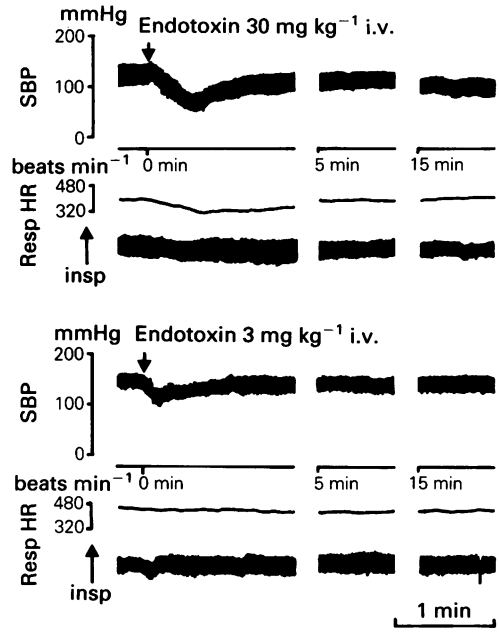

Figure 2 Typical tracings showing the changes in systemic blood pressure (SBP), heart rate (HR) and respiratory movement (Resp) after injection of endotoxin. Insp: direction of record of inspiration. The bar below the trace indicates 1 min.

decreased significantly (by 15% and 22% for 3 and 10 mg ml^{-1} endotoxin, respectively) (Figure 1c). However, the level of low-molecular-weight kininogen was not changed.

These results indicate that endotoxin from *E. coli* caused the release of bradykinin in rat plasma from high-molecular-weight kininogen by the conversion of plasma prekallikrein to the active plasma kallikrein.

Changes in systemic blood pressure induced by the intravenous injection of endotoxin

Figure 2 shows typical tracings of the systemic blood pressure, heart rates and respiratory movements induced by a bolus injection of endotoxin (30 or 3 mg kg^{-1}). When a solution of endotoxin was injected into the femoral vein of rats, the systemic blood pressure fell gradually. The drop in systemic blood pressure caused by injection of 3 or 30 mg kg^{-1} of endotoxin was completely reversed and blood pressure returned to the preinjection level 5 min after the injection of endotoxin.

Figure 3a shows the initial maximum decrease in the mean systemic blood pressure (−26.3, −44.3 and −46.9 mmHg for 3, 10 and 30 mg kg^{-1} endotoxin, respectively). As shown in Figure 3b, the haematocrits of the blood samples, which were collected 15 min after the injection of endotoxin increased in a dose-dependent manner to 104%, 109% and 123% of control values for 3, 10 and 30 mg kg^{-1} endotoxin, respectively. These values suggest that haemoconcentration due to plasma leakage, probably as a result of the formation of bradykinin, was induced in parallel with the decrease in blood pressure.

Changes in the levels of the components of the plasma kallikrein-kinin system after the intravenous injection of endotoxin

As shown in Figure 4b, plasma kallikrein activity was increased to 450% of the saline injected control 1 min after the injection of endotoxin (10 mg kg^{-1}).

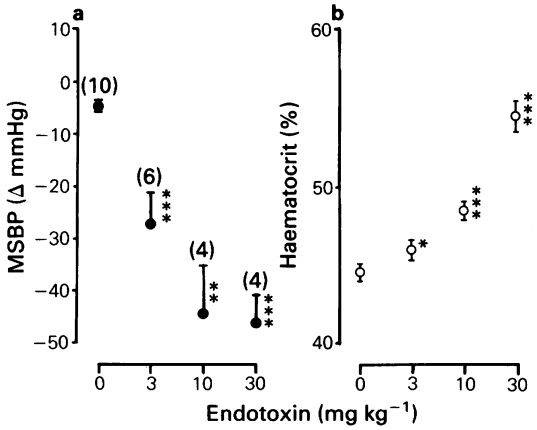

Figure 3 Changes of mean systemic blood pressure (MSBP, a) and haematocrit (%, b) after injection of endotoxin. The ordinate scale in (a) indicates the maximal decrease in mean systemic blood pressure. The numbers in parentheses indicate the numbers of experiments. Each point represents the mean value (s.e.mean shown by vertical bar) and is compared with the value of the saline control (no endotoxin). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

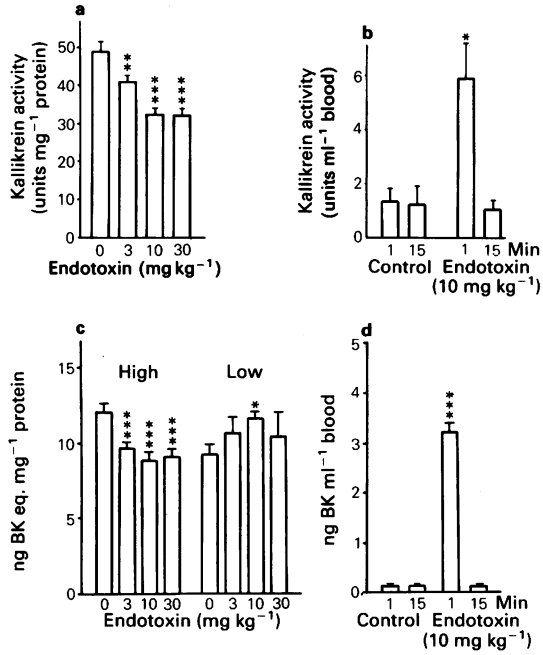

Figure 4 Changes in the levels of the components of the plasma kallikrein-kinin system in the blood collected after injection of endotoxin. This figure illustrates residual levels of plasma prekallikrein at 15 min (a), high-molecular-weight (High) and low-molecular-weight (Low) kininogen at 15 min (c), plasma kallikrein activity (b), and free bradykinin (d). The ordinate scale in (a) shows the arbitrary units mg^{-1} of plasma protein and that in (b) shows arbitrary units ml^{-1} of blood. The ordinate scale in (c) shows ng of bradykinin (BK) mg^{-1} of plasma protein and that in (d) shows ng of bradykinin ml^{-1} of blood. Control indicates the saline-injected group. Each column represents the mean of results from 4–8 experiments (s.e.mean shown by vertical bars) and is compared with the saline control (no endotoxin). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

However, 15 min after the injection of endotoxin, the level of plasma kallikrein returned to that in controls. However, the residual level of plasma prekallikrein in rat plasma remained significantly lower even 15 min after the injection of endotoxin and the extent of the reduction was increased by 17%, 34% and 34% for 3, 10 and 30 mg kg^{-1} endotoxin, as compared to values for controls (no endotoxin) (Figure 4a).

At the same time, free bradykinin was detectable (3.2 ± 0.2 ng ml^{-1} blood) only 1 min after the injection of endotoxin, when there was evidence of activated plasma kallikrein. Fifteen min after the injection of endotoxin, the levels of free bradykinin in the blood had returned to the preinjection level (Figure 4d). The residual level of high-molecular-weight kininogen was significantly reduced by 21%, 25% and 24% with 3, 10 and 30 mg kg^{-1} endotoxin, respectively, and these low levels persisted for at least 15 min after the injection. The residual level of low-molecular-weight kininogen was not reduced (Figure 4c).

These results indicate that, even in this model *in vivo*, the intravenous injection of endotoxin resulted in the activation of the plasma prekallikrein, so that free bradykinin could be detected together with active plasma kallikrein in the blood, but the active forms were found only immediately after the injection of endotoxin.

Detection of des-Phe^8-Arg^9-bradykinin after injection of endotoxin in vivo.

The results on the activation of plasma prekallikrein, with the generation of kinin, were further validated by the detection of des-Phe^8-Arg^9-bradykinin in the blood after intravenous injection of endotoxin.

As shown in Figure 5a, free bradykinin was detected only 1 min after injection of endotoxin (960 ± 450 pg ml^{-1}, $n = 5$). Three minutes later, the level of free kinin had returned to the control level (below 80 pg ml^{-1} blood). In contrast, des-Phe^8-Arg^9-bradykinin could be detected for at least 5 min (Figure 5b). Fifteen minutes later, when blood pressure was restored to normal levels, the level of this

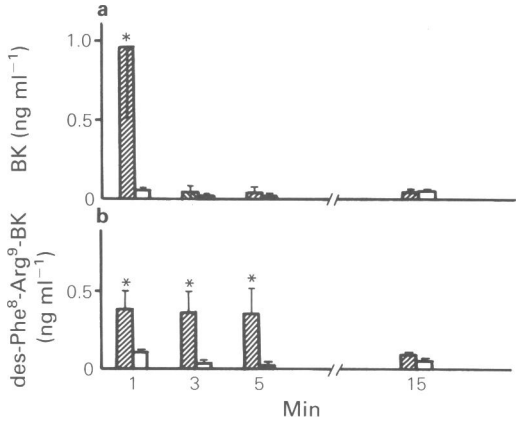

Figure 5 Time course of the increase in the levels of bradykinin (BK) and des-Phe^8-Arg^9-bradykinin (des-Phe^8-Arg^9-BK) in blood after injection of endotoxin. Open columns indicate results from saline-injected (1 ml kg^{-1}) rats and hatched columns indicate those from endotoxin-injected (10 mg kg^{-1}, i.v.) rats. Each column represents the mean of results from 3–6 experiments (s.e.mean shown by vertical bars) and is compared with the results from the saline-injected rats at each time point. $*P < 0.05$.

metabolite did not differ from the control value measured after injection of saline.

This result indicates that des-Phe^8-Arg^9-bradykinin is a long-lasting marker of the release of bradykinin in blood.

Inhibition of the immediate drop in blood pressure by soybean trypsin inhibitor

Intravenous infusion of soybean trypsin inhibitor (10 mg 20 min^{-1} per rat) clearly prevented the immediate hypotension caused by endotoxin, as shown in Figure 6a. The extent of hypotension was significantly reduced from 44.3 mmHg to 4.7 mmHg (mean systemic blood pressure) (Figure 6b). The infusion of this inhibitor also prevented release of bradykinin, as well as of des-Phe^8-Arg^9-bradykinin, in arterial blood as shown from measurements made 1 min and 3 min after the injection of endotoxin (Figure 7).

These results clearly indicate that bradykinin was formed as a result of the intravenous injection of endotoxin and that the immediate hypotension induced by endotoxin may be caused mainly by bradykinin formed.

Discussion

Bradykinin has been reported to have very potent hypotensive effects, to increase vascular permeability, and to heighten pain sensation (Johnson, 1979).

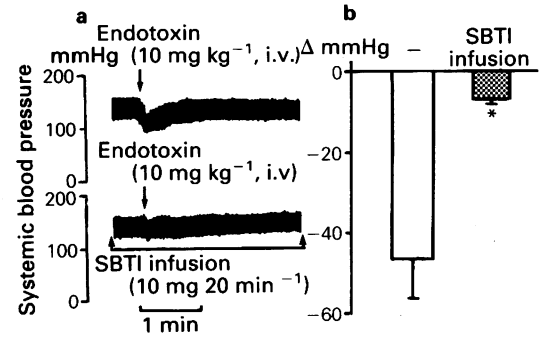

Figure 6 Inhibition of the endotoxin-induced immediate drop in systemic blood pressure by soybean trypsin inhibitor (SBTI): (a) shows typical tracings of changes in the systemic blood pressure. In (b), the immediate drop in the mean systemic pressure is seen to be significantly inhibited ($*P < 0.05$) during infusion of SBTI (stippled column), compared with the systemic pressure during infusion of saline (open column).

Thus, bradykinin has been claimed to be a major cause of hypotension in endotoxin shock (Nies *et al.*, 1968; Sardesai & Rosenberg, 1974; Robinson *et al.*, 1975; Gillimore *et al.*, 1978; Assen *et al.*, 1983; McConn *et al.*, 1983; Högström *et al.*, 1987). However, the evidence presented in previous papers in somewhat incomplete and fragmentary, and, thus, the involvement of bradykinin in endotoxin shock

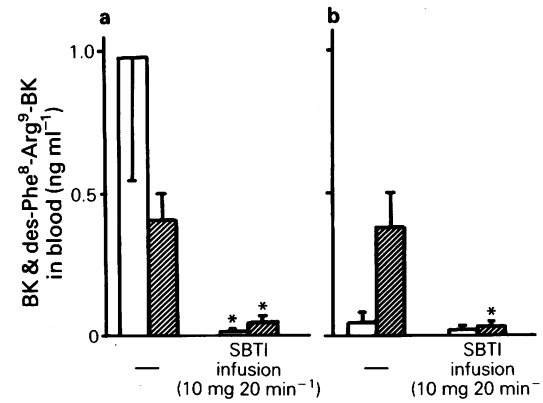

Figure 7 Inhibition of the generation of bradykinin (BK, open column) and des-Phe^8-Arg^9-bradykinin (des-Phe^8-Arg^9-BK, hatched column) in blood after injection of endotoxin (10 mg kg^{-1}) during infusion of soybean trypsin inhibitor (SBTI). The ordinate scale indicates ng of BK and des-Phe^8-Arg^9-BK ml^{-1} blood at 1 (a) and 3 min (b) after injection of endotoxin. Each column represents the mean of results from 3–6 experiments, vertical bars show s.e.mean. Values during infusion of SBTI were compared with those during infusion of saline. $*P < 0.05$.

has been a matter of debate. The controversy can be, in part, attributed to the rapid destruction of and the difficulties in the detection of bradykinin in pathological states. Plasma kallikrein is also readily inactivated by various types of inhibitor in plasma. Our experiments were designed to provide comprehensive and conclusive results. In the present study, free bradykinin and active plasma kallikrein could only be detected 1 min after the injection of endotoxin; 15 min later neither bradykinin nor plasma kallikrein were elevated (Figure 4). In the present experiments, the respective decreases in levels of precursors and products were clearly seen to be mirror images. In our experiment *in vitro*, the incubation of plasma with endotoxin resulted in the consumption of residual prekallikrein and high-molecular-weight kininogen, together with increases in levels of active plasma kallikrein and bradykinin (Figure 1). These mirror-image processes were corroborated *in vivo* after a bolus injection of endotoxin in rats. In this case, low-molecular-weight kininogen was not consumed since plasma kallikrein acts only on high-molecular-weight kininogen *in vivo*. In inflammatory models, a reduction in the residual levels of plasma prekallikrein and high-molecular-weight kininogen, and not of low-molecular-weight kininogen, has been taken as indirect evidence of involvement of bradykinin and interpreted as the activation of plasma kallikrein-kinin (Uchida *et al.*, 1983).

Endotoxin from *E. coli* is a sulphated polysaccharide (Morrison & Cochrane, 1974) and is analogous to carrageenin, which has been reported, by groups including ours, to activate factor XII (Erdös & Miwa, 1968; Morrison & Cochrane, 1974; Uchida *et al.*, 1983). It has been assumed, therefore, that endotoxin from *E. coli* can activate factor XII (Hageman factor) in plasma, resulting in plasma prekallikrein being converted to active plasma kallikrein.

The increase in the level of bradykinin in blood after injection of endotoxin was only transient but this brief generation of bradykinin was detectable via a long-lasting metabolite of bradykinin, des-Phe^8-Arg^9-bradykinin, present in blood for at least 5 min after the injection. We have developed an enzyme immunoassay for this metabolite in order to overcome the difficulties involved in the detection of bradykinin in body fluid (Katori *et al.*, 1988; Majima *et al.*, 1988a). Bradykinin is degraded to des-Arg^9-bradykinin by kininase I and to des-Phe^8-Arg^9-bradykinin by kininase II in rats and man (Majima *et al.*, 1988a). Since des-Phe^8-Arg^9-bradykinin is the first major degradation product that can be detected in rat plasma, it can be used as a marker for the previous formation of the kinin in pathological states; large amounts of this metabolite can be detected in pleural exudates in carrageenin-induced and kaolin-induced pleurisy in rats (Katori *et al.*, 1988; Majima *et al.*, 1988a, b; Majima *et al.*, 1989). Thus, the detection of des-Phe^8-Arg^9-bradykinin provides a useful tool for verification of the involvement of bradykinin in pathological states, even in cases of endotoxin shock, when there is rapid destruction of bradykinin. The detection of des-Phe^8-Arg^9-bradykinin in the blood for up to 5 min after injection of endotoxin provides further support for the involvement of bradykinin in hypotension during endotoxin shock and becomes a good marker for the formation of kinin (Figure 5).

Soybean trypsin inhibitor is a potent inhibitor of plasma kallikrein, but not of glandular kallikrein (Imanari *et al.*, 1974–76). The reduction in amounts of bradykinin as well as of des-Phe^8-Arg^9-bradykinin in the blood to undetectable levels after infusion of soybean trypsin inhibitor provides still further evidence for the generation of bradykinin by endotoxin from *E. coli*. Furthermore, abolition of the immediate endotoxin-induced hypotension by soybean trypsin inhibitor is strong evidence that the newly generated bradykinin plays a major role in this immediate hypotension. Increases in haematocrit after the injection of endotoxin, provide further, if indirect, support for the role of bradykinin in this immediate hypotension, since bradykinin is a potent effector of plasma leakage. Attenuation of the immediate drop in the blood pressure by an antagonist of bradykinin, namely, Lys-Lys-[Hyp^2, Thi^{5,8}, D-Phe^7]-bradykinin (B4148), which was reported by others (Weipert *et al.*, 1988) is further evidence in favour of this conclusion.

The protective effects of indomethacin (Anderson *et al.*, 1975; Katori, 1978) and of a specific antagonist of platelet-activating factor (Terashita *et al.*, 1985; Adnot *et al.*, 1986; Casala-Stenzel, 1987) suggest the possible involvement of prostaglandins and platelet-activating factor in endotoxin shock. However, the protective effects of these agents were implicated by survival ratios (Casala-Stenzel, 1987) or the late phase of a decrease in blood pressure (Anderson *et al.*, 1975; Katori, 1978; Terashita *et al.*, 1985), rather than the immediate decrease examined in our experiments. From our results, we can conclude that bradykinin is, without doubt, released during the very early stages and plays a major role in the immediate development of hypotension after the injection of endotoxin.

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