Comparative study of endotoxin-induced hypotension in kininogen-deficient rats with that in normal rats

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1 The aim of this study was to clarify the role of endogenous bradykinin (BK) in the hypotensive response induced by lipopolysaccharide (LPS) by comparing the degree of hypotension caused by LPS in a strain of specific pathogen-free (SPF) Brown Norway (B/N), kininogen-deficient mutant Katholiek rats with that of B/N normal Kitasato rats.

2 The dose-dependent hypotensive responses caused by intravenous injection of BK ($1\text{--}100\text{ nmol kg}^{-1}$) or platelet-activating factor (PAF, $0.003\text{--}1\text{ }\mu\text{g kg}^{-1}$), were not different in the two strains of rats used. However, there was a strong difference in the hypotensive response induced by LPS in kininogen-deficient and normal rats; in normal rats the hypotensive response was composed of two phases (15 min and 70–80 min after LPS injection), but in kininogen-deficient rats LPS caused a delayed (second phase), but not an acute (first phase) hypotension.

3 We demonstrate that Hoe 140 (1 mg kg^{-1} , i.v.) is a potent, selective, and long-lasting antagonist of the hypotensive effects of BK. Hoe 140 diminished the hypotension caused by LPS in normal rats to the level observed in kininogen-deficient rats, but had no effect on the hypotension caused by LPS in kininogen-deficient rats.

4 TCV309 (0.1 mg kg^{-1} , i.v.) selectively inhibited the hypotension caused by repetitive injection of PAF for up to 180 min. Pretreatment with TCV309 caused a near complete inhibition of the LPS-induced hypotension in kininogen-deficient and normal B/N rats.

5 In the normal rats, dexamethasone (0.5 mg kg^{-1} , i.p.) inhibited the second phase of the hypotension induced by LPS, but not the first phase of the hypotension.

6 A small amount of BK (0.1 nmol kg^{-1}) potentiated the hypotensive action of PAF ($0.01\text{ }\mu\text{g kg}^{-1}$), when they were injected simultaneously.

7 In conclusion, we demonstrate that formation of endogenous BK contributes primarily to the acute, but not to the delayed hypotension afforded by endotoxin in the rat. In contrast, formation of endogenous PAF contributes to both the acute and the delayed hypotension afforded by endotoxin *in vivo*.

Keywords: Endotoxin; kininogen-deficient rats; BK antagonist; PAF antagonist; blood pressure; hypotension

Introduction

Endotoxin (lipopolysaccharide, LPS), present in the outer membrane of gram-negative bacteria, can induce a wide range of systemic or local inflammatory responses, including septic shock (Nishijima *et al.*, 1973). Several animal models have been used to study the pathophysiological mechanisms of septic shock. In these models, the pathochemical alterations comprise of increase in the formation of platelet-activating factor (PAF) (Chang *et al.*, 1987; Dobrowsky *et al.*, 1991), prostaglandins and thromboxane (Kolsterhalfen *et al.*, 1992) as well as a release of cytokines including interleukins and tumour necrosis factor- α (Cannon *et al.*, 1990; Folhe *et al.*, 1991). The involvement of bradykinin (BK) in the pathophysiology of septic shock was indirectly shown by the partial consumption of factors of the kallikrein-kinin system (Nies, *et al.*, 1968; Herman *et al.*, 1974), such as prekallikrein and high-molecular-weight kininogen, and by the detection of BK or breakdown products of BK in the plasma of animals with septic shock (Katori *et al.*, 1989). As some of these mediators including BK (Kontos *et al.*, 1964) and PAF (Sybertz *et al.*, 1984) have potent hypotensive effects, it has been postulated that these mediators contribute to the hypotension caused by endotoxin *in vivo*. However, a rise in plasma BK or PAF following LPS injection to animals does not necessarily mean that these mediators account for the hypotension caused by endotoxin.

Furthermore, the interactions of these mediators during the time course of the LPS-induced hypotension are not well understood and need to be investigated.

Brown Norway (B/N) Katholiek rats are the only strain of rats that genetically lack high-molecular-weight kininogen as well as low-molecular-weight kininogen in their plasma, and also have a very low level of plasma prekallikrein (Damas & Adam 1980; Oh-ishi *et al.*, 1982; 1984; Hayashi *et al.*, 1984; 1992). B/N Katholiek rats originated from the Catholic University of Leuven (Belgium) (Damas & Adam, 1980; Danckwardt *et al.*, 1990), and some of their offsprings were obtained by Caesarean sections for two generations to become specific pathogen-free (SPF) animals. These rats are being maintained at Kitasato University, School of Pharmaceutical Sciences, along with SPF B/N Kitasato rats, which have a normal kallikrein-kinin system (Oh-ishi *et al.*, 1982). Furthermore, the inflammatory response to an inflammatory stimulus, such as carrageenin or kaolin, is significantly smaller in kininogen-deficient rats than in normal rats (Oh-ishi *et al.*, 1986; 1987). Thus, the potential involvement of BK in any pathophysiological event can be investigated by comparing the respective responses of kininogen-deficient rats with those of normal rats.

Recently, a new potent and long-lasting antagonist against BK, Hoe 140 (D-Arg-[Hyp³, Thi⁵, D-Tic⁷, Oic⁸]BK), was reported to attenuate the vasodilator responses elicited by BK when given as a bolus, intravenous injection (Hock *et al.*, 1991; Wirth *et al.*, 1991). Furthermore, a selective antagonist

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against PAF, TCV309 (3-bromo-5-[N-phenyl-N-[2-[[2-(1,2,3,4-tetrahydro-2-isoquinolylcarbonyl-oxy)ethyl]carbonyl]ethyl] carbamoyl]1-propylpyridinium nitrate), was also reported to exert beneficial haemodynamic effects in rodent (Terashita *et al.*, 1991) and canine (Yamanaka *et al.*, 1993) models of septic shock.

This study was designed to elucidate the potential role and relative contribution of BK to the hypotension caused by endotoxin *in vivo* by comparing the hypotension afforded by LPS in kininogen-deficient rats with those caused by LPS in normal rats. In addition, we have investigated the differences in the degree of hypotension caused by LPS in the presence or absence of antagonists of BK or PAF in kininogen-deficient rats and normal rats in order to elucidate the relative contribution as well as the potential interaction of BK and PAF in the hypotension elicited by endotoxin in the anaesthetized rat.

Methods

Measurement of systemic blood pressure

In this study, male SPF Sprague-Dawley (SD) rats (300–340 g, Nihon SLC Co., Hamamatsu, Japan) and male SPF B/N rats (300–350 g), genetically kininogen-deficient Katholiek rats and normal kitasato rats, were used for the measurement of systemic blood pressure. These rats were anaesthetized with pentobarbitone sodium (50 mg kg⁻¹, i.p.). Supplementary doses (15 mg kg⁻¹, i.p.) of anaesthetic were given as needed, to maintain a uniform level of anaesthesia. There was no difference between the strains of rats with respect to the amount of supplementary anaesthesia needed. The right femoral artery was cannulated with a polyethylene catheter (Hibiki No. 3) filled with physiological saline containing heparin (100 units ml⁻¹) to measure arterial blood pressure with a transducer (model TP 200T, Nihon Koden, Tokyo, Japan). The right femoral vein was also cannulated to enable the intravenous bolus injection of drugs. The systemic blood pressure was recorded on a polygraph system (model RM6000, Nihon Koden); and the heart rate, calculated from the ECG, was also monitored.

The drugs (in a volume of 0.1 ml per 100 g body weight; followed by 0.3 ml of sterile physiological saline to wash out the cannula) were administered following stabilization of the blood pressure and heart rate, which was achieved within 20–30 min after completion of the surgical intervention. The haemodynamic effects of LPS were expressed as the difference in the systolic, mean, and diastolic systemic blood pressure (SBP, MBP, and DBP, respectively) from the respective values just before the injection of LPS.

Experimental protocol

The first series of experiments was designed to examine the effect, specificity and duration of action of each of the two antagonists used in this study, which were Hoe 140 (1 mg kg⁻¹, i.v.), a potent BK₂ receptor antagonist, and TCV309 (0.1 mg kg⁻¹, i.v.), a selective PAF receptor antagonist. Each antagonist was tested by measuring its effect on the hypotensive responses to BK (3 or 10 nmol kg⁻¹, i.v.) and PAF (0.01 or 0.03 µg kg⁻¹, i.v.). BK or PAF was injected repeatedly 30 min before and every 30 min for 150 min after injection of each antagonist.

The second series of experiments was performed to estimate the interaction of BK and PAF on hypotension. The hypotensive response induced by simultaneous injection of BK (0.1 nmol kg⁻¹) and PAF (0.01 µg kg⁻¹) was compared with that induced by the same dose of BK or PAF alone.

The third series of experiments was performed to elucidate the involvement of BK in the hypotension induced by LPS (10 mg kg⁻¹, i.v.). The hypotensive effect of LPS in the B/N

kininogen-deficient Katholiek rats was compared with that in B/N normal Kitasato rats by observing both responses over a 120 min period after injection of LPS.

The fourth series of experiments examined the effects of the antagonists against the hypotensive response induced by LPS (10 mg kg⁻¹, i.v.) in both B/N kininogen-deficient and normal rats. Hoe 140 (1 mg kg⁻¹, i.v.) and TCV309 (0.1 mg kg⁻¹, i.v.) were administered 30 min before injection of LPS.

The fifth series of experiments tested the effect of dexamethasone (0.5 mg kg⁻¹, i.p.) on the hypotension induced by LPS (10 mg kg⁻¹, i.v.) in B/N normal rats. Dexamethasone was administered 2 h before injection of LPS.

Drugs

Nembutal (pentobarbitone sodium, 50 mg ml⁻¹) was purchased from Abbott Lab., North-Chicago, IL, U.S.A., LPS (*E. coli* 055:B5) was from Difco Lab., Detroit, MI, U.S.A. and was dissolved in pyrogen-free sterile physiological saline (Otsuka Pharmaceut. Co., Tokushima, Japan) at a concentration of 10 mg ml⁻¹ just before use. BK (Peptide Institute Co., Osaka, Japan) was kept as a stock solution of 100 nmol ml⁻¹ in sterile saline at -20°C until used. PAF (Funakoshi Co., Tokyo, Japan) was dissolved in filtered (0.45 µm) Tyrode solution containing 0.25% bovine serum albumin (BSA, fraction V, Sigma Chemical Co., St. Louis, MO, U.S.A.).

Hoe 140 was made available from Hoechst AG (Frankfurt, Germany). TCV309 was a generous gift from Takeda Pharmaceutical Ind., Osaka, Japan. These antagonists were dissolved in pyrogen-free sterile saline just before use.

Dexamethasone (Sigma Chemical Co., St. Louis, MO, U.S.A.) was suspended in 1% carboxymethylcellulose at a concentration of 0.5 mg ml⁻¹.

Statistical analysis

Values are presented as the means ± s.e.mean of the indicated number of animals. Statistical significance of differences between means was calculated by two-way analysis of variance followed by Student's *t* test or with paired *t* test where indicated. Probability values (*P*) of less than 0.05 were considered significant.

Results

Dose-response curves to BK and PAF in B/N rats

In SD rats, intravenous injections of BK (10 nmol kg⁻¹) or PAF (0.03 µg kg⁻¹) caused a substantial fall in systolic blood pressure of -46 ± 2 mmHg (*n* = 3) and -47 ± 3 mmHg (*n* = 3), respectively. Even when the injections of these two agents were repeated every 30 min, the degree of hypotension induced by either BK or PAF did not change throughout the 3.5 h observation period.

As shown in Figure 1, BK (1–100 nmol kg⁻¹) or PAF (0.003–1 µg kg⁻¹) caused dose-dependent hypotension in both strains of B/N rats, kininogen-deficient Katholiek rats and normal Kitasato rats. There were no significant differences between the hypotensive responses elicited by BK or PAF in either kininogen-deficient and normal rats.

Duration of the actions of antagonists and their specificities

When rats were pretreated with Hoe 140 (1 mg kg⁻¹, i.v.), the hypotension induced by BK (3 or 10 nmol kg⁻¹) when given immediately after injection of Hoe 140 was almost abolished, and this inhibition lasted for 150 min (Figure 2). However, the hypotension afforded by PAF was not influenced by Hoe 140.

The specificity of TCV309 as an inhibitor of the responses of PAF and the duration of this inhibition are also depicted in Figure 2. The hypotensive effects induced by PAF ($0.01\text{--}0.03\text{ }\mu\text{g kg}^{-1}$) were abolished for 150 min by treatment of rats with TCV309 (0.1 mg kg^{-1} , i.v.), while the hypotension induced by BK was not affected.

Potential of the action of PAF by BK

Injection of a small dose of BK (0.1 nmol kg^{-1} , i.v.) into five rats caused no significant hypotension ($-1.8 \pm 2.0\text{ mmHg}$), whereas PAF ($0.01\text{ }\mu\text{g kg}^{-1}$, i.v.) induced a considerable hypotension ($-27.0 \pm 2.0\text{ mmHg}$). However, when these doses of BK and PAF were administered simultaneously to the same rat, the hypotensive response was potentiated; i.e., the response to simultaneous injection of BK and PAF ($-38.6 \pm 1.8\text{ mmHg}$) was significantly ($P < 0.05$, paired *t* test) larger than the sum of the responses to BK alone and PAF alone (Table 1).

Difference between normal and kininogen-deficient B/N rats in hypotension induced by LPS.

The systolic and diastolic arterial blood pressures and the heart rate of B/N normal rats ($n = 16$) before injection of LPS were $138 \pm 8\text{ mmHg}$ (SBP), $95 \pm 5\text{ mmHg}$ (DBP) and $363 \pm 10\text{ beats min}^{-1}$ (HR), respectively. In B/N kininogen-deficient rats ($n = 7$), the respective control values were $147 \pm 9\text{ mmHg}$ (SBP), $101 \pm 5\text{ mmHg}$ (DBP), and $359 \pm 9\text{ beats min}^{-1}$ (HR). Thus, prior to starting the experiment (baseline), there were no significant differences in any of the haemodynamic parameters measured between the two strains of rats used.

LPS (10 mg kg^{-1} , i.v.) induced a hypotension in both B/N normal and kininogen-deficient rats. However, there was a difference in the time-course of the hypotensive response of kininogen-deficient rats from that of the normal rats. When LPS was injected into B/N normal rats (Figure 3), a severe biphasic hypotension was observed. An initial hypotension (first phase) was maximal at 15 min after LPS injection and was $-64 \pm 7\text{ mmHg}$ for SBP and $-46 \pm 5\text{ mmHg}$ for DBP; thereafter the blood pressure partially recovered and subsequently fell again (second phase) to $-50 \pm 4\text{ mmHg}$ for SBP and to $-47 \pm 4\text{ mmHg}$ for DBP at 70 min after LPS injection. In contrast, in kininogen-deficient rats, injection of LPS did not cause a substantial, immediate hypotension (first phase). Thus, the fall in blood pressure caused by endotoxin in kininogen-deficient rats within 15 min (acute phase) (at 15 min; $-15 \pm 4\text{ mmHg}$ for SBP and $-10 \pm 2\text{ mmHg}$ for DBP) was significantly ($P < 0.01$) smaller than the fall in blood pressure caused by LPS in normal rats. In kininogen-

deficient rats which had received endotoxin, there was, however, a secondary fall in blood pressure which was maximal after 70 min. This secondary fall in blood pressure elicited by LPS (70 min) was, however, still significantly ($P < 0.01$) smaller in kininogen-deficient rats than in normal rats. How-

Figure 2 The specificities and duration of action of Hoe 140 and TCV309 in inhibiting the hypotensive responses induced by repeated injections of bradykinin (BK) and platelet activating factor (PAF) into SD rats. Panels (a) and (b) show the effects of Hoe 140 (1 mg kg^{-1}) and TCV309 (0.1 mg kg^{-1}), respectively. These antagonists were injected at 0 min indicated on the abscissa scale, which shows the time course of the experiments. BK (3 nmol kg^{-1}) (open columns) and 10 nmol kg^{-1} (solid columns) and PAF $0.01\text{ }\mu\text{g kg}^{-1}$ (stippled columns) and $0.03\text{ }\mu\text{g kg}^{-1}$ (hatched columns) were injected intravenously 30 min before and every 30 min after the treatment with the antagonist. The ordinate scale indicates the change in mean arterial blood pressure (MAP) induced by injections of BK and PAF. Each column shows the mean value with s.e.mean obtained from 3 rats.

Figure 1 The dose-response curves to bradykinin (BK) (a) and platelet-activating factor (PAF) (b) in kininogen-deficient Katholiek rats (●) and normal Kitasato rats (○) of Brown Norway stain rats. BK ($1\text{--}100\text{ nmol kg}^{-1}$) and PAF ($0.003\text{--}1\text{ }\mu\text{g kg}^{-1}$) were injected intravenously into the right femoral vein. Each point shows the mean value with s.e.mean ($n = 3$) of the maximal difference from the mean arterial blood pressure (MAP) just before injection of BK or PAF.

Table 1 Interaction of bradykinin (BK) and platelet activating factor (PAF) in the hypotensive response

Rat No.	BK alone	PAF alone	BK plus PAF
1	+4.3	-24.9	-34.4
2	-0.0	-32.0	-43.5
3	-7.0	-21.2	-38.0
4	-5.6	-30.9	-41.6
5	-0.3	-26.2	-35.3
Mean	-1.8	-27.0	-38.6
s.e.mean	2.0	2.0	1.8

The values indicate the change (mmHg) in arterial mean blood pressure in five individual rats induced by injections of BK (0.1 nmol kg^{-1}) alone, of PAF ($0.01\text{ }\mu\text{g kg}^{-1}$) alone and by simultaneous injection of BK and PAF (BK plus PAF), respectively. When calculated with paired *t* test, the values obtained in the experiment with simultaneous injection of BK and PAF were significantly ($P < 0.05$) different from the sum of the values of BK alone and PAF alone.

ever, by 120 min, the fall in blood pressure in kininogen-deficient rats was almost the same as that in normal rats.

Effect of Hoe 140 on the hypotension induced by LPS in B/N rats

In Figure 3, the effect of pretreatment of B/N rats with Hoe 140 (10 mg kg^{-1} , i.v.) at 30 min prior to LPS injection is indicated. In this series of experiments, the values of blood pressure before LPS injection were $136 \pm 10 \text{ mmHg}$ for SBP

and $95 \pm 8 \text{ mmHg}$ for DBP in normal rats ($n = 4$) and $145 \pm 3 \text{ mmHg}$ for SBP and $101 \pm 8 \text{ mmHg}$ for DBP in kininogen-deficient rats ($n = 4$).

In normal rats, the treatment with Hoe 140 abolished the first phase of hypotension induced by LPS ($P < 0.001$, Figure 3a). At 70 min (second phase), the falls in both systolic and diastolic blood pressure were also significantly ($P < 0.05$) diminished. However, the blood pressure values at 120 min in rats with Hoe 140-treatment were identical to those observed in rats without Hoe 140-treatment. Thus, the time course of

Figure 3 Hypotensive responses induced by lipopolysaccharide (LPS, 10 mg kg^{-1} , i.v.) in Brown Norway normal rats (circles in a) and kininogen-deficient Katholiek rats (triangles in b) and the effects of Hoe 140 (1 mg kg^{-1} , closed symbols). The responses of control experiments in both B/N rats are shown with open symbols. The antagonist was administered intravenously 30 min before injection of LPS. The figures on the abscissa scale indicate the time (min) after injection of LPS. The upper curves of each panel indicate the changes in the systolic blood pressure (ΔSP) and the lower ones show the changes in the diastolic blood pressure (ΔDP). Each point ($n = 4-16$) represents the mean with s.e.mean.

Figure 4 Hypotensive responses induced by lipopolysaccharide (LPS, 10 mg kg^{-1} , i.v.) in Brown Norway normal rats (circles in a) and kininogen-deficient Katholiek rats (triangles in b) and the effects of TCV309 (0.1 mg kg^{-1} , closed symbols). The responses of control experiments in both B/N rats are superimposed with open symbols. The antagonist was administered intravenously 30 min before injection of LPS. The figures on the abscissa scale represent the time (min) after injection of LPS. The upper curves of each panel indicate the changes in the systolic blood pressure (ΔSP) and the lower ones show the changes in the diastolic blood pressure (ΔDP). Each point ($n = 5-16$) represents the mean with s.e.mean.

Figure 5 The effect of dexamethasone (0.5 mg kg^{-1} , i.p., ●) on the hypotension induced by lipopolysaccharide (LPS, 10 mg kg^{-1} , i.v.) in Brown Norway normal Kitasato rats. The responses obtained in control experiments with B/N rats are shown (○). Dexamethasone was administered 2 h before injection of LPS. The figures on the abscissa scale represent the time (min) after injection of LPS. The curves in (a) show changes in the systolic blood pressure (ΔSP) and in (b) the changes in the diastolic blood pressure (ΔDP). Each point ($n = 5-16$) represents the mean with s.e.mean.

the hypotension induced by LPS in normal rats treated with Hoe 140 showed the same pattern as that of kininogen-deficient rats which had not received Hoe 140.

In contrast, Hoe 140 had no significant effect on the hypotension caused by LPS in kininogen-deficient rats (Figure 3b).

Inhibitory effect of TCV309 on the hypotension induced by LPS in B/N rats

The baseline blood pressure values of B/N rats before injection of LPS in this experiment were not significantly different from those observed in the other experiments. Pretreatment with TCV 309 (0.1 mg kg^{-1} , i.v.) inhibited the hypotension induced by LPS in both strains of B/N rats (Figure 4).

The effect of dexamethasone on the hypotension induced by LPS in B/N rats

In Figure 5, the effect of pretreatment of B/N normal rats with dexamethasone (0.5 mg kg^{-1} , i.p.) at 2 h prior to injection of LPS is indicated. Dexamethasone inhibited the second phase, but not the first (acute) phase of hypotension induced by LPS.

Discussion

We demonstrate here that the acute hypotension elicited by endotoxin in kininogen-deficient Katholiek rats was significantly smaller than the hypotension caused by endotoxin in normal Kitasato rats, although the hypotension elicited by BK in these two strains of rats was similar. Thus, an enhanced formation of BK in response to endotoxin contributes to the acute hypotension (at 15 min), but only to

a minor extent to the delayed hypotension (second phase) observed between 70 and 120 min after injection of LPS.

The dose of Hoe 140 used in this study (1 mg kg^{-1} , i.v.) was sufficient to prevent the hypotension caused by BK during the 150 min observation period, but was not effective against the hypotension elicited by PAF. Similarly, Hoe 140 attenuates the bronchoconstriction caused by BK, but not by PAF (Sakamoto *et al.*, 1992). However, pretreatment with this dose of Hoe 140 did not affect the hypotension caused by LPS in kininogen-deficient rats. This result supports the notion that (i) the plasma kallikrein-kinin system in this species cannot be activated by LPS due to a lack of kininogens in plasma; and that (ii) BK is not involved in the hypotension caused by LPS in kininogen-deficient rats. On the other hand, the hypotensive response caused by LPS in normal rats was attenuated by Hoe 140 to the level of kininogen-deficient rats. As Hoe 140 is a competitive as well as non-competitive antagonist of BK_2 receptors (Griesbacher & Lembeck, 1992), our results in normal rats support previous work demonstrating that other competitive BK antagonists cause a partial inhibition of the hypotension induced by LPS in rats (Weipert *et al.*, 1988; Wilson *et al.*, 1989). Comparing the hypotension produced by LPS in kininogen-deficient rats with that in normal rats and the effect of Hoe 140 in both rats, our results demonstrate that an enhanced formation of endogenous BK contributes primarily to the acute, but not or to a much lesser degree to the delayed hypotension afforded by LPS in the rat. This conclusion is in line with previous reports demonstrating that enhanced plasma levels of BK can be demonstrated within minutes after injection of endotoxin in the anaesthetized rat (Katori *et al.*, 1989).

TCV309 (0.1 mg kg^{-1} , i.v.) blocked the hypotension induced by PAF, but not that induced by BK. Pretreatment with TCV309 almost completely inhibited the hypotension caused by LPS in both strains of B/N rats for over 120 min from the time just after injection of LPS. Thus, we propose that endogenous PAF makes an important contribution to the biphasic hypotension caused by LPS in the rat.

In addition, we demonstrate that BK and PAF synergise to cause hypotension in the rat, for a small dose of BK (which did not cause a significant fall in blood pressure) potentiated the hypotension caused by PAF. Thus, it is possible that trace amounts of BK released in response to LPS interact with endogenous PAF to cause the observed fall in blood pressure elicited by endotoxin.

We found that dexamethasone inhibited the second phase of the hypotension induced by LPS, but not the first phase. Dexamethasone is known to inhibit the induction of NO synthase (NOS, Knowles *et al.*, 1990; Radomski *et al.*, 1990) and of the inducible isoform of cyclo-oxygenase (DeWitt & Meade, 1993). Inhibition of NOS activity attenuates the hypotension induced by PAF and LPS in dogs (Kilbourn *et al.*, 1990; Takekoshi *et al.*, 1993), and the LPS-induced hypotension in the rat (Thiemermann & Vane, 1990). There is evidence that the enhanced formation of NO elicited by LPS in endothelial cells (Salvemini *et al.*, 1990) as well as the acute hypotension caused by endotoxin in the rat (Thiemermann & Vane, 1990; Szabo *et al.*, 1993a) is secondary to the release of endogenous BK (from endothelial cells) which is known to activate the constitutive NOS present in the vascular endothelium (Fleming *et al.*, 1992). PAF also activates the constitutive NOS in the rat thoracic aorta (Moritoki *et al.*, 1992) and endogenous PAF contributes to the induction of NOS caused by LPS *in vivo* (Szabo *et al.*, 1993b). PAF also activates the inducible prostaglandin synthase gene (Bazan *et al.*, 1994). Thus, the acute hypotension afforded by LPS in the rat is due to the release of BK and PAF, which in turn activate the constitutive NOS. The delayed hypotension (the second phase) elicited by LPS might be explained by the induction by LPS of NO synthase and/or cyclo-oxygenase; which is prevented by dexamethasone.

In conclusion, we demonstrate that an enhanced formation

of endogenous BK contributes primarily to the acute, but not to the delayed hypotension afforded by endotoxin in the rat. In contrast, an enhanced formation of endogenous PAF contributes to both the acute and the delayed hypotension afforded by endotoxin *in vivo*.

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