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Attenuation of arterial blood pressure fall in endotoxin shock in the rat using the competitive bradykinin antagonist Lys-Lys-[Hyp^2, Thi^{5,8}, DPhe^7]-Bk (B4148)

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The selective competitive bradykinin (Bk) antagonist, B4148 (Lys-Lys-[Hyp^2, Thi^{5,8}, DPhe^7]-Bk) infused at 100 $\mu g\,kg^{-1}\,min^{-1}$ into rats produced a significant inhibition of the hypotensive effect of Bk and had no effect against acetylcholine-induced responses. In a rat model of endotoxin shock, the fall in mean arterial blood pressure in response to an intravenous injection of lipopolysaccharide from *E. coli* was significantly attenuated by the same infusion of B4148 compared to controls. These findings suggest that kinins are involved in the hypotensive response to endotoxin shock in rats. The development of potent Bk antagonists offers a new experimental approach for evaluating the role of kinins in this and other disease states and potential therapy in such disorders.

Introduction Several studies have demonstrated that the fatal outcome in patients with severe septicaemia is accompanied by activation of the kallikrein-kinin system (Aasen *et al.*, 1983, McConn *et al.*, 1983). Similarly, in rat models of bacterial- (Högstrom *et al.*, 1987; McConn *et al.*, 1983) or endotoxin-induced (Kühne *et al.*, 1985) abdominal sepsis, decreased plasma prekallikrein and kininogen levels are seen which suggests that bradykinin (Bk) is also involved in the pathophysiology of septic shock in this species.

This study investigates the effects of the competitive selective Bk receptor antagonist B4148 (Whalley *et al.*, 1987) on the hypotensive response produced by the intravenous administration of endotoxin.

Methods Compound B4148 (Lys-Lys-Arg-Hyp-Pro-Gly-Thi-Ser-DPhe-Thi-Arg.TFA; Hyp = L-4-hydroxyproline; Thi = β-(2-thienyl)-L-alanine; TFA = trifluoracetic acid) was synthesized by R.J. Vavrek and J.M. Stewart, Denver, Colorado, U.S.A.

Male Sprague Dawley rats (Ivanovas, Kisslegg, FRG, 180–200 g) were fasted overnight and anaesthetized with pentobarbitone (40 mg kg^{-1}, i.p.). Polyethylene catheters (PE-50) were inserted into the carotid artery and into the external jugular vein. Arterial blood pressure was measured continuously with a Bently Trantec Model 800 transducer, an amplifier (Siemens, Sirecust 404, FRG) and a two channel recorder (Kipp & Zonen, BD 41, Netherlands).

Selectivity study The effect of bolus injections of Bk, 1 μg ($n = 6$) and 2.5 μg ($n = 6$) i.v., (bradykinin triacetate salt, Sigma, Munich, FRG) and acetylcholine (ACh), 500 ng ($n = 6$) i.v., (acetylcholine chloride, Merck, Darmstadt, FRG) on arterial blood pressure were studied before and during an infusion of B4148 (100 $\mu g\,kg^{-1}\,min^{-1}$, i.v.). The compound B4148 was dissolved in isotonic saline such that the volume of infusion was 0.1 $ml\,kg^{-1}\,min^{-1}$. The dose of 100 $\mu g\,kg^{-1}\,min^{-1}$ of B4148 was chosen from pilot studies which demonstrated that higher doses (200 $\mu g\,kg^{-1}\,min^{-1}$) produced a fall ($\approx$25 mmHg) of mean arterial pressure. The effect of B4148 against Bk and ACh was expressed as percentage inhibition of the maximum change in diastolic arterial pressure before and during the infusion of B4148.

Endotoxin shock study After the operation only rats with a MAP between 110 and 130 mmHg were included. For this reason 5 rats were excluded from the study; 14 rats were assigned into two groups: lipopolysaccharide (LPS) + B4148 ($n = 7$) receiving the antagonist B4148 infusion (100 $\mu g\,kg^{-1}\,min^{-1}$ dissolved in 0.1 $ml\,kg^{-1}\,min^{-1}$ isotonic saline) i.v. over 25 min; LPS + saline ($n = 7$) receiving an infusion of 0.1 $ml\,kg^{-1}\,min^{-1}$ isotonic saline. Five minutes after starting the infusions, endotoxin shock (designated time = 0) was induced by an i.v. bolus injection of LPS from *E. coli* (LPS 0111: B_4; Sigma, Munich, FRG) at a dose of 15 $mg\,kg^{-1}$ in a volume of 0.5 $ml\,kg^{-1}$ isotonic saline. MAP was recorded every minute from time −5 to 10 min and again at 15 and 20 min.

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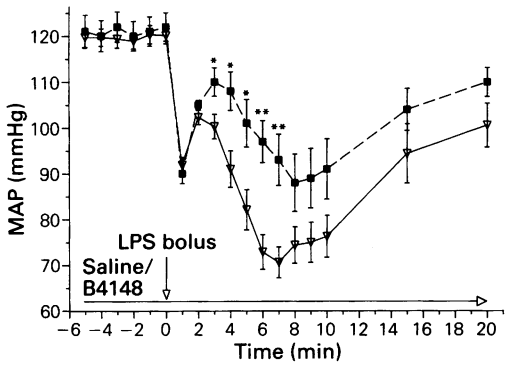

Figure 1 Reduction of lipopolysaccharide (LPS)-induced hypotension by i.v. infusion of the competitive bradykinin antagonist B4148 in rats. Mean arterial blood pressure (MAP) in mmHg is displayed on the ordinate scale (means with s.e. mean shown by straight line). B4148 was infused at a rate of $100\,\mu g\,kg^{-1}\,min^{-1}$ over 25 min starting 5 min before LPS ($n = 7$) (■). LPS was given as a bolus injection ($15\,mg\,kg^{-1}$) at time 0 ($n = 7$). Isotonic saline was infused over 25 min in the LPS + saline group (▽) $^{*}P < 0.05$, $^{**}P < 0.01$ (Student's t test).

Statistics The results are expressed as means ± s.e. mean. Statistical evaluation was made by use of paired (inhibitory capacity and selectivity) and unpaired (endotoxin shock) Student's t tests.

Results Bk at 1 μg and 2.5 μg bolus injection produced changes in diastolic blood pressure (mmHg) of -20 ± 1.3 and 38.7 ± 3.3 respectively in the absence, and -9.0 ± 0.5 ($P = 0.0002$) and -17.7 ± 2.1 ($P = 0.001$) in the presence of B4148 ($100\,\mu g\,kg^{-1}\,min^{-1}$). Responses to ACh (500 ng) were not significantly different in controls (-55.8 ± 2.8 mmHg) compared to B4148-infused animals (-53.7 ± 2.3 mmHg).

Before LPS injection, MAP in saline and B4148-treated animals were not significantly different, being 120.0 ± 1.8 and 122.0 ± 3.1 mmHg respectively (Figure 1). Immediately after injection of LPS a sharp fall of MAP was noted in the LPS + saline group (91.9 ± 1.7 mmHg). Thereafter, MAP recovered towards normal at time 2 min after which time a second less rapid fall in MAP was seen reaching 71.0 ± 3.4 mmHg at 7 min. Thereafter the MAP rose slowly, being 110.0 ± 3.2 mmHg at time 20 min. The effect of LPS injection during the infusion of B4148 was not significantly different from the saline group from time 0 to 2 min. However, the values for MAP from 3 to 7 min were significantly ($P < 0.05$) higher than the saline controls. The MAP remained higher in the B4148 group compared to saline controls from 8 to 20 min, but the values were not significantly different from each other.

Discussion The results from the present study have demonstrated that a single bolus injection of LPS consistently produced a biphasic hypotensive sequence of MAP response in all animals (Figure 1). The first hypotensive phase of the MAP after the injection of LPS was not affected by the administration of B4148, suggesting that kinins are not involved in this early response. Three minutes after the LPS bolus injection a second hypotensive phase of longer duration was seen. During this phase B4148 significantly reduced MAP fall in each animal compared to the LPS and saline group. It can be concluded that kinins are involved in this second hypotensive phase since the same dose of B4148 produced significant inhibition of the hypotensive effect of Bk, without having an effect against ACh responses.

Probably because over 90% of kinins present in the blood stream are inactivated during the first passage through the lung vasculature (Levine *et al.*, 1973) and the difficulties in measuring the concentrations of kinins (McConn *et al.*, 1983), the role of these peptides in human disease has been underestimated for a long time. Yet, minute amounts of kinins may suffice to depress blood pressure (McConn *et al.*, 1983). Therefore, the development of selective, competitive antagonists of Bk (Vavrek & Stewart, 1985) has resulted in a group of compounds that has allowed a more precise evaluation of the physiological and pathological roles and pharmacological actions of kinins (Whalley *et al.*, 1987).

It is not yet known exactly by which mechanisms LPS induces arterial hypotension in endotoxaemia. LPS fractions of *E. coli* were able to activate human prekallikrein *in vitro* (Kalter *et al.*, 1983). In addition, by exposing the negatively charged surfaces, underlying the endothelial cells, LPS can activate the contact-phase system. After such an endothelial lesion, binding and activation of plasma prekallikrein may result in kinin release via limited proteolysis of kininogen (Müller-Esterl & Fritz, 1984).

Our findings let us conclude that kinins are strongly involved in rat endotoxin shock and the use of specific, competitive Bk antagonists provides a promising approach for further investigations.

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References

AASEN, A.O., SMITH-ERICHSEN, N. & AMUNDSEN, E. (1983). Plasma kallikrein-kinin system in septicemia. *Arch. Surg.*, **118**, 343–345.

HÖGSTRÖM, H., CLAESON, G., LARSSON-BACKSTRÖM, C., LUNDBERG, D., WENNGREN, E. & HAGLUND, U. (1987). Septic shock in the rat: activation of plasma proteolytic systems and effect of a kallikrein inhibitor/bradykinin antagonist (S-2411). *Acta Chir. Scand.*, **153**, 161–164.

KALTER, E.S., van DIJK, W.C., TIMMERMAN, A., VERHOEF, J. & BOUMA, B.N. (1983). Activation of purified human prekallikrein triggered by cell wall fractions of *E. coli* and *S. aureus*. *J. Infect. Dis.*, **148**, 682–691.

KÜHNE, H., SCHAPER, U., LEHMANN, B., DASSLER, S. & SCHEUCH, D.W. (1985). Zur Aktivierung des Kallikrein-Kinin Systems durch Endotoxin im experimentellen Schockmodell der Ratte. *Z. Med. Labor. Diagn.*, **26**, 67–70.

LEVINE, B.W., TALAMO, R.C. & KAZEMI, H. (1973). Action and metabolism of bradykinin in dog lung. *J. Appl. Physiol.*, **34**, 821–826.

McCONN, R., WASSERMANN, F. & HABERLAND, G. (1983). The kallikrein-kinin system in the acutely-ill: (A) changes in plasma kininogen in acutely-ill patients. (B) the efficacy of pulmonary clearance of bradykinin. *Adv. Med. Biol.*, **156b**, 1019–1035.

MÜLLER-ESTERL, W. & FRITZ, H. (1984). Human kininogenes and their function in the kallikrein-kinin system. In *Proteases: Potential Role in Health and Disease*. ed. Hörl, W.H. & Heidland, pp. 285–290. A. New York: Plenum Publishing Corp.

VAVREK, R.J. & STEWART, J.M. (1985). Competitive antagonists of bradykinin. *Peptides*, **6**, 161–164.

WHALLEY, E.T., NWATOR, I.A.A., STEWART, J.M. & VAVREK, R.J. (1987). Analysis of the receptors mediating vascular actions of bradykinin. *Naunyn Schmiedebergs Arch. Pharmacol.*, **336**, 430–433.

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