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Delayed myocardial protection induced by endotoxin does not involve kinin B_1-receptors

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1 Endotoxin is known to confer a delayed protection against myocardial infarction. Lipopolysaccharide (LPS) treatment also induces the *de novo* synthesis of kinin B_1-receptors that are not present in normal conditions. The aim of this study was to evaluate whether LPS-induced B_1-receptors are implicated in the reduction of infarct size brought about by LPS.

2 Rabbits were submitted to a 30-min coronary artery occlusion and 3-h reperfusion sequence. Six groups were studied: pretreated or not (control animals) with LPS (5 μg kg^{-1} i.v.) 24 h earlier and treated 15 min before and throughout ischaemia–reperfusion with either the B_1-antagonist R-715 (1 mg kg^{-1} h^{-1}), the B_1-agonist Sar-[D-Phe^8]-des-Arg^9-bradykinin (15 μg kg^{-1} h^{-1}) or vehicle (saline). Infarct size and area at risk were assessed by differential staining and planimetric analysis.

3 The presence of B_1-receptors in LPS-pretreated animals was confirmed by a decrease in mean arterial pressure in response to B_1 stimulation. LPS-pretreatment significantly reduced infarct size ($6.4\pm1.7\%$, of area at risk vs $24.1\pm2.5\%$ in control animals, $P<0.05$). This protection was not modified by B_1-receptor antagonism ($7.4\pm2.2\%$, NS) or stimulation ($5.2\pm1.2\%$, NS). Neither antagonist nor agonist modified infarct size in control animals.

4 In conclusion, these data suggest that LPS-induced myocardial protection in the rabbit is not related to concomitant *de novo* B_1-receptor induction.
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Abbreviations: CTL, control; LPS, lipopolysaccharide; MAP, mean arterial pressure; MLA, monophosphoryl lipid A

Introduction

Endotoxin is known to depress myocardial function but can also induce a delayed myocardial protection against subsequent endotoxic shock (Meng *et al*., 1996) or infarction (Brown *et al*., 1989). In the rabbit, 72 h after bacterial lipopolysaccharide (LPS) treatment, the infarct size induced by a myocardial ischaemia–reperfusion sequence is reduced (Rowland *et al*., 1996). In the rat, pretreatment with LPS decreases the ischaemic insult thus improving cardiac function (Rowland *et al*., 1997) and reducing cell injury (Zacharowski *et al*., 1999), infarct size (Eising *et al*., 1996) and arrhythmia incidence (Song *et al*., 1994). This protection is related to protein synthesis since it is inhibited by cycloheximide (Meng *et al*., 1997). Various protective mechanisms have been proposed such as an increase in antioxidant enzyme activity (Brown *et al*., 1989; Maulik *et al*., 1995), synthesis of heat stress proteins (Rowland *et al*., 1996; Meng *et al*., 1996) and induction of nitric oxide synthase (McKenna *et al*., 1995).

B_1-receptors are not present in normal conditions but are induced by various inflammatory stimuli such as *in vitro* incubation (Regoli *et al*., 1977) and *in vivo* administration of LPS (Regoli *et al*., 1981) or cytokines (deBlois *et al*., 1989). Vascular B_1-receptors are present in the rabbit 5 and 20 h after LPS administration (Regoli *et al*., 1981). Their induction is prevented by pretreatment with protein synthesis inhibitors (deBlois *et al*., 1989). When stimulated, B_1-receptors induce a hypotension due to an endothelial-dependent vasodilation (Regoli *et al*., 1981; Pruneau & Bélichard, 1993). This could lead to improved cardiac function during myocardial ischaemia–reperfusion, especially by counteracting the no-reflow phenomenon. Another cardioprotective mechanism associated with B_1-receptor stimulation could be brought about by their ability to reduce noradrenaline release upon ischaemia–reperfusion (Chahine *et al*., 1993; Feng *et al*., 1997).

Kinins, which are generated during myocardial ischaemia–reperfusion (Pitt *et al*., 1970; Torstila, 1978), are known to participate in ischaemic preconditioning mostly by stimulating B_2-receptors (Brew *et al*., 1995; Parratt *et al*., 1995). However B_1-receptors have been implicated in the early protective effects on endothelial function (Bouchard *et al*., 1998).

The aim of this study was thus to investigate whether B_1-receptors, induced by LPS, could participate to the delayed myocardial protection conferred by this toxin. To evaluate this, we tested the effect of B_1-receptor blockade and stimulation on the development of infarct size in LPS-pretreated rabbits.

Methods

Surgical preparation

This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication No. 85-23, revised 1985).

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Specific pathogen-free male New-Zealand rabbits (ESD, France, $n=42$) weighing 2.6–3.8 kg, were anaesthetized with pentobarbitone sodium (40 mg kg^{-1}) administered *via* the marginal ear vein. Additional doses were given as required. Positive pressure respiration with room air was maintained by a pump (Roche-Kontron 3100S) connected to an endotracheal tube. Ventilation rate and tidal volume were respectively 35 breaths min^{-1} and 30 ml.

The right common carotid artery was cannulated with a polyethylene catheter and the arterial pressure was measured using a pressure transducer (Baxter 33-260, Healthcare Corp., U.K.). The right jugular vein was cannulated for intravenous drug administration. A left thoracotomy was performed at the 4th intercostal space and the pericardium was opened. A 3/0 silk thread was then placed around the first marginal branch of the circumflex artery and passed through a small polyethylene tube.

Heart rate and arterial pressure were continuously recorded on a computer using a data acquisition system (Power Lab, ADInstruments). An intravenous injection of 1000 I.U. of heparin (Choay) was performed at the beginning of the experimental protocol.

Experimental protocol

After the surgical preparation, a 15 min stabilization period was observed. The animals then received the various drug treatments starting 15 min before and throughout a 30-min occlusion and 3-h reperfusion period. Clamping the snare around the artery produced ischaemia; successful ligation was confirmed by myocardial cyanosis with bulging and reperfusion by the appearance of a myocardial blush (Marber *et al*., 1993).

Area at risk and infarct size measurement

At the end of the protocol, the heart was removed for infarct size assessment using a variant of the classic method described by Marber *et al*. (1993). The heart was retrogradly perfused *via* the aorta with a physiological solution adjusted to pH 7.4 containing (in mM): NaCl 118, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.22, KH_2PO_4 1.2, $CaCl_2$ 1.8, glucose 11. The coronary artery was re-occluded and a blue dye (Unisperse blue, Ciba Geigy) was perfused in the non-ischaemic zone. The left ventricle was frozen and cut in 2 mm slicesthat were incubated for 10 min at 37°C in phosphate buffer containing 1% triphenyl tetrazolium chloride (Sigma). The area at risk and infarct size were measured using a computerized planimetric technique (Minichromax, Biolab) and expressed as a percentage of the left ventricle and area at risk, respectively.

Peptide synthesis

Sar-[D-Phe^8]-des-Arg^9-bradykinin and AcLys-[D-βNal^7, Ile^8]-des-Arg^9-bradykinin (R-715) were synthesized with a peptide synthesizer (Applied Biosystems 430 A) using Merrifield-type resins with the first amino acid attached. Amino acids were activated by dicyclohexylcarbodiimide 1-hydroxybenzotriazole (Peptides International) on 1-methyl-2-pyrrolidinone. Peptides were cleaved from the resins with anhydrous hydrogen fluoride in the presence of appropriate scavengers. The resulting peptides were purified by medium-performance reversed-phase (C18) chromatography and if necessary by HPLC. Peptide purity and identity were confirmed by analytical HPLC and by ion-mass spectrometry respectively, as described by Drapeau & Regoli (1988).

Experimental groups

Endotoxin treatment, at a dose (5 μg kg^{-1}) known to induce B_1-receptors in the rabbit (Regoli *et al*., 1981), was produced by injecting LPS (from *E. coli* serotype 0111:B4, Sigma) *via* the marginal ear vein 24 h before the experimental protocol. Six groups of animals ($n=7$ in each) were studied in which control and LPS-pretreated animals were submitted to one of the following treatments administered intravenously at a rate of 5 ml h^{-1} 15 min before and throughout the occlusion–reperfusion period: (1) Saline perfusion; (2) Perfusion with the B_1-receptor antagonist R-715 at a dose of 1 mg kg^{-1} h^{-1} in saline; or (3) Perfusion with the B_1-receptor agonist Sar-[D-Phe^8]-des-Arg^9-bradykinin at a dose of 15 μg kg^{-1} h^{-1} in saline.

Statistical analysis of data

In order to compare data from experimental groups, three- and two-way analyses of variance followed by *post hoc* multiple comparison Tukey tests were performed when normality was respected. When it was not, non-parametric Kruskal-Wallis tests with multiple comparison Dunn's tests and Mann-Whitney Rank Sum tests were used for intra and inter-group analyses respectively (SigmaStat statistical software v.2.0, Jandel Scientifics). Statistical significance was set at $P \leqslant 0.05$.

Results

Haemodynamic measurements

All animals survived the LPS treatment and the ischaemia–reperfusion protocol. They were therefore all included in the study.

Pretreatment with LPS resulted in significantly decreased arterial pressure values before and during ischaemia but not upon reperfusion (Table 1). In LPS-pretreated animals, treatment with the B_1-agonist Sar-[D-Phe^8]-des-Arg^9-bradykinin significantly decreased both mean arterial pressure and heart rate in comparison to control animals (Figure 1A,B). Treatment with the B_1-antagonist R-715 slightly but significantly increased mean arterial pressure in LPS-pretreated

Table 1 Variations of mean arterial pressure before during and after coronary occlusion

		Time after occlusion (min)		*Time after reperfusion* (min)		
MAP (mmHg)	*Pre-Occ*	*1*	*30*	*1*	*60*	*180*
Control ($n=21$)	79±3	63±4#	72±3	67±4	68±4	63±4#
LPS ($n=21$)	55±3*	48±3#*	59±3*	58±3	56±4	51±3#

Mean arterial pressure (MAP) was lower in lipopolysaccharide (LPS)-treated animals (*$P<0.05$ vs Control animals) before (Pre-Occ) and during occlusion. In both groups, MAP values decreased (#$P<0.05$ vs Pre-Occ value) upon occlusion and at the end of the reperfusion period. Data are means±s.e.mean of pooled values from the variuos groups.

animals (Figure 1A). This was however not sufficient to bring the mean arterial pressure back to the control value. Indeed, neither B_1-agonist nor antagonist significantly modified the arterial pressure response to the ischaemia-reperfusion protocol.

Infarct size

There was no significant difference in the area at risk (expressed as a percentage of the left ventricle) of the various experimental groups (Figure 2A).

LPS-pretreatment brought about an important (about 75%) reduction in infarct size (Figure 2B). The administration of B_1-antagonist or agonist did not modify infarct size in either control or LPS-pretreated animals (Figure 2B).

Discussion

The present study demonstrates that kinin B_1-receptors are not implicated in the endotoxin-induced delayed protection against myocardial infarction in the rabbit.

In our experimental conditions, LPS treatment dramatically decreased infarct size in all groups studied. This is in accordance with other studies in the rat (Zacharowski *et al*., 1999) or in the rabbit (Rowland *et al*., 1996). LPS treatment has been shown to induce synthesis of various cell protective proteins such as heat shock proteins (Meng *et al*., 1996), antioxidant enzymes like superoxide dismutase, catalase and glutathione reductase (Maulik *et al*., 1995), inducible nitric oxide synthase (McKenna *et al*., 1995) or cyclo-oxygenase 2 (Breder & Saper, 1996) that could participate in its beneficial effects against myocardial ischaemia–reperfusion injury.

The LPS-pretreatment carried out in this study was effective in inducing B_1-receptors since, in accordance with previous studies, the administration of the B_1-agonist Sar-[D-Phe^8]-des-Arg^9-bradykinin decreased arterial pressure and heart rate in pretreated animals (Audet *et al*., 1997). The absence of B_1-receptors in control animals was confirmed by the lack of response to Sar-[D-Phe^8]-des-Arg^9-bradykinin or to R-715, both highly selective for this receptor (Gobeil *et al*., 1996; Audet *et al*., 1997). The hypertensive response seen in LPS-pretreated animals following B_1-receptor blockade with R-715 suggests a basal stimulation of B_1-receptors by endogenous agonists such as des-Arg^9 derivatives. However, treatment with R-715 was not sufficient to bring the arterial pressure back to its control value. The hypotension observed after endotoxin treatment could rather be due to the vasodilatation associated with induction of nitric oxide synthase (Forfia *et al*., 1998).

We have shown that LPS was able to simultaneously, (1) induce B_1-receptor synthesis and (2) afford a protection against myocardial ischaemia, 24 h after its administration. However, blockade or stimulation of B_1-receptors throughout the ischaemia–reperfusion sequence did not modify infarct size in both LPS-pretreated and control animals. Thus B_1-receptors do not appear to influence the development of cell injury during myocardial ischaemia-reperfusion.

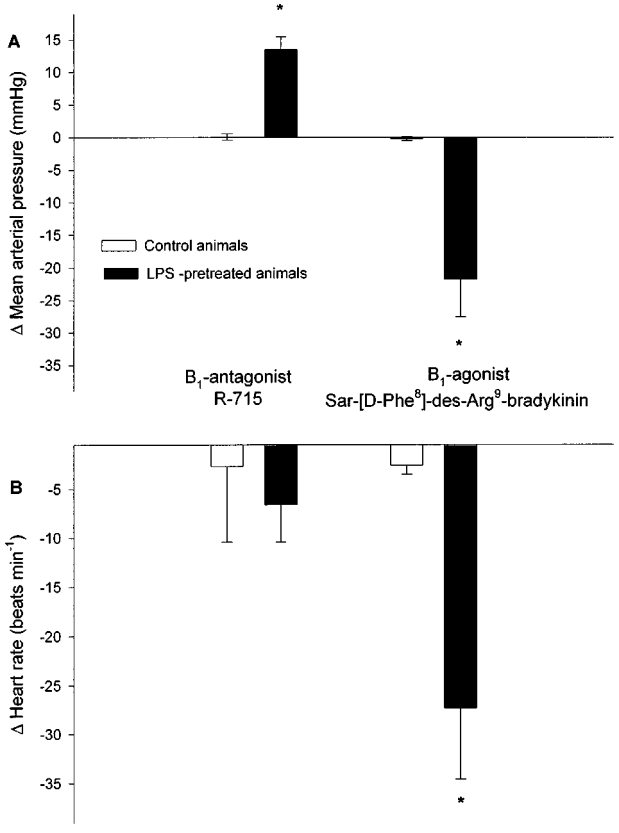

Figure 1 Decreased mean arterial pressure (A) and heart rate (B) (mean ± s.e.mean) 15 min after the beginning of Sar-[D-Phe^8]-des-Arg^9-bradykinin administration in lipopolysaccharide (LPS)-pretreated animals ($n=7$) and increased mean arterial pressure 15 min after R-715 administration. *$P<0.05$ vs corresponding control animals ($n=7$).

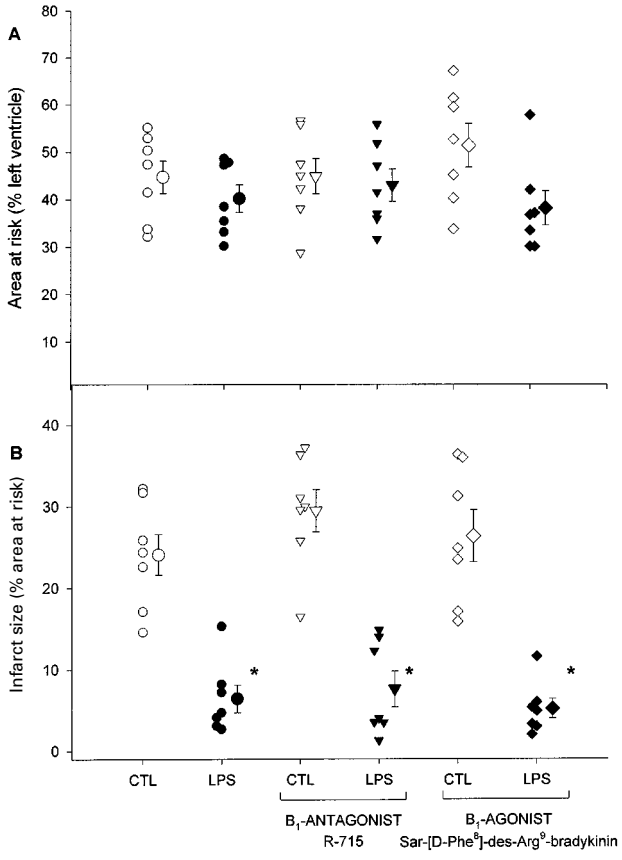

Figure 2 (A) Area at risk (expressed as a percentage of the left ventricle) was identical in all experimental groups. (B) Infarct size (expressed as a percentage of the area at risk) was reduced in the LPS-pretreated animals, independently of B_1-receptor blockade or stimulation. Data are individual values and means ± s.e.mean of CTL = control animals, LPS = lipopolysaccharide-pretreated animals. *$P<0.05$ vs corresponding CTL value.

Monophosphoryl lipid A (MLA) is a derivative of LPS presenting the same protective effects against myocardial ischaemia–reperfusion without other side effects (Elliott, 1998). We have recently demonstrated that MLA fails to induce B_1-receptors 24 h after administration (time at which it is effective for myocardial protection) (Mazenot *et al*., 1999). This is another observation showing that induction of B_1-receptors does not appear to be necessary for this type of cardioprotection. Moreover, a common property of LPS and MLA that could be responsible for their cardioprotective effects is their ability to induce nitric oxide synthase (McKenna *et al*., 1995; Zhao *et al*. 1997). Indeed, inducible nitric oxide synthase appears to be essential for MLA cardioprotection (Xi *et al*., 1999). Finally, the hypotension observed 24 h after LPS pretreatment could, by itself, have cardioprotective actions.

Although B_1-receptors do not appear to affect the development of infarct size, they could present other protective effects in the setting of myocardial ischaemia–reperfusion. Indeed, they have been shown to decrease ischaemia-induced noradrenaline release in the rat (Chahine *et al*., 1993; Feng *et al*., 1997) but not in other species (Hatta *et al*., 1999). This discrepancy could be due to the lack of specific induction of B_1-receptors in both cases. Further studies are thus needed to evaluate the exact role of B_1-receptors in the context of myocardial ischaemia–reperfusion with special emphasis on their specific effects on noradrenaline release or inflammation.

In conclusion, this study provides evidence that B_1-receptors are not involved in LPS-induced protection against myocardial ischaemia–reperfusion injury in the rabbit.

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