Endothelial kinin B_1-receptors are induced by myocardial ischaemia–reperfusion in the rabbit

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1. Kinin B_1-receptors are induced by various inflammatory stimuli. Since myocardial ischaemia–reperfusion results in inflammation, we questioned whether it could induce B_1-receptor-dependent responses to des-Arg^9-bradykinin (DBK).

2. Thirty-six rabbits were submitted either to a 30 min coronary occlusion followed by a 3 h reperfusion or to a sham operation. The response to DBK was then tested *in vivo* on mean arterial pressure (MAP) and *in vitro* on isolated hearts and arterial rings.

3. DBK induced a dose-dependent decrease in MAP in the ischaemia–reperfusion group (DBK, $10\ \mu g\ kg^{-1}$, intra-arterial: -12 ± 2 *vs.* -5 ± 2 mmHg in the sham group, $P < 0.02$), which was significantly antagonised by [Leu^8]-des-Arg^9-bradykinin (LBK), a B_1-receptor antagonist. Following ischaemia–reperfusion, isolated hearts responded to DBK by a decrease in coronary perfusion pressure greater than that of the sham group. DBK dose-dependently decreased the isometric force of isolated carotid rings (DBK, 10^{-5} M: -9 ± 2 *vs.* -1 ± 2 % in the sham group, $P < 0.02$) and mesenteric arteries (DBK, 10^{-6} M: -38 ± 7 % *vs.* -3 ± 2 % in the sham group, $P < 0.001$). The vascular effects of DBK seen after ischaemia–reperfusion were significantly antagonised by LBK. The presence of B_1-receptors in ischaemia–reperfusion animals was confirmed by immunolocalisation and Western blot analysis.

4. This study demonstrates that myocardial ischaemia–reperfusion induces a global induction of functional kinin B_1-receptors in the endothelium.

B_1-receptors are inducible receptors. They were first described by Regoli *et al.* (1977) who showed that large rabbit arteries, which normally do not respond to des-Arg^9-bradykinin (DBK), gradually increased their sensitivity to this B_1-receptor agonist when incubated *in vitro* in normal conditions. These authors also demonstrated that intravenous administration of bacterial lipopolysaccharide (LPS) 5 or 20 h earlier is able to induce hypotension or vasorelaxation in response to DBK by promoting the *de novo* formation of B_1-receptors (Regoli *et al.* 1981). Various inflammatory stimuli are able to induce B_1-receptors (Marceau *et al.* 1997). In particular, cytokines such as interleukin-1 (IL-1) have been shown to enhance the development of the response to DBK (de Blois *et al.* 1988) and could be one of the mediators of the effect of LPS. The induction of B_1-receptors is also enhanced by angiotensin converting enzyme (ACE) inhibition or bradykinin (BK) administration, possibly because of the inflammatory effect of the increase in kinin concentrations (Nwator *et al.* 1989).

Ischaemia–reperfusion is known to induce an inflammatory response that contributes to tissue injury; it is associated with the activation of monocytes and neutrophils, and the production of cytokines (Entman *et al.* 1991). In particular, IL-1 is released by endothelial cells upon hypoxia–reoxygenation (Ala *et al.* 1992), and its mRNA expression is increased in ischaemic myocardium (Herskowitz *et al.* 1995). Also, kinins are released during ischaemia in different species (Hashimoto *et al.* 1978; Matsuki *et al.* 1987; Baumgarten *et al.* 1993) and could contribute to the inflammatory response. Thus, the aim of this study was to investigate whether myocardial ischaemia–reperfusion could induce B_1-receptor-dependent responses. The effect of DBK following myocardial ischaemia–reperfusion was evaluated *in vivo* in anaesthetised rabbits and *in vitro* on isolated rabbit hearts and arterial rings. The presence of cardiovascular B_1-receptors was also confirmed by Western blot analysis and immunohistochemical localisation.

METHODS

Ischaemia–reperfusion model

Surgical preparation

This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1985) and was given local ethical commitee approval.

Male New Zealand White rabbits ($n = 36$) weighing between 2.5 and 3.5 kg were used in this study. They were anaesthetised with intravenous pentobarbital sodium (40 mg kg^{-1}) administered via the marginal ear vein. Additional doses were given as required (10 mg kg^{-1} h^{-1}). Positive pressure respiration with room air was maintained by a pump (Roche-Kontron 3100S) connected to an endotracheal tube. The ventilation rate was 35 breaths min^{-1} and tidal volume was 30 ml. The animals were placed on a heating pad. Sterile solutions were used in order to limit the inflammatory reaction to the surgical preparation.

The right common carotid and right femoral artery for the *in vivo* study were cannulated with polyethylene catheters (internal and external diameter of 1 and 2 mm, respectively) inserted into the aorta. Arterial pressure was measured, using a pressure transducer (Baxter 33-260, Healthcare Corp., UK). 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. Lead II of the surface electrocardiogram, heart rate and arterial pressure were continuously recorded on a computer (Macintosh Power PC) using a data acquisition system (MacLab 8 channels, ADInstruments). Monophasic action potentials were measured with a Franz epicardial electrode (model 225, EP Technologies). An intravenous injection of 2500 i.u. of heparin (Choay) was performed at the beginning of the protocol.

Experimental protocol

After the surgical preparation, rabbits were allowed a 15 min stabilisation period to reach a steady state. Coronary occlusion was produced in the ischaemia–reperfusion group by clamping the snare around the artery. Myocardial ischaemia was confirmed by elevation of the ST segment of the electrocardiogram and by monophasic action potential shortening. If ventricular fibrillation occurred, animals were defibrillated by myocardial massage. After 30 min ischaemia, the ligation was loosened and there was a 3 h reperfusion period. Rabbits from the sham group were submitted to the same surgical and experimental protocol but ischaemia was not performed. All animals survived the experimental protocol and were included in the study. Thereafter, animals from both groups were submitted to either of the following protocols for the assessment of B$_1$-receptors.

B1-receptor assessment

In vivo protocol

The presence of B$_1$-receptors was evaluated *in vivo* by measuring the arterial pressure response to several drug injections. DBK (2.5, 5 and 10 μg kg^{-1}, Bachem) was administered through the femoral artery. The effects of DBK (10 μg kg^{-1}) were also assessed 10 min after the beginning of an intra-arterial perfusion of 10 μg kg^{-1} min^{-1} of [Leu^8]-des-Arg^9-bradykinin (LBK) (Bachem), a B$_1$-antagonist, and 10 min after an intravenous injection of 2 μg kg^{-1} of HOE 140 (Icatiban, Bachem), a B$_2$-antagonist.

Isolated perfused heart protocol

Hearts were removed, placed in cold perfusion medium and rapidly perfused retrogradely in the Langendorff mode at a constant flow using a pump (Minipuls 3, Gilson). The flow was adjusted at the beginning of the experiment to achieve an initial perfusion pressure of 80 mmHg measured by a pressure transducer placed just before the heart. After adjustment, coronary flow was similar in both groups (37 ± 3 *vs.* 41 ± 3 ml min^{-1} in the sham group). The perfusion medium was a modified Krebs-Henseleit solution containing (mM): NaCl 118, NaHCO$_3$ 25, KCl 4.7, MgSO$_4$ 1.22, KH$_2$PO$_4$ 1.2, CaCl$_2$ 1.8, glucose 11, gassed with 95% O$_2$, 5% CO$_2$ and adjusted to pH 7.4. The cardiac temperature was measured continuously by a thermometer probe (model T200KC, Digitron) inserted into the left ventricle and adjusted to 37 °C. A water-filled latex balloon (No. 12. Hugo Sachs) connected to a pressure transducer was placed through the left atrium into the left ventricle, and adjusted to a left ventricular end-diastolic pressure of 10 mmHg. Heart rate (HR) and left ventricular developed pressure (LVDP) were recorded continuously.

After a 15 min stabilisation period, variations in perfusion pressure were measured following increasing concentrations of DBK from 10^{-9} to 10^{-6} M added to the perfusion medium. The heart was then perfused with DBK (10^{-6} M) along with LBK (10^{-6} M) and finally with acetylcholine (10^{-6} M) (Sigma) and sodium nitroprusside (10^{-4} M) (Sigma). All drugs were perfused for 10 min, a time sufficient to attain a stable response.

Thereafter, the coronary artery was re-occluded and the area at risk and infarct size induced by the ischaemia–reperfusion protocol were determined using standard coloration techniques with blue dye (Unisperse blue, Ciba Geigy) and triphenyl tetrazolium chloride (1%, Sigma). The area at risk and infarct size were measured using a computerised planimetric technique (Minichromax, Biolab) and expressed as percentage of left ventricle and area at risk, respectively.

Isolated arteries protocol

Isolated carotid and aortic rings.
Portions of the left carotid artery and thoracic aorta were removed and placed in cold Krebs solution. The arteries were dissected free of connective tissue, cut into 3 mm wide rings and mounted in 10 ml tissue baths containing buffered Krebs solution (mM: NaCl 130, NaHCO$_3$ 14.9, KCl 3.7, CaCl$_2$ 1.6, KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, glucose 11, Hepes 2.38) at 37 °C and gassed with 95% O$_2$ and 5% CO$_2$. In some arteries, the vascular endothelium was rubbed off with a polyethylene tube introduced into the vessel. Changes in isometric force were measured with a transducer (model UF-1, Pioden) and recorded continuously. Carotid and aortic rings were stretched gradually to a passive force of 2 g and 4 g, respectively (tensions at which the response to KCl, 60 mM, was maximal). After a 1 h equilibration period, arterial rings were contracted with phenylephrine (5×10^{-7} to 5×10^{-6} M, Sigma) in order to reach 40–60 % of the maximal contraction. The response to cumulative concentrations of DBK (10^{-8} to 10^{-5} M) was then recorded followed by the effect of LBK (10^{-5} M). Finally, acetylcholine (10^{-6} M) was added to verify the functionality of the endothelium, and sodium nitroprusside (10^{-4} M) to evaluate the maximal vasodilatation.

Isolated mesenteric arteries.
Segments of second-order mesenteric arteries (approx. 200 μm external diameter) were trimmed free of fat and adhering connective tissue, and mounted in a myograph (Marty Technology) containing buffered Krebs solution, according to the technique of Mulvany & Halpern (1977). Isometric force was recorded as previously described. Each vessel was placed under an optimal stretch, equivalent to an *in vivo* arterial blood pressure of approximately 50 mmHg. Vessels were allowed to equilibrate for 30 min. Tissue contractility was then assessed by three exposures to KCl (60 mM). Vessels were contracted with phenylephrine as previously described. The response to cumulative concentrations of DBK (10^{-10} to 10^{-6} M) and the effect of LBK (10^{-5} M), acetylcholine (10^{-6} M) and sodium nitroprusside (10^{-4} M) were then recorded.

Immunological assays

Peptide synthesis and production of anti B₁-receptor antisera.
Peptides derived from the various intracellular (ID) ID2–ID4 and extracellular (ED) ED1–ED3 domains of the human B₁-receptor sequence were synthesised by the solid phase method using fluorenylmethyloxycarbonyl (Fmoc) chemistry (Table 1). The peptides were used for immunisation without prior conjugation to carrier protein except for ENI-17 and SLR-22, which were previously coupled to keyhole limpet haemocyanin by 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (Abd Alla *et al.* 1993). Balb/c mice were immunised using standard procedures. For Western blotting and immunocytochemistry, a mixture of equal volumes from each of the corresponding antisera was used.

Western blot assay. Vessels and heart segments were sampled at the end of the ischaemia–reperfusion protocol as well as 5 h after LPS treatment (10 μg kg^{-1} I.V.) to use as a positive control. The tissues were frozen and pulverised under liquid nitrogen. The powders were re-suspended in ice-cold lysis buffer (20 mM Tris-HCl, pH 7.5, 5 mM EGTA, 150 mM NaCl, 20 mM glycerophosphate, 10 mM NaF, 1 mM sodium orthovanadate, 1% Triton X-100, 0.1% Tween 20, 1 μg ml^{-1} aprotinin, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM *N*-tosyl-L-phenylalanine chloromethyl ketone, 0.5 mM *N*(−)*p*-tosyl-L-lysine chloromethyl ketone) at a ratio of 0.3 ml/10 mg wet weight. Extracts were incubated on ice for 15 min and then centrifuged (12 000 *g*, 15 min, 4°C). The detergent-soluble supernatant fractions were retained.

Lysates were subjected to electrophoresis on a 9% polyacrylamide gel and transferred to nitrocellulose membranes. Membranes were blocked with 10% non-fat dry milk in TBST (20 mM Tris, pH 8.0, 150 mM NaCl, and 0.1% Tween 20) for 20 min and were then incubated with mouse anti-B₁-receptor antibodies at a dilution of 1:2000 in TBST for 1 h. An enhanced chemiluminescence system (ECL plus, Amersham) was used as the detection method. Blots were washed and subjected to autoradiography. Molecular weights of proteins were estimated by using pre-stained markers (Bio-Rad, 161-0324).

Immunohistochemistry analysis. Segments of the aorta, carotid and mesenteric arteries were sampled at the end of the ischaemia–reperfusion protocol. They were mounted in embedding medium (OCT 4583, Sakura), frozen in isopentane previously cooled in liquid nitrogen and stored at −80°C. Transverse cross-sections (7 μm thin) were obtained at −20°C from the frozen embedded segments. Sections were incubated for 30 min at 37°C with the mouse anti-B₁-receptor antibody previously described, and then with an anti-mouse antibody conjugated to biotin (Amersham) which was amplified by streptavidin–Texas red reagent (Amersham). Nuclei were also stained (Hoescht bis-benzidine, Sigma). Fluorescence staining was visualised using a microscope (Leica) equipped with an epifluorescence system. The anti-B₁-receptor antibody was used at a dilution of 1:200. Control experiments were performed in which the second or the first antibody alone were used. In both cases, no autofluorescence was observed.

Statistical analysis of data

Kolmogorov-Smirnov tests (with Lilliefors' correction) were used in order to assess normality prior to statistical analysis. Data were normalised when necessary (perfusion pressure in isolated hearts and isometric force of isolated mesenteric arteries) using the arcsin square root computation. Data from control and ischaemia–reperfusion groups were compared using repeated-measures two-way analyses of variance followed by Tukey's *post hoc* multiple comparison tests (SigmaStat statistical software v. 2.0, SPSS Science). Statistical significance was set at $P \leqslant 0.05$.

Table 1. Synthetic peptides used for the production of antibodies to the B1 receptor

Antigen[a]	Domain[b]	Positions	Conjugation[c]
ELQ-30	ED1	9–38	nil
ENI-17	ED2	95–111	KLH, EDC
RSI-30	ED3	176–205	nil
SQD-27	ID2	132–158	nil
SLR-22	ID3	228–249	KLH, EDC
RFL-29	ID4	303–331	nil

[a] Peptides are identified by their three terminal amino acid residues using the one-letter code, followed by the total number of residues constituting the peptide. [b] ED, extracellular domain; ID, intracellular domain. [c] Unconjugated peptides were used for immunisations except for ENI-17 and SLR-22 which were previously coupled to keyhole limpet haemocyanin (KLH) by 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC).

RESULTS

Myocardial ischaemia–reperfusion

In our experimental conditions, occlusion–reperfusion of the proximal branch of the circumflex artery induced an area at risk of 40 ± 6% of the left ventricle and an infarct size of 45 ± 8% of the area at risk ($n = 5$).

In vivo measurements

At the end of the protocol the mean arterial pressure was 52 ± 4 mmHg in the ischaemia–reperfusion group *vs.* 69 ± 4 mmHg in the sham group ($P < 0.05$); the heart rate was similar in the two groups (284 ± 14 *vs.* 303 ± 18 beats min^{-1}, respectively).

Intra-arterial injection of DBK caused significant hypotension and bradycardia in both groups. The response observed in the ischaemia–reperfusion group was greater than that of the sham group (Fig. 1). The effects observed with DBK (10 μg kg^{-1}) were antagonised by LBK in the ischaemia–reperfusion group only (Fig. 1) but were not affected by HOE 140 (MAP: −12 ± 2 *vs.* −9 ± 3 mmHg; HR: −23 ± 4 *vs.* −25 ± 5 beats min^{-1} before and after HOE 140, respectively). In both groups, LBK did not affect baseline haemodynamic parameters.

In vitro measurements

Isolated hearts

Baseline left ventricular developed pressure was lower in the ischaemia–reperfusion group (73 ± 7 mmHg) than in the sham group (102 ± 10 mmHg, $P < 0.05$). Heart rate was similar in the two experimental groups (162 ± 2 beats min^{-1} in the ischaemia–reperfusion group *vs.* 167 ± 9 beats min^{-1} in the sham group).

Perfusion with DBK caused a concentration-dependent decrease in perfusion pressure (Fig. 2) that was statistically significant in the ischaemia–reperfusion but not in the sham group. LBK tended to reverse the effect

of DBK but this was not significant. The responses to acetylcholine and sodium nitroprusside were the same in both groups (-15 ± 6 *vs.* -17 ± 6 mmHg and -22 ± 4 *vs.* -23 ± 4 mmHg in ischaemia–reperfusion and sham groups, respectively). DBK did not significantly affect heart rate or LVDP.

Isolated aorta, carotid and mesenteric arteries

In both groups, DBK induced a dose-dependent constriction of intact and de-endothelialised aorta that was statistically greater in de-endothelialised rings. LBK significantly inhibited this effect in de-endothelialised rings only (Fig. 3). There was no statistical difference between sham and ischaemia–reperfusion groups. DBK caused a dose-dependent relaxation of intact carotid arteries in the ischaemia–reperfusion but not in the sham group and this was significantly inhibited by LBK (Fig. 4). In both groups, DBK did not affect the isometric force of de-endothelialised carotid arteries.

Finally, DBK caused a dose-dependent relaxation of mesenteric arteries in the ischaemia–reperfusion but not in the sham group (Fig. 5). This effect was significantly antagonised by LBK.

Myocardial ischaemia–reperfusion did not affect the response of any type of artery to acetylcholine or sodium nitroprusside.

Immunolocalisation of cardiovascular B_1-receptors

Western blot analysis allowed us to verify that our antibody was specifically bound to the B_1-receptor protein. Indeed, there was only one band on the autoradiogram and it corresponded to a 58 kDa protein. This band was prominent on lines corresponding to the heart, aorta, carotid and mesenteric arteries from ischaemia–reperfusion and LPS-pre-treated animals. Figure 6 shows a characteristic Western blot obtained with cardiac tissue isolated from the ischaemic zone; this is also representative of all vascular tissues.
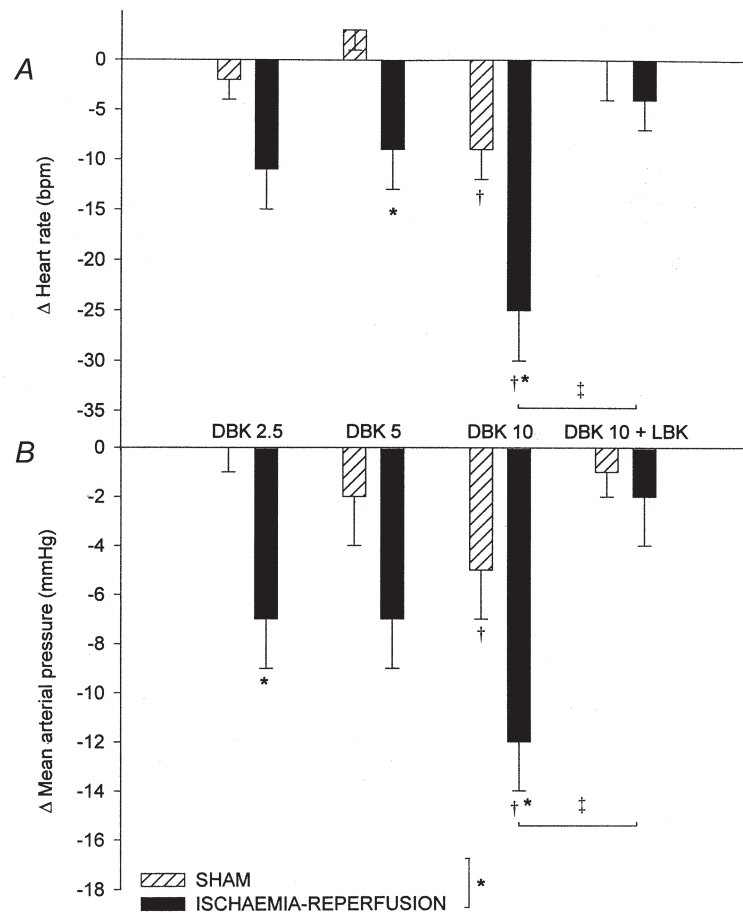

Figure 1

Variation of heart rate (*A*, beats min^{-1}) and mean arterial blood pressure (*B*) (means $\pm$ S.E.M.) after I.A. injection of des-Arg^9-bradykinin at 2.5 μg kg^{-1} (DBK 2.5), 5 μg kg^{-1} (DBK 5) and 10 μg kg^{-1} (DBK 10) and DBK 10, 10 min after [Leu^8]-des-Arg^9-bradykinin (10 μg kg^{-1} min^{-1}) (DBK 10 + LBK) in the sham ($n = 6$) and ischaemia–reperfusion ($n = 5$) groups. *$P < 0.05$ *vs.* sham group, †$P < 0.05$ *vs.* control value, ‡$P < 0.05$ *vs.* corresponding value.
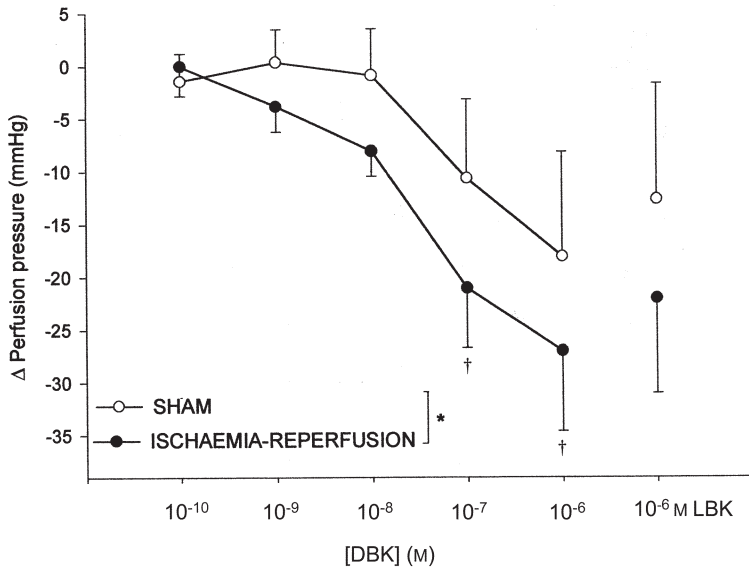
Figure 2

Cumulative decrease in perfusion pressure (means ± S.E.M.) of isolated hearts with increasing concentrations of des-Arg^9-bradykinin (DBK; 10^{-10} to 10^{-6} M), and the effect of DBK (10^{-6} M) after [Leu^8]-des-Arg^9-bradykinin (LBK; 10^{-6} M) in the sham ($n = 5$) and ischaemia–reperfusion ($n = 5$) groups. *$P < 0.05$ *vs.* sham group, †$P < 0.05$ *vs.* control value.
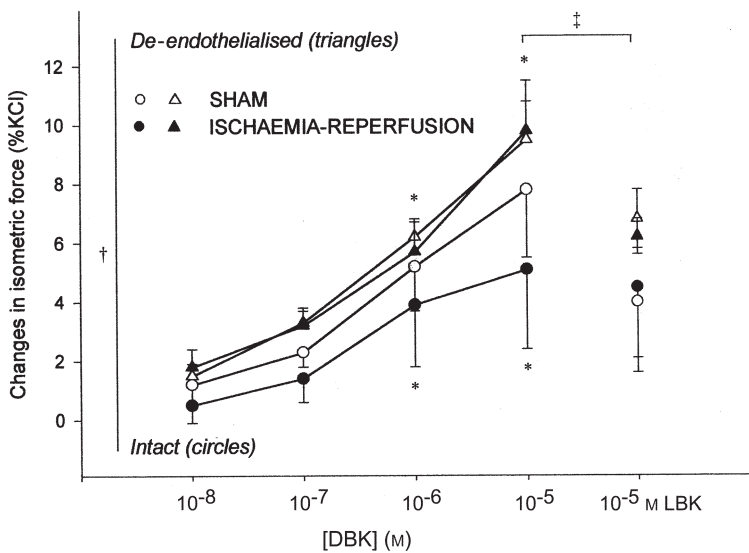

Figure 3

Changes in the isometric force of aortic rings (means ± S.E.M., percentage of maximal KCl contraction) after des-Arg^9-bradykinin (DBK; 10^{-8} to 10^{-5} M), and [Leu^8]-des-Arg^9-bradykinin (LBK; 10^{-5} M) in the sham ($n = 6$) and ischaemia–reperfusion ($n = 7$) groups. *$P < 0.05$ *vs.* control tension for pooled values from both groups, †$P < 0.05$ intact *vs.* de-endothelialised vessels; ‡$P < 0.05$ LBK *vs.* DBK (10^{-5} M). Comparison between intact (circles) and de-endothelialised (triangles) rings.
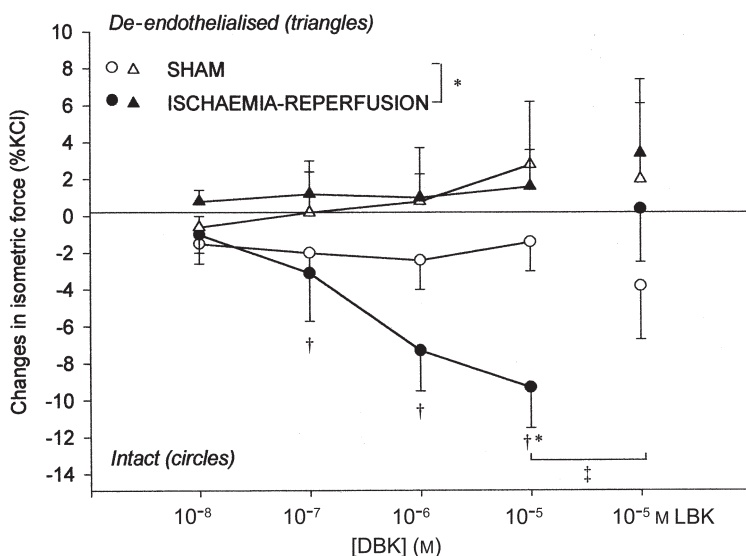

Figure 4

Changes in isometric force of carotid rings (means ± S.E.M., percentage of maximal KCl contraction) after des-Arg^9-bradykinin (DBK; 10^{-8} to 10^{-5} M), and [Leu^8]-des-Arg^9 bradykinin (LBK; 10^{-5} M) in the sham ($n = 6$) and ischaemia–reperfusion ($n = 7$) groups. *$P < 0.05$ *vs.* sham group, †$P < 0.05$ *vs.* control tension. ‡$P < 0.05$ *vs.* corresponding value. Comparison between intact (circles) and de-endothelialised (triangles) rings.
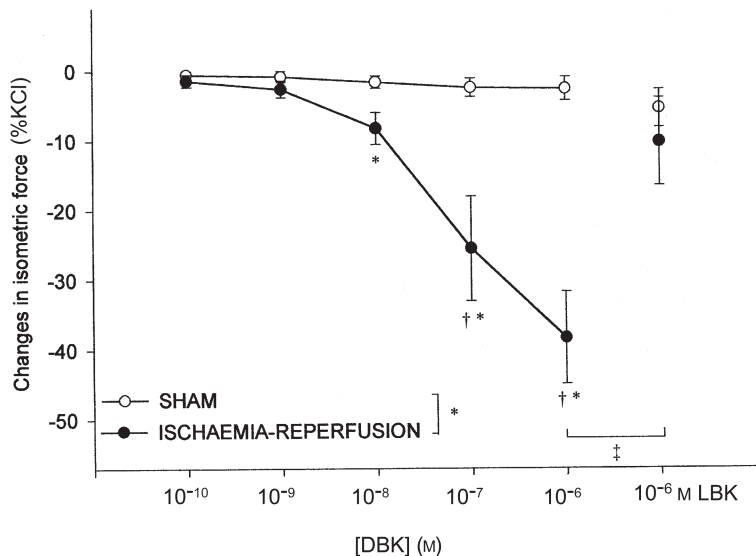
Figure 5

Changes in isometric force of intact mesenteric arteries (means ± S.E.M., percentage of maximal KCl contraction) after des-Arg^9-bradykinin (DBK; 10^{-10} to 10^{-6} M) and [Leu^8]-des-Arg^9-bradykinin (LBK; 10^{-6} M), in the sham ($n = 5$) and ischaemia–reperfusion ($n = 6$) groups. *$P < 0.05$ *vs.* sham group, †$P < 0.05$ *vs.* control tension, ‡$P < 0.05$ *vs.* corresponding value.

The immunohistology assay showed fluorescence in the endothelium and adventitia of all arteries studied and some fluorescence in the aortic media; this corresponded to the presence of B$_1$-receptors in these territories after myocardial ischaemia–reperfusion (Fig. 7).

DISCUSSION

The objective of this work was to evaluate whether myocardial ischaemia–reperfusion was able to induce a *de novo* synthesis of B$_1$-receptors in the rabbit. The presence of B$_1$-receptors was evaluated pharmacologically by assessing the effects of des-Arg^9-bradykinin, the classical B$_1$-receptor agonist (Marceau *et al.* 1998) and by immunolocalisation of the B$_1$-receptor protein. Our results clearly demonstrate that a myocardial ischaemia–reperfusion sequence can induce cardiac as well as vascular B$_1$-receptor expression. This confirms the recent study of Tschöpe *et al.* (2000) that demonstrates an increase in cardiac RNAs coding for B$_1$-receptors after myocardial infarction. In addition, we have shown that myocardial ischaemia–reperfusion could induce B$_1$-receptors at the cardiac level but also at a distance in systemic vessels and that these receptors were functionally active.

In vivo, after myocardial ischaemia–reperfusion, des-Arg^9-bradykinin induced a bradycardia and a decrease in the mean arterial pressure. These effects were due to B$_1$-receptors since they were antagonised by [Leu^8]-des-Arg^9-bradykinin and unaffected by HOE 140. The small response to DBK observed in the sham group could be explained by a weak inflammatory response to combined anaesthesia and surgical stress. However, since it is not significantly antagonised by LBK, we cannot exclude a B$_1$-receptor-independent effect of DBK injection. The observed hypotension could be due to a decrease in vascular resistance as well as in cardiac output. Since no effect on heart rate was seen in isolated hearts, this suggests that the bradycardia seen *in vivo* could be due to a central effect of des-Arg^9-bradykinin.

Since [Leu^8]-des-Arg^9-bradykinin had no effect on baseline arterial pressure or heart rate, B$_1$-receptors do not appear to play an important role in the control of haemodynamic parameters after myocardial ischaemia–reperfusion. However, this effect was measured 3 h after reperfusion rather than at the beginning of reperfusion when kinin outflow from the ischaemic zone is maximal (Lamontagne *et al.* 1995). Moreover, in the isolated rat heart model, des-Arg^9-bradykinin has been shown to be released from the coronary circulation after myocardial ischaemia–reperfusion only when ACE is blocked (Lamontagne *et al.* 1995). Indeed, inhibition of the most important degradation pathway of bradykinin could lead
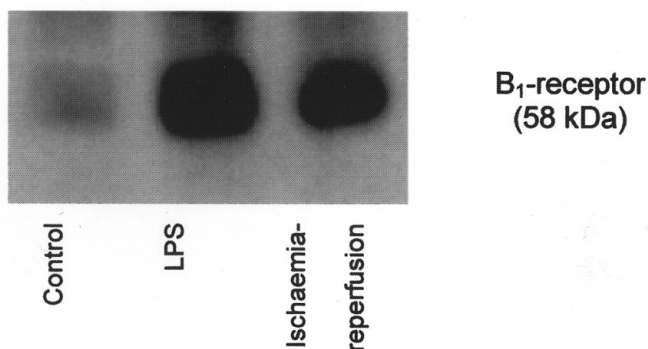

B$_1$-receptor (58 kDa)

Figure 6

Western blot analysis for B$_1$-receptors in tissue extracted from the ischaemic zone.

to an increased activity of the carboxypeptidase responsible for the synthesis of des-Arg^9 fragments (Marceau, 1995). This suggests that, in patients treated with ACE inhibitors, the presence of a B_1-agonist along with B_1-receptors could lead to B_1-receptor-dependent haemodynamic responses after myocardial ischaemia–reperfusion.

In vitro, the isolated aorta, the classic model for the study of B_1-receptors (Regoli *et al.* 1977), did not show a B_1-receptor induction by myocardial ischaemia–reperfusion. Isolated aortic rings from both groups responded to des-Arg^9-bradykinin by a constriction. This could also be explained either by the previously mentioned weak inflammatory response and/or by an *in*
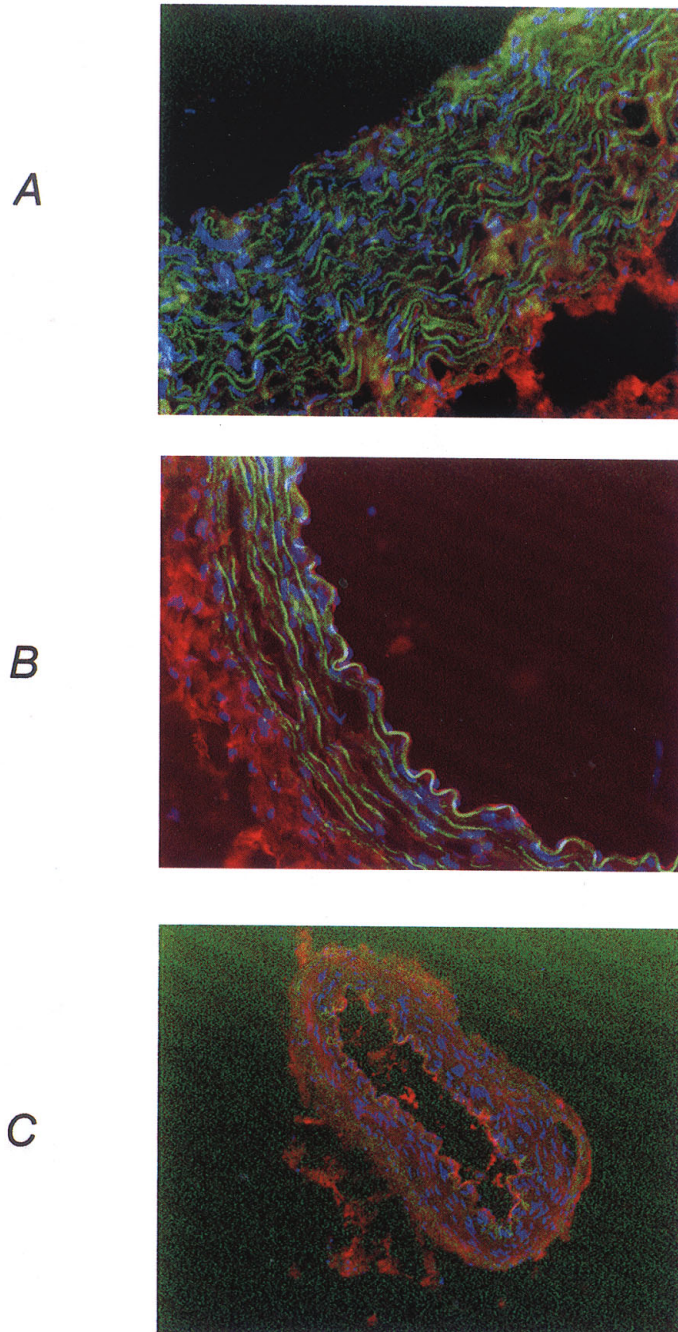

Figure 7

Immunohistological fluorescence photograph of aorta (*A*), carotid (*B*) and mesenteric (*C*) arteries from an animal submitted to a myocardial ischaemia–reperfusion sequence. Red, B_1-receptors; blue, nuclei; green, autofluorescence of elastin.

vitro induction by incubation, since the response to des-Arg^9-bradykinin was evaluated approximately 1.5 h after isolation. Indeed, although a 1 h incubation time is not sufficient to induce aortic B_1-receptors in control animals (Bouthillier *et al.* 1987), after surgical stress this delay could be reduced. De-endothelialised aortic rings responded more intensely than intact ones to des-Arg^9-bradykinin. This suggests that vasodilatory endothelial B_1-receptors in the aorta could counteract the smooth muscle-induced vasoconstriction. B_1-receptors leading to smooth muscle-dependent vasoconstriction were first characterised in isolated aortic strips (Regoli *et al.* 1977), which do not permit the study of endothelial function. Thereafter, the use of aortic rings showed that the effect of des-Arg^9-bradykinin on the intact aortic wall was a vasoconstriction (Levesque *et al.* 1995). To our knowledge, this is the first study using de-endothelialised and intact aortic rings to evaluate the effect of des-Arg^9-bradykinin on the aortic endothelium. Our results suggest that it induces an endothelial vasodilatation that is masked by a more pronounced media-induced vasoconstriction. This is confirmed by the immunolocalisation of B_1-receptors on both endothelium and media in the aorta. A strong fluorescence was also seen in the adventitia of all the vessels studied. Specific experiments will be needed to determine the role of these adventitial B_1-receptors.

Three hours after myocardial ischaemia–reperfusion but not after sham operation, we observed a dose-dependent and endothelium-dependent vasodilatation in response to des-Arg^9-bradykinin on isolated carotid rings. Indeed, immunohistochemical staining showed the presence of B_1-receptors in the endothelium of carotid arteries with little fluorescence in the media. Our results are in accordance with those of Pruneau & Bélichard (1993), who reported that, after induction of B_1-receptors by 5 h *in vitro* incubation, isolated rabbit carotid arteries respond to des-Arg^9-bradykinin by a vasodilatation. These authors have also shown that acutely denuded carotid arteries do not respond to des-Arg^9-bradykinin (Pruneau *et al.* 1994). The mechanisms involved in the B_1-receptor-dependent relaxation are known to implicate NO (Pruneau & Bélichard, 1993) and prostaglandin (de Blois *et al.* 1987) synthesis.

The effect of des-Arg^9-bradykinin on large arteries cannot explain the decrease in arterial pressure observed *in vivo*. We thus tested its effect on small resistance arteries such as the coronary bed of the isolated heart and isolated mesenteric arteries. After myocardial ischaemia–reperfusion, isolated hearts responded to des-Arg^9-bradykinin by a decrease in perfusion pressure demonstrating the presence of B_1-receptors at the site of ischaemia. Sham-operated hearts tended to respond to des-Arg^9-bradykinin, although not significantly. This suggests that, in the heart, a small proportion of B_1-receptors could have been induced by inflammation brought about by surgical stress, in particular the silk

thread passed around the coronary artery, as well as by the isolated perfusion protocol, which can be considered as an *in vitro* incubation. The inflammation related to the surgical protocol could also explain the tendency of sham animals to respond to des-Arg^9-bradykinin.

After myocardial ischaemia–reperfusion, small mesenteric arteries responded to des-Arg^9-bradykinin by an almost complete relaxation. The immunohistochemical study showed a preferential distribution of B_1-receptors at the endothelial level in mesenteric arteries after myocardial ischaemia–reperfusion. The fact that B_1-receptors are present in systemic vessels is an interesting finding since a local stress (myocardial ischaemia) was able to induce B_1-receptors at a distance and not only locally as previously shown (Tschöpe *et al.* 2000). Thus, the induction of endothelial B_1-receptors on resistance arteries could (along with the bradycardia) explain the hypotension observed *in vivo*. After myocardial ischaemia–reperfusion we did not observe a decreased endothelial function in the vessels studied. This is in agreement with other authors. Indeed, no endothelial dysfunction is seen in the aorta, femoral and mesenteric arteries following myocardial ischaemia (Brandes *et al.* 1998). However, ischaemia–reperfusion is associated with an endothelial dysfunction in vessels submitted to ischaemia, as reported for coronary arteries isolated distally from the occlusion site (Richard *et al.* 1994), or for isolated hearts submitted to a global low-flow ischaemia (Bouchard *et al.* 1998). Our results are in accordance with this since no decreased response to acetylcholine was seen in vessels not submitted to ischaemia. In the heart, we did not observe an endothelial dysfunction since it was evaluated globally and not only in the previously ischaemic zone.

B_1-receptors, which were first demonstrated to be induced by *in vitro* incubation (Regoli *et al.* 1977), are also promoted by various pro-inflammatory substances such as LPS, IL-1, IL-2, IL-8, adjuvant and bradykinin or by inflammatory pathological processes (immune complex arthritis, balloon catheter injury, septicaemia or UV irradiation) (Marceau, 1995). This is due to a *de novo* synthesis of the receptor since the induction is prevented by inhibitors of RNA synthesis (Marceau, 1995) and of protein synthesis or trafficking (Audet *et al.* 1994). IL-1 appears to play a primordial role since all substances responsible for B_1-receptor induction *in vivo* or *in vitro* can induce IL-1 synthesis or release (Marceau, 1995). There is a strong body of evidence that inflammation occurs during myocardial ischaemia and reperfusion (Hansen, 1995). The role of cytokines in mediating inflammation in this situation seems to be particularly important since their expression is increased during ischaemia and early reperfusion, particularly in coronary vessels (Galea *et al.* 1996). Moreover, IL-1 is released by endothelial cells submitted to hypoxia–reoxygenation (Ala *et al.* 1992). The production of IL-1 in the vascular bed of ischaemic myocardium could participate in the

induction of B_1-receptors in coronary arteries and also, as demonstrated by our results, in systemic vessels. Finally, although the involvement of kinins in the induction of B_1-receptors remains to be confirmed (Nwator *et al.* 1989; Marceau, 1995), their release upon myocardial ischaemia could also contribute to the induction observed in this study. Some studies have shown that B_1-receptors present in the isolated heart model can decrease noradrenaline outflow during ischaemia (Chahine *et al*, 1993; Feng *et al.* 1997). Others report that they can mimic the effect of ischaemic preconditioning in protecting the coronary endothelium (Bouchard *et al.* 1998). Taken together with our results showing that myocardial ischaemia–reperfusion is able to promote induction of bradykinin B_1-receptors, this indicates that under ischaemic conditions kinins could interact with B_1-receptors and have an eventual protective role. Finally, a combined action of IL-1 and kinins has been shown to stimulate angiogenesis through the activation of B_1-receptors (Hu *et al.* 1993). Thus, the implication of these receptors in the neovascularisation occurring after myocardial ischaemia–reperfusion should also be investigated and could have clinical relevance, especially in patients treated with ACE inhibitors. In conclusion, our study is the first to provide evidence that myocardial ischaemia–reperfusion induces a global induction of functional kinin B_1-receptors in the endothelium.

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