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ARTICLE

ET_B Receptor and Nitric Oxide Synthase Blockade Induce BQ-123–Sensitive Pressor Effects in the Rabbit

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Abstract: *Abstract* Endothelin-1 (0.25 nmol/kg, injected into the left cardiac ventricle) induces a protracted increase of mean arterial pressure that is significantly reduced by the selective ET_A receptor antagonist BQ-123 (1 and 10 mg/kg) in the anesthetized rabbit. The sole administration of the selective ET_B antagonist BQ-788 (0.25 mg/kg) induces a pressor response abolished by BQ-123 (1 mg/kg). Concomitant to the increase in mean arterial pressure, BQ-788 induces a significant increase in plasma levels of endothelin-1 and its precursor big endothelin-1. The nitric oxide synthase inhibitor *N*^ω-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg) also increases arterial blood pressure, and the response is reduced dose-dependently by BQ-123 (1 and 10 mg/kg). In addition, the administration of BQ-788 in the presence of L-NAME induced a further increase in arterial blood pressure. The duration of the pressor response to L-NAME is also significantly reduced by an endothelin-converting enzyme inhibitor, phosphoramidon (10 mg/kg). Finally, L-NAME induces an increase in plasma levels of big endothelin-1 but not endothelin-1. Our results illustrate that blockade of either nitric oxide synthase or ET_B receptors triggers a raise in plasma levels of endothelin-1 or its precursor. These later moieties are suggested to be significantly involved, through the activation of ET_A receptors, in the pressor effects of L-NAME and BQ-788 in the anesthetized rabbit.

Key Words: receptors, endothelin ■ arterial pressure ■ rabbits ■ endothelin-1 ■ endothelin-converting enzyme ■ nitric oxide

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The protracted pressor response to ET-1 is strongly modulated by endogenous factors such as prostacyclin and EDRF.^{1 2} Later studies have further shown that EDRF might be importantly involved in the regulation of the expression and the release of ET-1, in cultured endothelial cells and in intact porcine aortas.³

Recent advances in the development of highly potent and metabolically stable ET antagonists, such as bosentan,⁴ reveal a significant role for ET-1 in the control of vascular tone in pulmonary hypertension and chronic heart failure as demonstrated in animal models and in humans.^{5 6 7}

In general, the ET_A receptor activation has been associated with vasoconstriction, whereas ET_B receptors located on the vascular endothelium when stimulated are responsible for the release of the physiological antagonist nitric oxide.⁸ However, in some animal species as well as in lower resistance vessels (veins) in humans, ET_B receptors have also been suggested to be involved in the increase of vascular resistance induced by ET-1.^{9 10} Furthermore, ET-1–induced constriction of human veins is predominantly modulated by prostacyclin rather than nitric oxide.¹¹

These observations have prompted the development of mixed ET_A/ET_B receptor antagonists that have been shown to be useful in some vascular dysfunctions involving ET-1.⁷ However, whether the ET_B receptors located on the endothelium should be left unaltered, as we have recently suggested based on in vitro models,^{2 12} advocating the preferential use of selective ET_A receptor antagonists, remains to be fully investigated in vivo.

Recent evidence shows the important ET_B-dependent modulation of the pressor response to ET-1 by selective ET_B or mixed ET_A/ET_B antagonists in the rabbit.^{2 12} Furthermore, Allcock et al¹³ and Clozel et al¹⁴ have shown in the rat model, where ET_B receptors participate up to 40% in the pressor response to ET-1, that the predominant role of the ET_B receptor in the circulation is to limit the pressor effect of ET-1. Interestingly, antagonism of the ET_B receptor has also been associated with an increase in plasma ET levels¹⁵; this latter event may have deleterious effects in circuits particularly sensitive to ETs such as the kidney.¹⁶

We have recently reported that marked hyperresponsiveness to ET-1 was observed in the perfused rabbit kidney treated with the mixed ET_A/ET_B antagonist BQ-928, an effect attributed to the irreversible interference with ET_B receptors.¹² This further suggested that when systemic concentrations of a mixed ET_A/ET_B receptor antagonist, with a higher affinity for ET_B than for ET_A receptors, falls under its minimal effective level, a rebound pressor effect due to high plasma ET levels may be observed. On the other hand, ET_A or mixed ET_A/ET_B antagonists with higher affinity for the former receptor type may, in some cases, efficiently block the effects of increased plasma ET and act as marker of an effective ET_B receptor blockade.¹⁷

It has been shown that plasma levels of both ET-1 and big ET-1 correlate directly with the extent of increased pulmonary pressure in patients with chronic heart failure.⁷ In these conditions, the acute administration of the mixed ET_A/ET_B receptor antagonist bosentan acutely improved the hemodynamic parameters in these patients, although plasma ET levels were increased.⁷

On the other hand, Love et al recently showed that an ET_A receptor antagonist caused vasodilatory effect and an ET_B receptor antagonist a vasoconstrictor response in brachial arteries of both healthy individuals and patients with congestive heart failure.¹⁸ These results would suggest that ET_B receptors, located on the endothelium rather than on the smooth muscle, are importantly involved in the modulation of systemic vascular tone in humans.^{18 19}

We have therefore attempted to demonstrate the important role of endogenous ET-1 in an animal model in which the ET_A receptors are predominantly (>90%) involved in the pressor effects of the peptide. Because the rabbit may better reflect the hemodynamic responses found in humans after ET receptor activation,¹⁹ the contribution of the endogenous pressor peptide after acute ET_B receptor blockade or acute NO synthase inhibition was investigated in that animal species.

Methods

Hemodynamic Studies

Experiments were performed on New Zealand White rabbits of either sex weighing 1.3 to 1.6 kg. All experimental procedures conformed to the guiding principles for animal experimentation as enunciated by the Canadian Council on Animal Care and approved by the Ethical Committee on Animal Research of the Université de Sherbrooke Medical School.

The animals were anesthetized with an intramuscular injection of a mixture of ketamine (50 mg/kg) and xylazine (5 mg/kg) and supplemented as needed. A heparinized catheter (PE-90) was passed into the left ventricle via the right carotid artery for administration of the various agents and for blood sampling.

MAP and heart rate were monitored via a cannula in the left femoral artery linked to a pressure transducer. The animals were ventilated (6 mL air/kg, 40 strokes/min) via a third cannula inserted into the trachea and connected to a respirator (model 683, Harvard Apparatus). MAP and heart rate were recorded by an automated computer data acquisition system, Blood Pressure Analyzer (BPA-200A, Micro-Med). The system was linked to a DMSI-200/8 Digi-Med System Integrator on a DTK Pentium 100 MHz computer for data collection. Throughout the experiments, all of the above-mentioned parameters were constantly monitored.

Pharmacological responses to the various agents (ET-1, 0.25 nmol/kg; BQ-788, 0.025 and 0.25 mg/kg; or L-NAME, 10 mg/kg) were assessed for at least 30 minutes after a single bolus dose. In some experiments, phosphoramidon (5 mg/kg or 10 mg/kg) or BQ-123 (0.25, 1, or 10 mg/kg) was administered 5 minutes before BQ-788, L-NAME, or ET-1. In other experiments, BQ-788 (0.25 mg/kg) was administered 10 minutes after the L-NAME (10 mg/kg) injection.

Sample Preparation

Blood samples (750 μ L), taken before and 1, 2, 5, 10, 15, and 30 minutes after injection of the drugs, were collected in trisodium citrate (3.5%) in a 9:1 ratio (vol/vol). Samples were centrifuged for 1 minute at 15 000g for plasma separation and stored at -80°C until assayed. After they were thawed, 400 μ L plasma samples were acidified by the addition of 400 μ L of 1% TFA, and clarified by centrifugation for 5 minutes at 19 000g at room temperature. The resulting supernatants were purified through methanol-activated 100 mg Amprep C18 disposable cartridges (Amersham).

Unbound materials were washed from the columns with 3 mL of 0.1% TFA and discarded. Immunoreactive ET and big ET-1 were eluted with a subsequent 2 mL of 100% methanol. Eluates were evaporated to dryness in polypropylene tubes using a Savant Speed Vac Concentrator. The dessicated residue was redissolved in RIA buffer. The recoveries of ET-1 and big ET-1 were 53% and 67%, respectively ($n=4$). Plasma immunoreactive peptide concentrations are shown uncorrected for extraction recovery.

HPLC Analysis

Plasma samples (1.5 mL) for HPLC analysis were obtained at 2 minutes from four different rabbits. Samples were acidified with 375 μ L of hydrochloric acid 2N and centrifuged for 1 minute at 19 000g. Supernatants were applied to activated 500 mg Amprep C2 columns (Amersham), eluted with 80%-20% methanol-acetonitrile, and subsequently evaporated to a volume of 100 μ L. Samples were

diluted to a final volume of 1 mL with 10% acetonitrile/1% TFA and separated on a reversed-phase HPLC using a Waters C18 Delta-Pak column. The samples were then eluted with a 30-minute linear gradient of 28% to 40% acetonitrile in 0.1% TFA at a flow rate of 1 mL/min using a Waters model 510 pump. Fractions (1 mL) were evaporated to dryness and analyzed by RIA. For calibration, synthetic ET-1, -2, and -3 were run separately, and the elution position of the standards was determined by RIA.

RIA

IR-ET and IR-big-ET-1 were measured by a double-antibody assay. IR-ET assay kit (RPA 555) was purchased from Amersham, and assay was performed according to protocol. IR-big-ET-1 assay was done according to Löffler et al.²⁰ Cross-reactivities of the ET antiserum for ET-1, -2, and -3 were 100%, 144%, and 52%, respectively. Cross-reactivity of the ET antiserum with big ET-1 (1-38) was 0.4%, and less than 0.0033% with big ET-1 (22-38) (Amersham) and BQ-788. Cross-reactivity of the big-ET-1 antiserum with human big ET-1 (22-38) was 14%,²⁰ and no cross-reactivity up to 100 pmol/tube was detected, with ET-1, ET-2, ET-3, big ET-2, and big ET-3, BQ-788, BQ-123, and L-NAME.

Drugs and Solution

ET-1 was purchased from American Peptide. BQ-788 was generously supplied by Dr Masaki Ihara of Banyu Chemical (Tsukuba, Japan) and BQ-123 synthesized by Dr Witold Neugebauer (Department of Pharmacology, Université de Sherbrooke). Phosphoramidon was purchased from Peptides International, and L-NAME and heparin from Sigma Co. ¹²⁵I–big ET-1 was purchased from Peninsula Laboratories.

All drugs were dissolved in phosphate buffered saline (pH 7.4) except for BQ-788, which was diluted in 10% dimethyl sulfoxide and subsequently in phosphate buffered saline.

Statistical Analysis

Statistical analysis was done by ANOVA followed by Dunnett's test for multiple comparisons to evaluate the variation compared with basal values, whereas Student-Newman-Keuls posttest was used to evaluate the variation between doses (Fig 1A only). Comparisons between groups were made by Student's *t* test. Values of *P* < .05 were considered significant.

Results

Hemodynamic Effects of BQ-788 and L-NAME

Baseline MAP averaged 71.7±1.9 mm Hg in the anesthetized rabbit. Left cardiac ventricular (IC) administration of ET-1 (0.25 nmol/kg) induced a biphasic response characterized by a transient (1 minute) depressor response (16.6±4.0 mm Hg) followed by a sustained hypertensive phase (28.8±2.8 mm Hg) (Fig 1A). Pretreatment with the ET_B antagonist BQ-788 (0.25 mg/kg) 5 minutes before ET-1 administration resulted in the abolition of the depressor phase (*P* < .05) and potentiation of the hypertensive phase (from 28.8±2.8 to 49.5±3.5 mm Hg; *P* < .05). The ET_A antagonist BQ-123 did not affect the ET-1–induced depressor response but significantly and dose dependently reduced its hypertensive effect at 1 mg/kg (from 28.8±2.8 to 12.4±1.3 mm Hg; *P* < .001) and 10 mg/kg (from

28.8±2.8 to 5.0±1.0 mm Hg; $P<.001$ versus control and $P<.05$ versus BQ-123 at 1 mg/kg) (Fig 1A). The highest dose of BQ-123 was inactive against angiotensin II (0.1 nmol/kg) (control, 24.2±2.2 mm Hg; in the presence of BQ-123, 10 mg/kg, 22.4±1.8 mm Hg) (results not shown).

The sole administration of BQ-788 (0.25 mg/kg, IC) resulted in a mild yet significant increase in MAP (from 69.5±2.0 to 75.9±1.7 mm Hg; $P<.05$, $n=7$) (Δ MAP changes illustrated in Fig 1B) that lasted for 10 minutes. BQ-788 at a dose of 0.025 mg/kg or vehicle alone did not affect MAP (results not shown). A 5-minute pretreatment of the animal with BQ-123 (1 mg/kg) abolished this BQ-788–induced increase in MAP ($n=4$, $P<.01$) (Fig 1B). BQ-123 per se (1 and 10 mg/kg) induced a nonsignificant decrease in MAP (1 mg/kg: from 68.2±1.8 to 65.9±2.2 mm Hg; 10 mg/kg: from 71.7±3.3 to 70.4±2.8 mm Hg; $n=5$ each, $P>.05$; results not shown).

L-NAME (10 mg/kg) induced a protracted increase in MAP (Δ MAP: 17.4±1.5 mm Hg; $n=22$), which lasted for at least 30 minutes (Fig 2A and 2B) and on which BQ-123 at 0.25 mg/kg had no effect ($n=4$, results not shown). At 1 mg/kg of BQ-123, the pressor response to L-NAME was significantly reduced at times 10, 15, and 30 minutes ($n=7$, $P<.05$; Fig 2A) without affecting the peak maximal pressor effect. A higher dose of BQ-123 (10 mg/kg) significantly reduced the peak pressor response (5 minutes) to the NO synthase inhibitor (10 mg/kg) (from 17.4±1.5 to 10.4±0.6 mm Hg; $n=4$, $P<.05$). In another series of experiments, phosphoramidon at 10 mg/kg, but not 5 mg/kg, also affected the duration of the pressor effect of L-NAME at time points 15 and 30 minutes ($n=4$, $P<.05$; Fig. 2B). The same dose of phosphoramidon markedly reduced the pressor response to big ET-1 (1 nmol/kg) (control: 57.4±4.7 mm Hg; in the presence of phosphoramidon: 7.0±2.8 mm Hg; $n=3$, $P<.05$; results not shown).

Effect of BQ-788 and L-NAME on Plasma ET and Big ET

Plasma IR-ET levels were determined after treatment with BQ-788 (0.25 mg/kg). IR-ET levels were significantly elevated when compared with basal levels at the 2-minute time point (basal level: 7.2±1.7 fmol/mL; 2 minutes: 11.9±2.4 fmol/mL; $n=5$, $P<.05$; Fig 3A). Plasma levels returned to basal values within 10 minutes. Concomitant analysis of IR-big-ET-1 showed that, after the ET_B antagonist administration, plasma IR-big-ET-1 concentrations were significantly higher at time points 1 and 2 minutes postinjection (basal level: 17.8±6.7 fmol/mL; 2 min: 46.2±11.0 fmol/mL; $n=5$, $P<.05$; Fig 3B). BQ-123 at 1 and 10 mg/mL did not affect plasma ET or big ET levels (results not shown).

With the use of reversed-phase HPLC and the specific RIA for ETs, the ET isoforms in the plasma samples were further identified. The elution profile of the plasma samples revealed one major peak with the same retention time as the authentic ET-1 standard (Fig 4). This peak was increased after treatment of the rabbit with BQ-788 (0.25 mg/kg).

L-NAME (10 mg/kg) elicited an elevation of at least twice the basal level of plasma IR-big-ET-1 at time periods 1 and 2 minutes (basal level: 23.8±5.8 fmol/mL; 2 min: 58.1±9.9 fmol/mL; $n=7$, $P<.05$; Fig 5B) that returned gradually toward basal values. In contrast, in the same rabbits L-NAME did not affect plasma ET levels at any time points (basal level: 9.3±1.2 fmol/mL) (Fig 5A).

Effect of L-NAME on BQ-788–Induced Hemodynamic Effects and Plasma ET Level Increases

BQ-788 (0.25 mg/kg) applied at the peak maximal response to L-NAME (10 mg/kg) at 5 minutes still induced a significant increase in MAP when compared with L-NAME treatment alone (Δ MAP: L-

NAME, 14.9 ± 2.1 mm Hg; L-NAME+BQ-788, 20.1 ± 1.3 mm Hg; $n=4$, $P<.05$) (Fig 6).

The Table shows that in the same experiments as above, L-NAME also had no effect on the increase of plasma IR-ET levels induced by BQ-788 (0.25 mg/kg). BQ-788 still induced a similar increase in plasma IR-ET levels in the presence or in the absence of L-NAME. However, L-NAME (10 mg/kg) pretreatment prevented a further increase in IR-big-ET-1 levels induced by BQ-788 (0.25 mg/kg) (Table); the ET_B antagonist was administered 10 minutes after L-NAME treatment when levels of IR-big-ET-1 had returned to basal values as shown in Fig 5B.

Discussion

As illustrated in the present study, the pressor responses to the selective ET_B antagonist BQ-788 are associated with an increase in the plasma levels of immunoreactive ET and are sensitive to a selective ET_A receptor antagonist. Two possible mechanisms may account for this BQ-788–induced pressor response. First, it is possible that BQ-788 efficiently interferes with the activation of the ET_B-dependent release of EDRF by endogenous ET, resulting in an increase of endothelium-derived production or release of ET-1.³ Second, the selective ET_B antagonist may displace endogenous ET-1 from the ET_B clearance receptor.^{21 22}

An increase in plasma levels of ET after antagonism of the ET_B receptor had been reported previously in the rat.¹⁵ However, in that particular animal model, it is difficult to correlate elevated plasma ET-IR with changes in MAP since the ET_B receptors blocked by BQ-788 might be found on the endothelium as well as on the underlying vascular smooth muscle.⁹ The opposite effect of ET_B receptor activation may explain the lack of pressor response to the ET_B antagonist per se in the rat.²³

In the rabbit, the pressor effects induced by BQ-788 may be considered mild. However, it has been clearly demonstrated that subpressor doses of ET produce a marked decrease in renal blood flow,²⁴ a condition in which only slight increases in IR-ET plasma levels were reported.

On the other hand, the pressor response after NO synthase inhibition has been shown to be partly mediated by the release of ET in the anesthetized rat.^{25 26} In our study L-NAME not only induced pressor responses but raised the plasma levels of big ET-1 in the rabbit. After the inhibition of NO synthase, the increase of plasma IR-big-ET-1 concomitant to the BQ-123–sensitive increase of MAP implies that there is an extracellular conversion of the precursor of ET-1 through the endothelin-converting enzyme and subsequent activation of ET_A receptors.

Although both ET_B receptor blockade and NO synthase inhibition induce acute BQ-123–sensitive pressor responses, they differ in their ability to release ET-1 and its precursor big ET-1. BQ-788 triggers the release of ET-1 and big ET-1, NO synthase inhibition only raises the plasma levels of IR-big-ET-1. We explain these different properties by the fact that BQ-788 displaces ET-1 from the endothelial ET_B receptors or reduces its ET_B-dependent clearance from the plasma.^{21 22} In contrast, L-NAME does not affect the clearance receptors but would readily interfere with the polarized secretion of ET-1 under the form of its precursor big ET-1. The increase in plasma big ET-1 concentrations induced by BQ-788 also implies the blockade of the ET_B-dependent NO secretion and the release of readily stored big ET-1.

Interestingly, Plumpton et al did not observe any increase in plasma big-ET-1, in contrast to plasma ET levels, after the administration of the mixed ET_A/ET_B receptor antagonist TAK-044 in

humans.²⁷ However in the later study, the monitoring of ET and big-ET-1 levels was performed 15 minutes after the start of TAK-044 infusion. In the rabbit we observed a release of big ET-1 only shortly (first 2 minutes) after BQ-788 bolus administration, suggesting a rapid and short-lasting release of readily stored big ET-1.

One may also consider that during NO synthase inhibition, the ET_B receptors are not saturated and may still act as clearance receptors and mask any increase in plasma ET-1 concentration. Because the pressor effects of big ET-1 in the anesthetized rabbit are abolished by phosphoramidon, we suggest that the precursor per se must be converted to its active metabolite ET-1 through the specific action of a phosphoramidon-sensitive endothelin-converting enzyme. However, phosphoramidon does not reduce the maximal pressor effect induced by L-NAME, in contrast to BQ-123. Hence after NO synthase inhibition one cannot exclude intracellular conversion of the ET precursor that is not inhibited by phosphoramidon, considering its poor ability to penetrate cell membrane.²⁸

Finally, we have shown that BQ-788 can still induce an increase in blood pressure in the presence of L-NAME and simultaneously increase the plasma levels of ET-1 but not big ET-1. Thus, the BQ-788–induced pressor effect is not only caused by an increase of big ET-1 secretion but also the reduced ET_B-dependent clearance of endogenous ET-1, which will activate ET_A receptors on the underlying smooth muscle.

In summary, our study shows that ET_B receptor blockade and NO synthase inhibition both trigger a pressor response that is importantly mediated by the release of endogenous ET and ET_A receptor activation in the rabbit.

Considering the fact that ETs appear to be importantly involved in the maintenance of MAP in humans,¹⁹ our results support the postulate that ET_B receptors and NO synthase should not be blocked as they may play a protective role in pathological conditions associated with elevated plasma levels of ET, such as in chronic heart and renal failures, cyclosporine-induced nephrotoxicity, and pulmonary hypertension.^{6 7 29} The further validation of the latter hypothesis must, however, await a better understanding of the human physiopathology of ET_B receptors in the kidney and elsewhere in the vasculature.

Selected Abbreviations and Acronyms

ΔMAP	=	variations in mean arterial pressure
EDRF	=	endothelium-derived relaxing factor
ET	=	endothelin
ET-1	=	endothelin-1
HPLC	=	high-performance liquid chromatography
IR-big-ET-1	=	big endothelin-1 immunoreactivity
IR-ET	=	endothelin immunoreactivity
L-NAME	=	<i>N</i> ^ω -nitro-L-arginine methyl ester
RIA	=	radioimmunoassay
TFA	=	trifluoroacetic acid

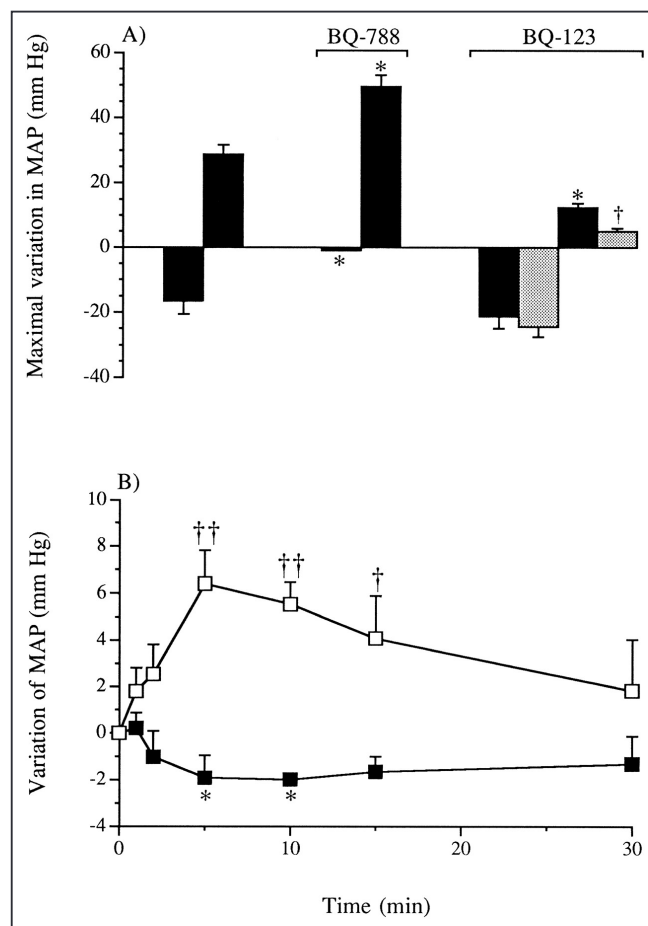


Figure 1. A, Effects of the ET_A antagonist BQ-123 (1.0 mg/kg, closed columns; 10.0 mg/kg, shaded columns) and the ET_B antagonist BQ-788 (0.25 mg/kg) on the maximal changes, depressor followed by the pressor effect, in MAP induced by ET-1 in the anesthetized rabbit (0.25 nmol/kg). Each column represents the mean $\pm$ standard error of at least three experiments. * P <.05 vs control, † P <.05 vs 1 mg/kg of BQ-123. Basal MAP: 72.4 $\pm$ 3.6 mm Hg, n =34. B, Time course of the effect of BQ-788 (0.25 mg/kg) on MAP alone (open squares) or in presence of BQ-123 (1 mg/kg) (closed squares). Each point represents mean $\pm$ standard error of at least four experiments. † P <.05 and †† P <.01 vs basal MAP, * P <.05 vs effect of BQ-788 alone.

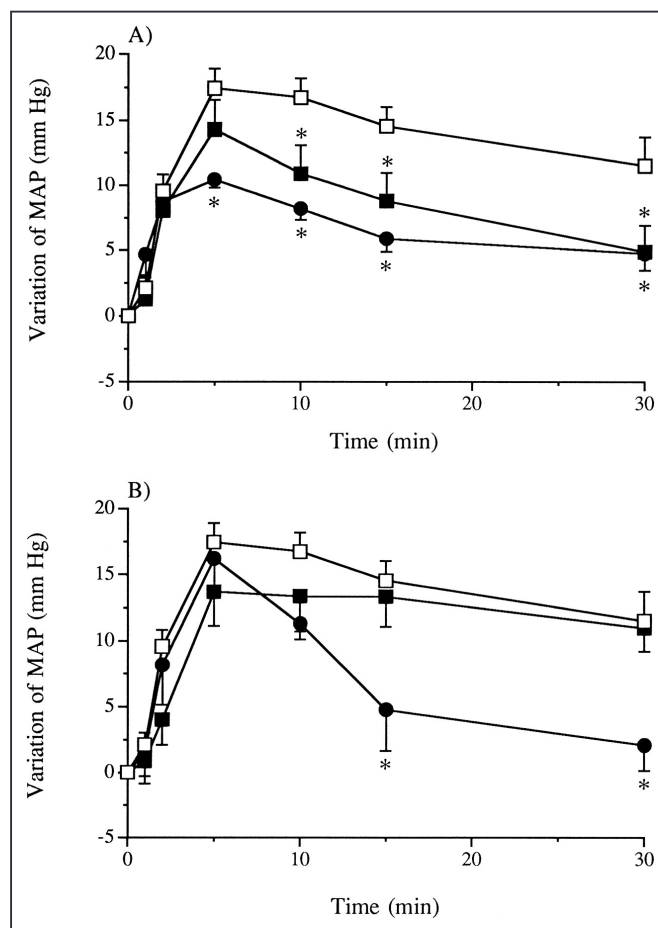


Figure 2. Time course of the effect of L-NAME (10 mg/kg) on the MAP of the anesthetized rabbit alone (open squares) in presence of BQ-123 (A) (1 mg/kg, closed squares; 10 mg/kg, closed circles) or in presence of phosphoramidon (B) (5 mg/kg, closed squares; 10 mg/kg, closed circles). Each point represents mean $\pm$ standard error of at least four experiments. * $P < .05$.

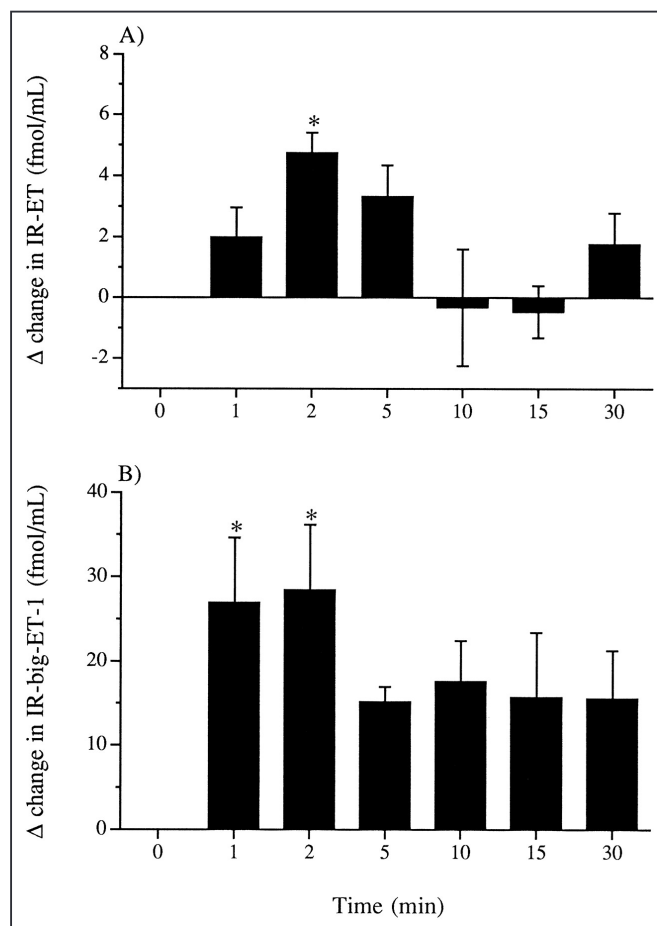


Figure 3. Time course of the effect of BQ-788 (0.25 mg/kg) on plasma IR-ET (A) and IR-big-ET-1 (B) concentrations in the anesthetized rabbit. Values are expressed as variations (Δ) from basal levels (basal levels IR-ET: 7.2 ± 1.7 fmol/mL; IR-big-ET-1: 17.8 ± 6.7 fmol/mL). Each column represents the mean $\pm$ standard error of at least five experiments. * $P < .05$.

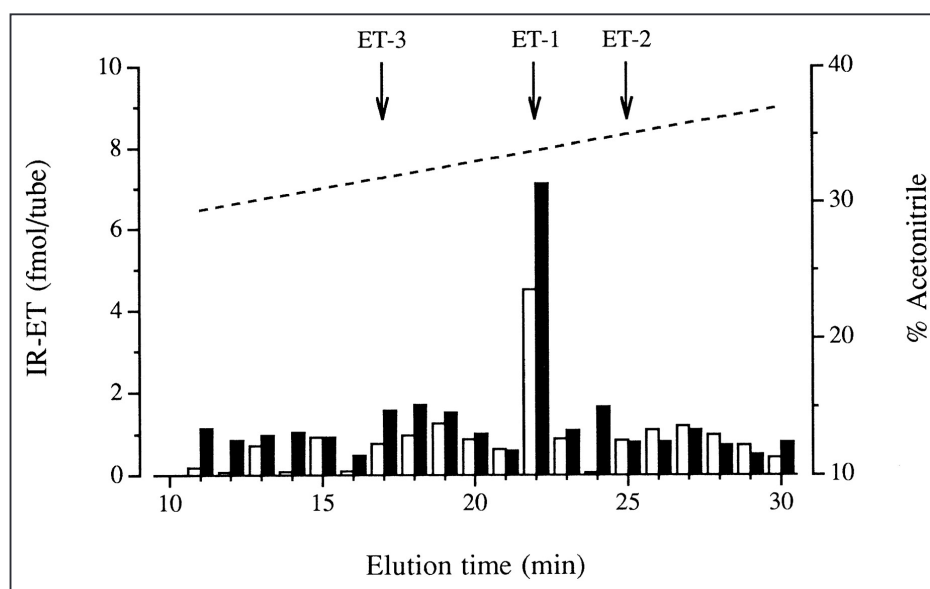


Figure 4. Reversed-phase HPLC elution profile of IR-ET extracted from plasma from control (white column) or BQ-788 (0.25 mg/kg)–treated (black columns) anesthetized rabbit. Each column represents plasma pooling of four experiments. Dashed line represents elution gradient of acetonitrile. Arrows indicate the retention times of synthetic ET-1, ET-2, and ET-3.

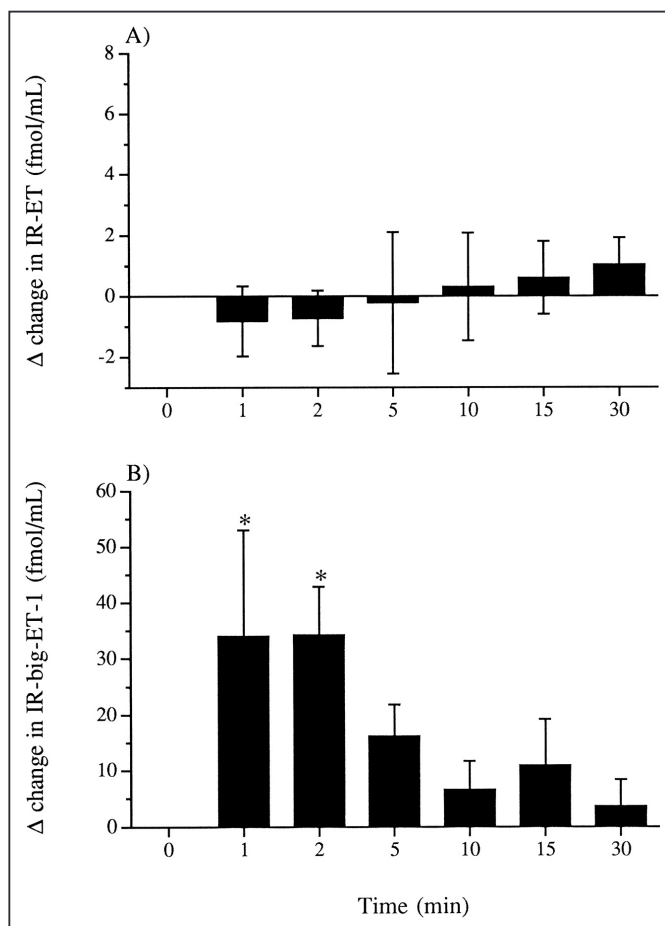


Figure 5. Time course showing the effect of L-NAME (10 mg/kg) on plasma IR-ET (A) and IR-big-ET-1 (B) concentrations in the anesthetized rabbit. Values are expressed as variations (Δ) from basal levels (basal levels IR-ET: 9.3 ± 1.2 fmol/mL; IR-big-ET-1: 23.8 ± 5.8 fmol/mL). Each column represents the mean $\pm$ standard error of at least five experiments. * $P < .05$.

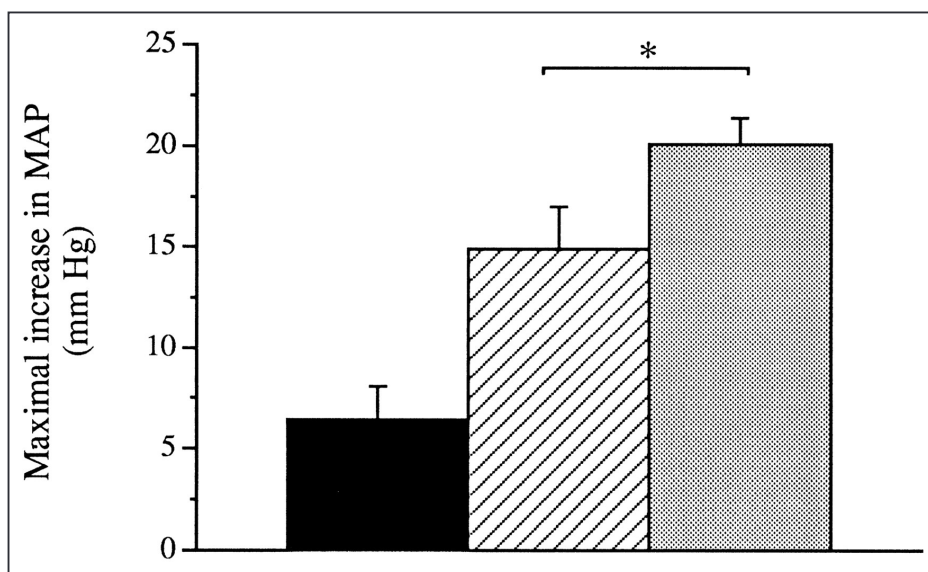


Figure 6. Maximal pressor effect induced by BQ-788 (0.25 mg/kg) (closed column), L-NAME (10 mg/kg) (hatched column), and a mixture of both (shaded column) in the anesthetized rabbit. Each column represents the mean $\pm$ standard error of at least four experiments. * $P < .05$.

Table 1. Effect of L-NAME Treatment on the Increase of Plasma Endothelin and Big ET-1 Levels Induced by BQ-788 in Anesthetized Rabbits ([Table view](#))

Peptides	Control	L-NAME
IR-ET, fmol/mL		
Basal	7.2±1.6	6.0±1.4
BQ-788 (0.25 mg/kg)	12.6±1.9 ¹	12.0±1.8 ¹
IR-big-ET-1, fmol/mL		
Basal	17.8±6.7	22.6±8.3
BQ-788 (0.25 mg/kg)	46.2±11.0 ¹	24.1±9.2

Values are expressed as mean±SEM.

¹ *P*<.05 vs basal levels.

Article Information

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