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ENDOTHELIAL RECEPTOR A BLOCKADE ALTERS THE HEMODYNAMIC RESPONSE
TO NITRIC OXIDE INHIBITION IN THE RAT

BY

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ABSTRACT

We have examined the extent to which the systemic and renal vasoconstriction induced by nitric oxide (NO) inhibition *in vivo* are mediated by endothelin (ET). We examined the effects of BQ610, a specific ET-A receptor antagonist, following NO inhibition with L-NAME in the anesthetized rat. Mean arterial pressure (MAP) increased following L-NAME infusion from 107 ± 2 to 133 ± 3 mmHg ($p < 0.05$ vs baseline period) and then fell to 115 ± 3 mmHg following administration of BQ610 ($p < 0.05$ vs L-NAME and baseline periods). Systemic vascular resistance (SVR) increased from 1.26 ± 0.06 to 2.17 ± 0.18 mmHg/ml/min/300g following L-NAME ($p < 0.05$ vs baseline period) and then fell to 1.69 ± 0.12 mmHg/ml/min/300g after BQ610 ($p < 0.05$ vs L-NAME and baseline periods). The increase in renal vascular resistance (RVR) from 6.4 ± 0.4 to 13.7 ± 1.4 mmHg/ml/min/300g induced by L-NAME ($p < 0.05$ vs baseline period) was reduced to 11.1 ± 1.0 mmHg/ml/min/300g by BQ610 ($p < 0.05$ vs L-NAME and baseline periods). The extent to which BQ610 reversed the L-NAME induced increases in RVR and SVR was comparable (RVR by $40 \pm 9\%$; SVR by $52 \pm 7\%$). GFR and renal blood flow (RBF) were both reduced by L-NAME, but neither value increased following BQ610, possibly because the renal vasodilation induced by ET-A blockade was offset by the concomitant reduction in MAP and renal perfusion pressure. In summary, ET, acting via the ET-A receptor, partially contributes to the systemic and renal hemodynamic vasoconstrictor and hypertensive effects of NO inhibition.

Key words: Nitric oxide; endothelin; ET-A receptor; L-NAME; BQ610; blood pressure; renal function; vascular resistance

INTRODUCTION

Endothelin (ET), a 21 amino acid peptide with potent vasoconstrictor properties, was described in 1988 by Yanagisawa et al. (28) and subsequently found to comprise a family of pharmacologically distinct isoforms, ET-1, ET-2 and ET-3 (11). Two receptors for ET, ET-A and ET-B have been identified and cloned (14). The ET-A receptor is highly specific for ET-1 and is widely distributed in vascular smooth muscle cells. ET-1 stimulation of the ET-A receptor mediates most of the vasoconstrictor response to ET (6,14,21). The ET-B receptor, unlike ET-A, responds equally to all three isoforms of ET, is present largely on endothelial cells and mediates endothelium-dependent vasodilation by stimulating NO formation (6,14).

In contrast to endothelin, the production of nitric oxide (NO) by endothelial cells plays an important role in inducing smooth muscle relaxation. Inhibition of NO production *in vivo* using analogues of arginine such as N ω -nitro-L-arginine methyl ester (L-NAME) results in a substantial increase in blood pressure and a fall in renal blood flow and GFR (3,9,19,22). These studies indicate that the constitutive production of NO contributes to the modulation of basal vascular tone in the resistance vessels of the kidney and other organs and plays an essential role in the regulation of blood pressure and renal function.

It has become evident that a complex counterregulatory relationship exists between NO and endothelin. NO not only opposes ET action by independently causing vasodilation (19), but also plays an important role in directly modulating the production and vasoconstrictor effects of ET-1. NO, produced by endothelium or provided exogenously by NO donors such as sodium nitroprusside, inhibits the production of endothelin by endothelial cells at a transcriptional level (5,15). Goligorsky et al. (10) have recently provided evidence that NO also regulates the interaction of ET-1 with its receptor. NO is able to terminate the ET-1 induced rise in intracellular calcium both by directly displacing bound ET-1 from its ET-A receptor

and by interfering with postreceptor pathways involved in calcium mobilization (10). These findings are consistent with prior observations by Lerman et al. (16) that NO inhibition with L-NMMA enhanced vasoconstriction induced by exogenously administered ET-1 in the renal, systemic, pulmonary and coronary circulation. Thus, in addition to the independent role of NO as a vasodilator, NO may also influence vascular tone by regulating the production (5,15) and vasoconstrictor action (10) of endothelin.

If NO plays an important role in negatively modulating ET-1 production and activity, a reduction in NO availability would be expected to increase ET-1 production and/or activity which in turn should contribute additively to the vasoconstrictor effects associated with a reduction in NO production. We have shown, using an inhibitor of the ET-A receptor, that ET-1 contributes to the systemic and intrarenal hemodynamic effects induced by L-NAME in pentobarbital anesthetized rats.

METHODS

1. Surgical procedures: Male Sprague-Dawley rats, weighing between 300 and 350g fed regular Purina rat chow (Purina Mills, Chicago, IL) and allowed free access to water were used for all experiments. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg/100g body wt) followed by a constant intravenous infusion of pentobarbital sodium (91 ug/min) as previously described (18). Body temperature was monitored via a temperature probe in the carotid artery and maintained between 36 and 38°C. Both femoral arteries were cannulated with PE-50 tubing, one for blood pressure monitoring and the other for blood sampling. A bladder catheter (PE-90) was placed for urine sampling. The right internal jugular vein was cannulated with three catheters of PE-50 tubing. One catheter was used to infuse inulin, PAH and pentobarbital sodium, the second was used for the infusion of L-NAME (Sigma, St Louis, MO.), and the third catheter for the administration of the

ET-A receptor antagonist *N,N*-hexamethylene carbamoyl-Leu-D-Trp(CHO)-D-Trp (BQ610)(Peptide International, Louisville, KY). The right atrium was catheterized via the left jugular vein with PE-20 tubing.

Cardiac output (CO) was measured using the thermodilution method. The accuracy and reliability of this technique for measuring CO is well established (8). The determination of CO was performed by a Cardiomax II-R computer (Columbus Instruments Corp., Columbus, OH.) as previously described (24). Briefly, CO was measured by rapidly injecting 200ul of a cold solution of dextrose water via the left atrial catheter. The Cardiomax II-R determines CO by calculating the time taken for the cold solution to reach a temperature sensitive thermodilution microprobe directed through the carotid artery to the level just above the aortic valve (24). The Cardiomax II-R also provides a constant read out of heart rate and mean arterial pressure (MAP) via the femoral artery catheter and body temperature via the temperature sensitive probe placed in the aortic arch. Stroke volume is calculated by the Cardiomax-II-R from the measured cardiac output and heart rate (24).

Glomerular filtration rate (GFR) and effective renal plasma flow (RPF) were determined by the clearance of inulin-carboxyl [Carboxyl- ^{14}C] and aminohippuric acid P-[glycyl-2- ^3H] respectively (New England Nuclear, Boston, MA). The inulin was infused at a rate of 0.06 $\mu\text{Ci}/\text{min}$ and the PAH at a rate of 0.23 $\mu\text{Ci}/\text{min}$. Aliquots of urine as well as plasma samples (obtained at the midpoint of each clearance period) were counted using a Packard Tri-carb (1600TR) liquid scintillation counter. The technique of full spectrum dual DPM (disintegrations per minute) counting (13) was used to separate the spectra of the two radio-isotopes. The accuracy of the dual label counting technique was verified by us by adding varying amounts of ^{14}C -inulin and ^3H -PAH (over a range from 1×10^2 to 1×10^6 DPM) to separate scintillation vials as well as combined within the same vials. The same DPM was obtained for each radio-

isotope counted either individually or together with the other isotope over this entire concentration range.

2) Protocols: a) Experimental protocol

Effect of endothelin receptor blockade with BQ610 following nitric oxide inhibition with L-NAME (n=12).

Baseline period: After preparation of the rats as outlined above, an infusion of inulin and PAH was begun. After an equilibration period of 30 min, three 15 minute urine collections were obtained for measurement of baseline inulin and PAH clearance. MAP and heart rate were monitored continuously throughout this period.

L-NAME period: At the end of the baseline period a constant infusion of L-NAME (0.12mg/kg/min) was begun and continued until the end of the experiment. After a 30 minute equilibration period, three 15 minute collections of urine were obtained for determination of inulin and PAH clearances as well as MAP and heart rate.

L-NAME + BQ610 period: A bolus of 100ug/kg of BQ610 was administered slowly, followed by a constant infusion of BQ610 (4ug/kg/min) which was continued for the duration of the experiment. After a further 30 minute equilibration period, three 15 minute clearance periods were obtained for clearance measurements, MAP and heart rate.

Duplicate determinations of cardiac output, were made during the baseline, L-NAME and L-NAME + BQ periods and the duplicate results averaged. The measurements of GFR and effective renal plasma flow obtained during the three clearance periods were also averaged.

b) Control protocols

i) Effect of BQ610 following administration of the L-NAME vehicle (n=5)

The protocol for this study was identical to the experimental protocol except that the vehicle for L-NAME (5g/100ml dextrose water infused at a rate of 0.01ml/min) was given instead of L-NAME. The purpose of this protocol was to

determine if ET-A blockade altered systemic or renal resistance in the absence of prior NO inhibition with L-NAME.

ii) Effect of BQ vehicle following L-NAME (n=4)

This protocol was the same as the experimental protocol except that the vehicle for BQ610 (bolus of 250ul of normal saline followed by a constant infusion of normal saline infused at 0.01ml/min) was given after L-NAME instead of the BQ610. The purpose of this protocol was to provide a time control for the effects of L-NAME infusion.

c) Efficacy of BQ610 as an ET-1 antagonist

Rats were treated with BQ610 (in the dose stated above)(n=4) or with the BQ610 vehicle (n=4). Thirty minutes later the blood pressure response to a bolus infusion of endothelin (ET-1 Peptide International, Louisville, KY.) (1.0nM/kg body weight) was compared in the two groups

3) Calculations

Renal plasma flow (RPF) was calculated from the clearance PAH (effective plasma flow) assuming a PAH extraction of 80% (18). The filtration fraction (FF), renal blood flow (RBF), renal vascular resistance (RVR), systemic vascular resistance (SVR) were calculated using standard formulae. Measurements of GFR, RPF, RBF, CO, stroke volume, RVR and SVR were normalized to 300g body weight.

The extent (in %) to which blockade of the ET-A receptor by BQ610 reversed the changes in MAP, CO, SVR and RVR induced by L-NAME was calculated as follows:

- i) Fall in MAP (%): $(\text{MAP during L-NAME period}) - (\text{MAP during BQ610+L-NAME period}) \div (\text{MAP during L-NAME period}) - (\text{MAP during baseline period}) \times 100$
- ii) Fall in SVR (%): $(\text{SVR during L-NAME period}) - (\text{SVR during BQ610+L-NAME period}) \div (\text{SVR during L-NAME period}) - (\text{SVR during baseline period}) \times 100$
- iii) Fall in RVR (%): $(\text{RVR during L-NAME period}) - (\text{RVR during BQ610+L-NAME period}) \div (\text{RVR during L-NAME period}) - (\text{RVR during baseline period}) \times 100$

iv) Increase in CO (%): $(\text{CO during BQ610 period}) - (\text{CO during L-NAME period})$
divided by $(\text{CO during baseline period}) - (\text{CO during L-NAME period}) \times 100$

4) Statistics: All data are expressed as means $\pm$ SE. All comparisons were made using the Student's t test. Whenever more than two groups of data were compared the Bonferroni correction was used to adjust for the comparison of multiple means.

RESULTS

1. Efficacy of BQ610 in antagonizing the hypertensive effect of ET-1

In rats administered endothelin following administration of the BQ610 vehicle, MAP rose from 107 ± 3 to 135 ± 1 mmHg ($p < 0.01$) ($n=4$). In rats pretreated with BQ610, the administration of endothelin thirty minutes later increased MAP from 110 ± 4 to 113 ± 3 mmHg ($p < 0.01$). Thus, BQ610 resulted in an 91% reduction in the ET-1 induced increase in MAP ($p < 0.01$ vs endothelin in the absence of BQ610 pre-treatment).

2. Effect of BQ610 on the systemic and renal hemodynamic effects of L-NAME

MAP rose from 107 ± 2 (baseline period) to 133 ± 3 mmHg following infusion of L-NAME ($p < 0.01$). Subsequent infusion of BQ610 resulted in a fall in MAP to 115 ± 3 mmHg ($p < 0.01$ vs baseline and L-NAME periods) (Figure 1). Cardiac output fell from 90 ± 5 to 66 ± 5 ml/min/300g with L-NAME and then increased to 71 ± 5 ml/min/300g after BQ610 ($p < 0.01$ vs baseline as well as L-NAME periods) (Figure 1). Stroke volume fell with L-NAME from 260 ± 13 ml to 201 ± 14 ml/beat/300g ($p < 0.01$) and remained unchanged during endothelin blockade with BQ610 (196 ± 11 ml/beat/300g) ($p = \text{NS}$ vs L-name period; $p < 0.01$ vs baseline period). Heart rate fell from 350 ± 11 to 320 ± 6 beats/min with L-NAME ($p < 0.01$) and returned to values comparable to control (348 ± 10 beats/min) with BQ610 ($p < 0.01$ compared to L-NAME period). Systemic vascular resistance rose from 1.27 ± 0.06 to 2.17 ± 0.18 mmHg/ml/min/300g in response to L-NAME ($p < 0.01$) and fell to 1.69 ± 0.12 mmHg/ml.min/300g after BQ610 ($p < 0.01$ vs L-NAME and baseline periods) (Figure 1).

GFR fell from 3.0 ± 0.2 to 2.6 ± 0.2 ml/min/300g with L-NAME ($p < 0.01$) but remained unchanged (2.6 ± 0.1 ml/min/300g) following BQ610 (Figure 2). The filtration fraction rose from 32.0 ± 1.0 to $45.0 \pm 2.0\%$ ($p < 0.01$) but was unaltered by BQ610 ($45 \pm 3.0\%$). Urine flow rate rose from 10 ± 3 to 44 ± 9 μ l/min with L-NAME ($p < 0.01$) and then fell to 25 ± 4 μ l/min following BQ610 ($p < 0.01$ compared to baseline and L-NAME periods). RPF fell following L-NAME from 9.5 ± 0.6 to 5.9 ± 0.6 ml/min/300g ($p < 0.01$) while RBF (Figure 2) fell from 17.1 ± 1.2 to 10.8 ± 0.9 ml/min/300g ($p < 0.01$). BQ610 did not alter either RPF (6.3 ± 0.5 ml/min/300g) or RBF (0.8 ± 0.9 ml/min/300g) (Figure 2). Renal vascular resistance rose from 6.4 ± 0.4 to 13.7 ± 1.4 mmHg/ml/min/300g with L-NAME ($P < 0.01$) and then fell to 11.0 ± 0.9 mmHg/ml/min/300g following BQ610 ($P < 0.01$ compared to baseline and L-NAME periods)(Figure 2).

The proportion of the alterations in MAP, SVR and cardiac output induced by L-NAME that were reversed by BQ610 were calculated as described in the methods section. The BQ610 induced reversal of the L-NAME induced rise in SVR ($52 \pm 7\%$) was substantially greater than the reversal of the L-NAME induced fall in cardiac output ($31 \pm 9\%$) associated with ET-A blockade ($p < 0.05$) (Figure 3). As a result, BQ610 reversed the L-NAME associated hypertension to a proportionately greater extent ($76 \pm 10\%$) than the L-NAME associated increase in SVR ($p < 0.01$)(Figure 3). BQ610 reversed the L-NAME induced increases in SVR (by $52 \pm 7\%$) and RVR (by $40 \pm 9\%$) to a comparable extent.

3. Effect of BQ610 on systemic and renal hemodynamic effects when administered in the absence of prior L-NAME infusion.

Systemic and renal hemodynamics as well as renal function were unchanged when BQ610 was administered following administration of the L-NAME vehicle (Table 1).

4. Effect of the BQ610 vehicle following administration of L-NAME

When L-NAME infusion was followed by administration of the BQ610 vehicle, the systemic and renal hemodynamic alterations induced by L-NAME remained unchanged (Table 2).

DISCUSSION

The systemic and renal effects of NO synthase inhibition reported in this study were comparable to those reported in many other studies conducted *in vivo* in both conscious (3,9) and anesthetized animals (1, 22,20,23).

L-NAME caused systemic vasoconstriction, increasing total peripheral resistance and markedly elevating MAP into the hypertensive range (Figure 1). However, the MAP did not increase in proportion to the increase in peripheral resistance because of an associated profound fall in cardiac output (Figure 1) that was due to a decrease in both stroke volume and heart rate. Comparable changes in cardiac output, stroke volume and heart rate have been previously reported in response to L-NAME in conscious rats (9). The fall in heart rate in response to NO inhibition has been ascribed to a reflex baroreceptor response to the hypertension (1,9,23). The factor/s responsible for the substantial fall in stroke volume are less certain since the effects of NO on myocardial function remain incompletely understood and controversial (9,17). In addition to its systemic effects, L-NAME caused marked intrarenal vasoconstriction and a consequent fall in GFR as well as renal plasma flow and blood flow (Figure 2).

We have used (N,N,hexamethylene)carbamoyl-L-Leu-D-Trp(CHO)-D-Trp-OH (BQ610), a specific antagonist of the ET-A receptor (12), to determine the extent to which ET-1 mediates the vasoconstrictor and hypertensive effects of L-NAME. BQ610 is a linear derivative of the cyclic pentapeptide BQ123(cyclo[D-Trp-D-Asp-Pro-D-Val-Leu]) (4) which is also an ET-A selective antagonist. BQ123 has been successfully

used to demonstrate the role of endothelin in mediating intrarenal vasoconstriction in a number of pathophysiologic states of the kidney (14). BQ610 has been reported to be a more potent and selective ET-A receptor antagonist than BQ123 (12). We have demonstrated that the hypertensive effect of endothelin was reversed by the ET-A specific antagonist BQ610, data that argues against any important role for ET-B receptors in the hypertensive response to ET.

In the absence of prior NO inhibition, BQ610 had no effects on renal or systemic hemodynamics (Table 1) suggesting that ET, acting via the ET-A receptor, does not substantially modulate renal or systemic hemodynamics in the euvoletic rat. Also, administration of the BQ610 vehicle after NO inhibition did not alter the systemic or renal hemodynamic changes induced by L-NAME (Table 2).

However, ET-A blockade with BQ610 had marked effects on systemic and renal hemodynamics when administered after L-NAME. BQ610 resulted in a substantial fall in the L-NAME induced increase in both MAP and SVR (Figure 1). These data indicate for the first time that endothelin, acting via the ET-A receptor, contributes to the systemic vasoconstriction induced by NO inhibition.

Interestingly, the fall in MAP associated with BQ610 was proportionately greater than the reduction in systemic vascular resistance (SVR) (Figure 3) because cardiac output did not increase in proportion to the fall in SVR following ET-A receptor blockade (Figure 3). The increase in cardiac output following BQ610 was due entirely to a reversal of the reflex fall in heart rate induced by L-NAME while the depression in stroke volume was unchanged by ET-A receptor blockade. These data suggest that the fall in stroke volume induced by L-NAME, unlike the peripheral vascular constriction, is mediated by mechanisms independent of ET acting via the ET_A receptor.

BQ610 also resulted in a fall in renal vascular resistance (RVR) (Figure 2) suggesting that ET-1 induced vasoconstriction contributes substantially to the

intrarenal vasoconstriction associated with L-NAME. The extent to which BQ610 reduced the L-NAME induced increases in RVR ($40 \pm 9\%$) and SVR was comparable ($52 \pm 7\%$). However, we cannot determine the extent to which changes in RVR in response to BQ610 are due to direct effects of ET-A blockade versus a reflex autoregulatory response to the BQ610 associated fall in blood pressure (2).

Recent evidence suggests that ET-B receptors are present on some smooth muscle cells and that the intrarenal vasoconstriction induced by ET in the rat may be mediated in part by ET-B as well as ET-A activation (27). In contrast to this finding, Chan et al. (7) reported that BQ123, a specific ET-A antagonist, completely reversed the intrarenal vasoconstrictor effects of endothelin in the isolated perfused rat kidney. While the role played by ET-B receptors in mediating intrarenal vasoconstriction remains controversial, we cannot exclude the possibility that we may have underestimated the contribution of endothelin to the intrarenal vasoconstriction induced by L-NAME in this study because we used an ET-A receptor antagonist rather than a nonspecific antagonist of both ET-A and ET-B receptor subtypes (27).

The renal vasodilation induced by BQ610 was not associated with any change in GFR or renal blood flow (Figure 2). This lack of change in renal function or blood flow despite BQ610 induced renal vasodilatation is likely due, at least in part, to the concurrent fall in MAP (Figure 1), and therefore of renal perfusion pressure.

In summary, this study demonstrates for the first time that endothelin, acting via the ET-A receptor, plays an important role in contributing to the systemic and intrarenal effects of inhibition of NO *in vivo*. These data are consistent with the hypothesis that endothelin contributes to the vasoconstriction associated with a number of pathophysiologic states in which endothelial production of NO is compromised. These disease states include hypertension, diabetes mellitus,

atherosclerosis (20) as well as acute ischemic injury to heart, kidney and other organs (7,19,26).

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LEGENDS

Figure 1

The effect of L-NAME followed by BQ610 on mean arterial pressure cardiac output and systemic vascular resistance

(n=12)

*=p<0.01 compared to baseline period; †=p<0.01 compared to L-NAME period

Figure 2

Effect of L-NAME followed by BQ610 on glomerular filtration, renal blood flow and renal vascular resistance

(n=12)

*=p<0.01 compared to baseline period; †=p<0.01 compared to L-NAME period

Figure 3

Proportion of the L-NAME induced changes in MAP, SVR and CO that were reversed by ET-A receptor blockade.

The L-NAME increase in SVR was reversed by BQ610 to a greater extent than the L-NAME associated fall in CO . As a results fall in MAP was substantially greater than the fall in SVR induced by BQ610.

*=p<0.01 compared to MAP

†=p<0.05 compared to SVR

TABLE 1

Effect of the ET_A receptor antagonist BQ610 administered following administration of the L-NAME vehicle

	Baseline	L-NAME vehicle	L-NAME vehicle ± BQ610
Mean arterial pressure (mmHg)	114 ± 2	113 ± 3	112 ± 4
Cardiac output (ml/min/300g)	93 ± 7	88 ± 5	95 ± 5
Glomerular filtration rate (ml/min/300g)	3.0 ± 0.2	3.1 ± 0.3	2.7 ± 0.2
Renal blood flow (ml/min/300g)	17.4 ± 1.2	15.6 ± 0.88	15.0 ± 1.1
Vascular resistance (mmHg/ml/min.300g)			
Renal	6.7 ± 0.7	7.3 ± 0.5	7.8 ± 0.7
Systemic	1.3 ± 0.1	1.3 ± 0.1	1.1 ± 0.1

TABLE 2
Effect of the BQ610 vehicle following administration of L-NAME

	<u>Baseline</u>	<u>L-NAME</u>	<u>L-NAME ±</u> <u>BQ VEHICLE</u>
Mean arterial pressure	110 ± 3	147 ± 6*	142 ± 7*
Cardiac output (ml/min/300g)	104 ± 4	63 ± 4*	61 ± 2*
Glomerular filtration rate (ml/min/300g)	3.3 ± 0.2	2.3 ± 0.2*	2.5 ± 0.2*
Renal blood flow (ml/min/300g)	21.7 ± 1.2	10.7 ± 1.3*	9.3 ± 1*
<u>Vascular resistance (mmHg/ml/min.300g)</u>			
Renal	5.1 ± 0.2	14.4 ± 2*	15.7 ± 1.0*
Systemic	1.2 ± 0.1	2.4 ± 0.2*	2.4 ± 0.2*

*=p<0.01 compared to baseline period

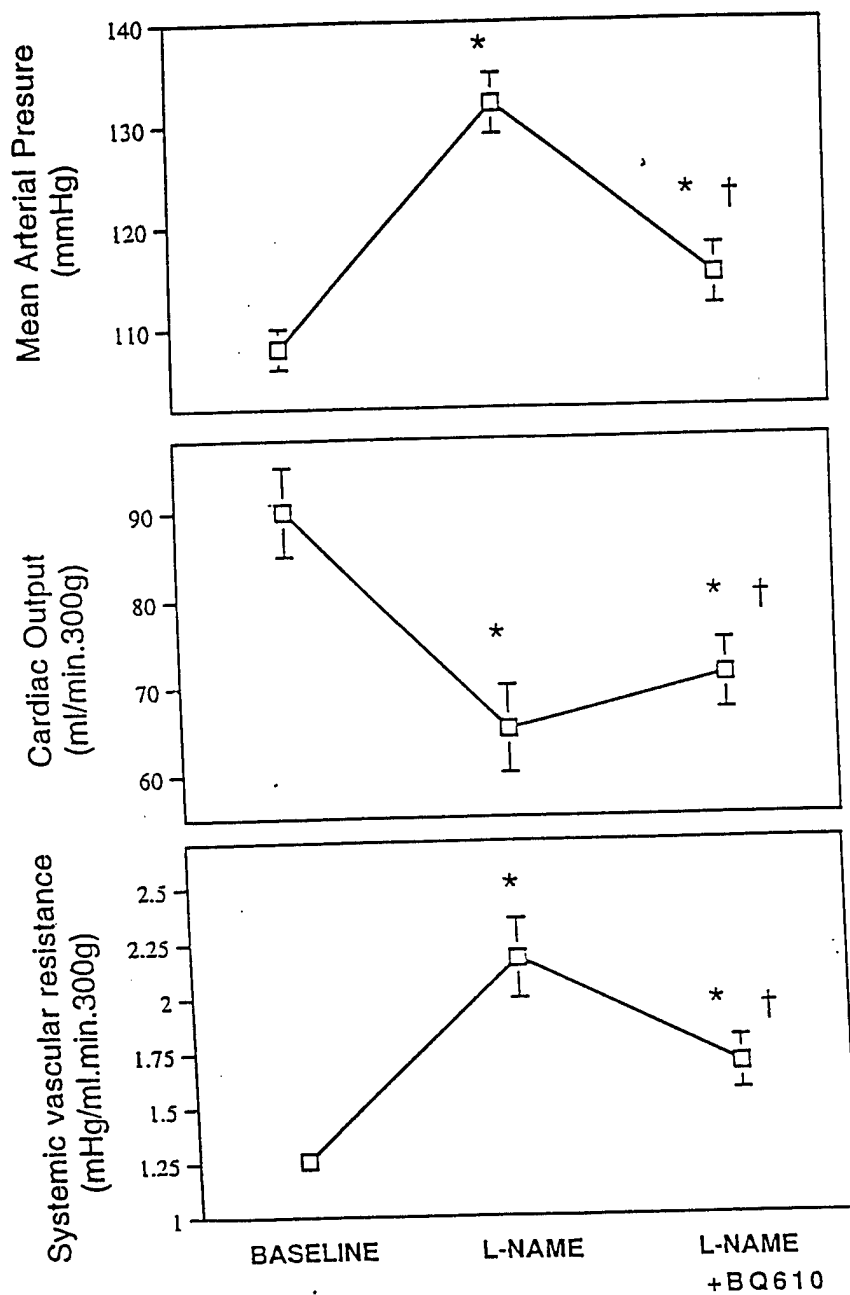
FIGURE 1

FIGURE 2

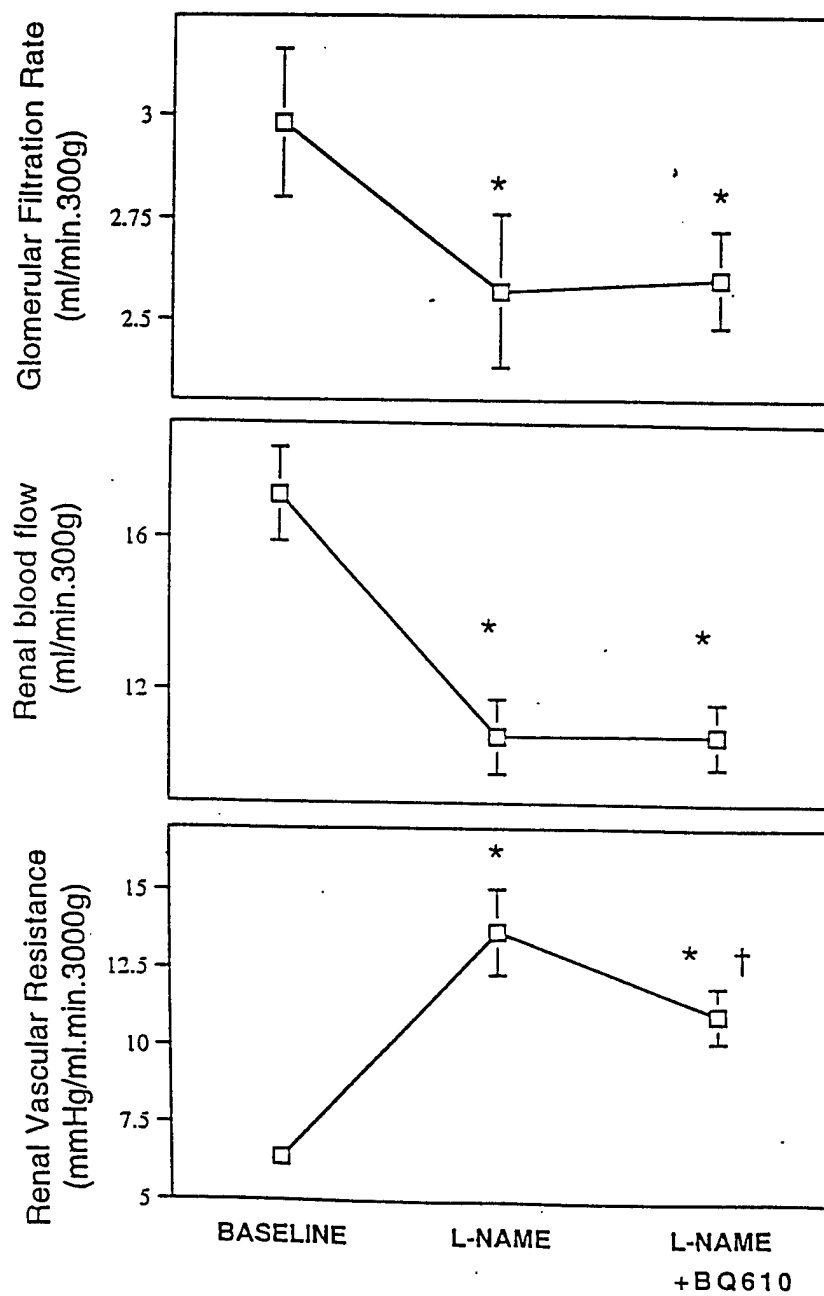


FIGURE 3