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Research Article

Studies were designed to examine the effect of a selective endothelinA (ETA) receptor antagonist, BQ123, on severe postischemic acute renal failure (ARF) in Sprague-Dawley rats. Severe ARF was induced in uninephectomized, chronically instrumented rats by 45-min renal artery occlusion. BQ123 (0.1 mg/kg.min) or vehicle was infused intravenously for 3 h on the day after ischemia. Measurements before infusion (24 h control) showed a 98% decrease in glomerular filtration rate (GFR), increase in fractional excretion of sodium from 0.6 to 39%, and in plasma K+ from 4.3 to 6.5 mEq/liter. All vehicle-treated rats died in 4 d because of continuous deterioration of renal function, resulting in an increase of plasma K+ to fatal levels (> 8 mEq/liter). Infusion of BQ123 significantly improved survival rate (75%) by markedly improving tubular reabsorption of Na+ and moderately increasing GFR and K+ excretion. Plasma K+ returned to basal levels by the 5th d after ischemia. Improved tubular function was followed by gradual recovery in GFR and urinary concentrating mechanism. Additional data from renal clearance studies in rats with moderate ARF (30-min ischemia) and in normal rats with intact kidneys showed that ETA receptor blockade increases Na+ reabsorption and has no effect on renal hemodynamics. These results indicate that in the rat, the ETA receptor subtype mediates tubular epithelial function, and it plays a significant role […]

Reversal of Postischemic Acute Renal Failure with a Selective Endothelin$_A$ Receptor Antagonist in the Rat

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Abstract

Studies were designed to examine the effect of a selective endothelin$_A$ (ET_A) receptor antagonist, BQ123, on severe postischemic acute renal failure (ARF) in Sprague-Dawley rats. Severe ARF was induced in uninephectomized, chronically instrumented rats by 45-min renal artery occlusion. BQ123 (0.1 mg/kg · min) or vehicle was infused intravenously for 3 h on the day after ischemia. Measurements before infusion (24 h control) showed a 98% decrease in glomerular filtration rate (GFR), increase in fractional excretion of sodium from 0.6 to 39%, and in plasma K^+ from 4.3 to 6.5 mEq/liter. All vehicle-treated rats died in 4 d because of continuous deterioration of renal function, resulting in an increase of plasma K^+ to fatal levels (> 8 mEq/liter). Infusion of BQ123 significantly improved survival rate (75%) by markedly improving tubular reabsorption of Na^+ and moderately increasing GFR and K^+ excretion. Plasma K^+ returned to basal levels by the 5th d after ischemia. Improved tubular function was followed by gradual recovery in GFR and urinary concentrating mechanism.

Additional data from renal clearance studies in rats with moderate ARF (30-min ischemia) and in normal rats with intact kidneys showed that ET_A receptor blockade increases Na^+ reabsorption and has no effect on renal hemodynamics. These results indicate that in the rat, the ET_A receptor subtype mediates tubular epithelial function, and it plays a significant role in the pathogenesis of ischemia-induced ARF. Treatment with the selective ET_A receptor antagonist reverses deteriorating tubular function in established ARF, an effect of possible therapeutic significance. (*J. Clin. Invest.* 1994. 93:900–906.) Key words: ischemia • moderate and severe acute renal failure • endothelin$_A$ receptor • BQ123

Introduction

Endothelin (ET)[1] (a 21–amino acid peptide), initially isolated from the endothelial cells, is the most potent vasoconstrictor known to date (1). Endothelin mediates its responses by binding to specific cell surface receptors. Based on the binding profiles and pharmacology of ET peptides, two types of ET receptors (ET_A and ET_B) have been identified, cloned, and sequenced (2, 3). There is accumulating evidence in the literature regarding the possible role of endothelin in the pathogenesis of acute renal failure (ARF) after ischemia. Some of the evidence is circumstantial, such as increased plasma level of ET-1 in patients with ARF (4), elevated tissue level of ET in postischemic kidney (5), or the time-dependent increase in the affinity of renal ET receptors during reperfusion after ischemia (6). More direct evidence has been obtained from studies with ET antibody (5, 7, 8) or receptor antagonist (9). To date, the results indicate that ischemia-induced ARF is attenuated by the infusion of ET antibody or receptor antagonist during ischemia or the early phase of reperfusion.

The profile of changes in renal tissue and plasma levels of ET and in the affinity of renal ET receptors during the postischemic period suggested to us that ET may also be an important factor during the maintenance phase of ARF. The current studies were designed to assess such a possible role. A selective ET_A receptor antagonist, BQ123 (10), was infused 24 h after ischemia in rats with moderate and severe ARF. The effect of the blocker on normal renal function was also evaluated in rats with intact kidneys. The results show that blockade of ET_A receptor 24 h after ischemia effectively reverses severe renal damage. Furthermore, they also indicate that the acute effect of ET_A receptor blockade involves improved tubular reabsorption of Na^+, suggesting that ET may be a significant factor in the genesis of ARF by its contribution to renal tubular dysfunction.

Methods

Animal preparation

All procedures were approved by the Institutional Animal Care and Use Committee (SmithKline Beecham Pharmaceuticals) and were in accordance with National Institutes of Health Guidelines for the care and use of animals.

Young (6 wk old) male Sprague-Dawley rats were obtained from Charles River Labs (Wilmington, MA). They were housed in a light controlled room with a 12-h light/dark cycle and were allowed ad libitum access to food and water. The right kidney was removed from all rats under ketamine hydrochloride (60 mg/kg) and acepromazine (0.3 mg/kg) anesthesia. After uninephectomy, a 2-wk period was allowed for recovery. Vascular catheters were implanted into the abdominal aorta and vena cava via the femoral vessels of all rats 4–5 d before the experiments. In addition, a silastic-covered stainless steel cannula was sewn in the urinary bladder of rats partaking in renal clearance studies. Details of surgery, as well as pre- and postsurgical care were previously described (11). The rats were housed individually after the surgery, first in their home cage and then in metabolic cages. To estab-

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1. *Abbreviations used in this paper:* ARF, acute renal failure; BQ123, selective endothelin$_A$ receptor antagonist; BUN, blood urea nitrogen; ET, endothelin; FE_{Na^+}, fractional excretion of sodium; GFR, glomerular filtration rate; PAH, *p*-aminohippurate; RBF, renal blood flow.

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lish basal values, urine was collected for 24 h, and plasma samples were taken on the day before ischemia.

For the occlusion of the renal artery, the rats were anesthetized with pentobarbital (40 mg/kg i.v.), and the left renal artery was exposed though a small flank incision and occluded with a nontraumatic clamp. Body temperature was maintained at 37°C throughout the occlusion. Occlusion lasted 30 min for the moderate ARF group and 45 min for the severe ARF group. The incision was closed after the removal of the clamp, and the rats were returned to their metabolic cage to recover.

Experimental groups

Prolonged effects of BQ123 treatment. The experiments were performed in the rats exposed to 45-min renal ischemia (severe ARF). They were housed in metabolic cages before ischemia, for 7 d and again on the 14th d after ischemia. Starting at 24 h after ischemia, vehicle or BQ123 was infused for 3 h via the intravenous catheter at a rate of 0.1 mg/kg · min, a dose that effectively (50%) blocks the pressor response to a maximum dose of ET-1. Urine was collected under oil, and daily blood samples (500 μl) were drawn via the arterial line. GFR in this group was calculated from the clearance of endogenous creatinine.

Acute effects of BQ123 treatment. Renal clearance studies were performed in conscious rats with moderate ARF (30-min occlusion). On the day after ischemia, the rats were placed in an experimental cage and set up for renal clearance study as previously described (11). Briefly, throughout the experiment, isotonic saline containing 2.5% inulin (Iso-Tex Diagnostics, Friendswood, TX) and 0.25% *p*-aminohippurate (PAH) (Merck Sharp & Dohme, West Point, PA) was infused intravenously at a rate of 20 μl/min. Urine was collected from the indwelling bladder cannula. 1-h equilibration was followed by two 30-min urine collections (control). Then vehicle or BQ123 (0.1 mg/kg · min i.v.) was infused for 3 h. Six additional 30-min urine collections were performed. A blood sample (300 μl) was drawn at every hour from the arterial line. The plasma was separated, and the cells were resuspended in saline and returned to the rats.

Renal effects of BQ123 in normal rats. The effects of BQ123 on normal renal function were evaluated in two groups of conscious Sprague-Dawley rats with intact kidneys. Total sodium infusion was 1 μEq/min · 100 g in the first group ($n = 5$), 3 μEq/min · 100 g body wt ($n = 6$) in the second group of rats during the renal clearance protocol. Inulin and PAH concentrations of the infusate were 10% and 1%, respectively. After 1-h equilibration and two 30-min control urine collections, BQ123 was infused at 0.1 mg/kg · min i.v., the same rate as in the rats with ARF. Urine collection was resumed 20 min later and two more 30-min collections were obtained. Blood samples were taken at the midpoint of each urine collection.

Analyses

Urinary and plasma concentrations of inulin and PAH were determined by the anthrone method (12) and by the method of Smith et al. (13), respectively. Creatinine, blood urea nitrogen (BUN), and electrolytes were measured using a Synchon AS8 clinical analyzer, osmolality by a model 2430 Precision osmometer. Clearance and excretion data are given as absolute values, expressed per hour for the prolonged study (group 1), per minute for the acute studies (groups 2 and 3). Fractional excretion of electrolytes was calculated as percent of creatinine or inulin clearance.

Values are expressed as means±SEM. ANOVA, Tukey compromise, Fisher's exact (to compare survival), and Wilcoxon (nonparametric) tests were used, and the differences were considered significant at $P < 0.05$.

Materials

BQ123 was synthesized in the Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals (King of Prussia, PA).

Results

Prolonged effects of BQ123 treatment: severe ARF. The pertinent data are presented in Table I (only for the BQ123-treated group) and in Figs. 1 and 2 for both vehicle and BQ123-treated groups. In unilaterally nephectomized, chonically catheterized rats, 45-min occlusion of the renal artery leads to severe ARF, with a mortality rate of 95–100% in 3–4 d after ischemia. As illustrated in Fig. 1 *A*, all vehicle-treated rats in this study died within 3 d after treatment. In contrast, all rats treated with BQ123 survived for 4 d and 75% fully recovered. GFR was barely detectable (1.8 ml/h, 1.5% of basal) on the day after ischemia (24 h control; Table I, Fig. 1 *B*). BQ123 treatment elicited gradual improvement in GFR with a significant increase by the first day after treatment. However, GFR was still 75% below control level on the 6th d, but fully recovered in 2 wk. The time course of changes in plasma creatinine (Fig. 1 *C*) and BUN (not shown) also indicate a slow improvement in renal hemodynamic function.

Table I. Recovery of Renal Function from Severe ARF After Treatment with BQ123

			Days after treatment with BQ123						
	Baseline	24 hr after ischemia	1	2	3	4	5	6	14
Number of rats	8	8	8	8	8	8	7	6	6
Body weight (g)	313±6	289±6‡	254±5*	245±5*‡	227±6*‡	219±6*‡	223±5*‡	228±12*‡	328±8*
P creatinine (mg %)	0.5±0.04	3.66±0.04‡	5.03±0.25*‡	5.06±0.5*‡	4.41±0.6‡	3.09±0.7‡	2.49±0.6*‡	1.45±0.4*‡	0.6±0.04*
GFR (ml/h)	118±8	1.80±0.5‡	3.86±0.9*‡	5.43±1.3*‡	9.1±2.9*‡	14.1±4.3*‡	16.9±4.8*‡	29.8±7.0*‡	133.4±18*
Na^+ excretion (μEq/h)	107±7	79.7±12‡	98.5±8	36.2±5*‡	32.3±4*‡	23.3±5*‡	40.6±5*‡	53.6±8*‡	131.2±19*
K^+ excretion (μEq/h)	258±12	47.8±9‡	79.3±5*‡	95.8±5*‡	89.9±8*‡	88.4±8*‡	98.9±9*‡	140.3±14*‡	267.7±24*
FE of sodium (%)	0.6±0.02	38.5±2‡	21.3±3.1*‡	5.6±1.1*‡	4.5±1.4*‡	2.4±1.1*‡	2.1±0.8*‡	1.7±0.6*‡	0.76±0.13*
FE of potassium (%)	55.6±4.9	441±13‡	366±20*‡	361±37*‡	293±45*‡	247±53*‡	195±44*‡	136±40*‡	50±9*
Uosm (mosmol/kg H_2O)	1187±51	379±14‡	471±11*‡	511±11*‡	566±18*‡	604±25*‡	644±22*‡	729±27*‡	1073±76*

Values are means±SEM. FE, fractional excretion; Uosm, urine osmolality.
* Significantly different from 24 h control; ‡ Significantly different from basal values ($P < 0.05$).

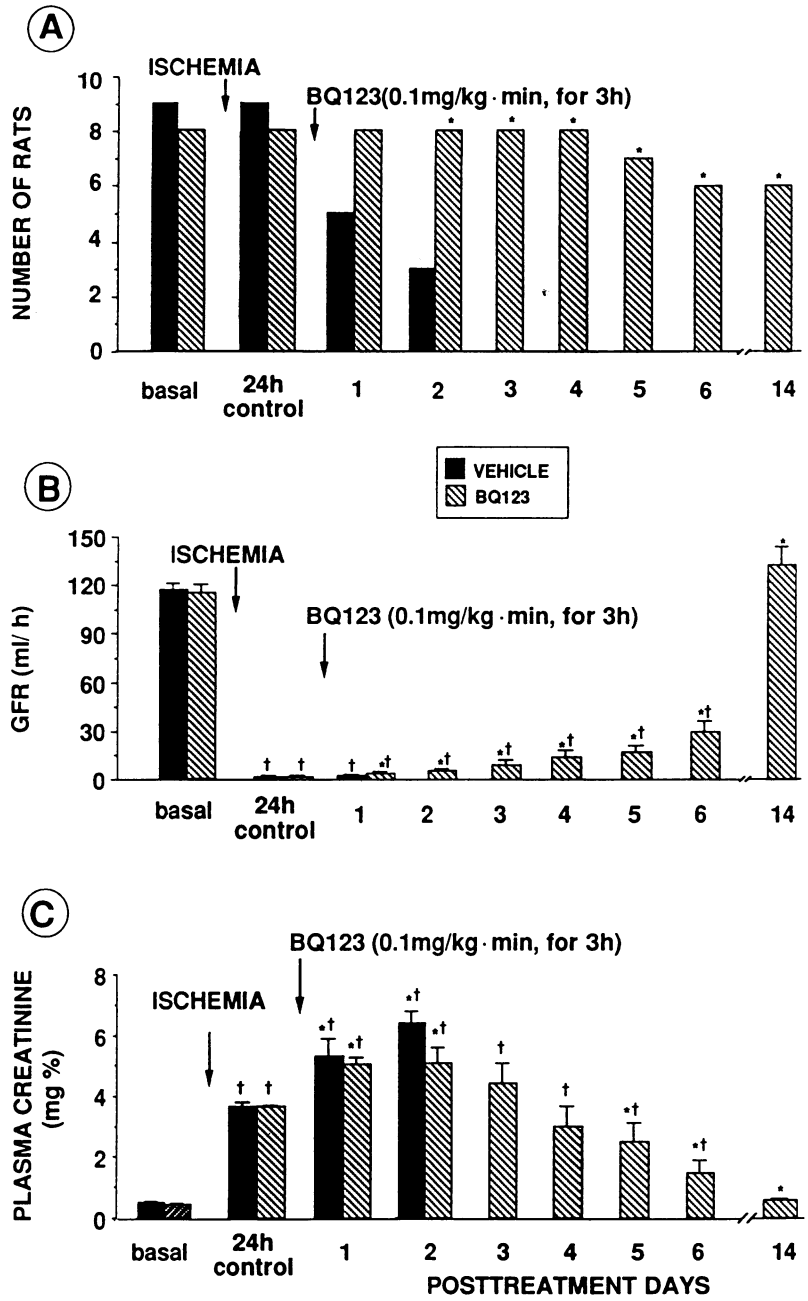

Figure 1. Effects of vehicle or BQ123 treatment on survival rate (*A*), GFR (*B*), and plasma creatinine (*C*) in rats with severe postischemic ARF. Vehicle or BQ123 was infused for 3 h on the day after ischemia. Basal values represent data from the day before ischemia; 24 h control values serve as pretreatment control. *Significant difference from vehicle-treated group in *A*, from 24 h control in *B* and *C*. †Significantly different from basal values, $P < 0.05$.

As shown in Fig. 2 *A* and in Table I, fractional excretion of sodium (FE_{Na^+}) increased from 0.6±0.02 to 38.5±2% during the first 24 h after 45-min renal ischemia. Thus in spite of minimal GFR, absolute Na^+ excretion decreased by only 26%, from 107±7 to 79.7±12 μEq/h. Excretion of K^+ was down by 85±3% (Fig. 2 *B*), resulting in a significant increase in the level of plasma K^+ (Fig. 3 *C*). These changes in tubular function were irreversible in the vehicle-treated rats, resulting in a fatal concentration of plasma K^+ (> 8 mEq/liter) by the 2nd or 3rd d after ischemia (Table I).

BQ123 treatment elicited a rapid improvement in tubular function. Na^+ reabsorption improved markedly, FE_{Na^+} decreased significantly during the 1st d after treatment (Fig. 2 *A*). The improvement continued at a fast rate during the next few days, in contrast to the slow recovery in GFR. Concomitant with the twofold increase in GFR, there was a similar increase in K^+ excretion on the first day after BQ123 treatment (Table I, Fig. 2 *B*). The increase in plasma K^+ was reversed and returned to basal level by the 4th d after treatment (Fig. 2 *C*). All other measured parameters of renal function returned to normal by the end of 2 wk after ischemia.

Acute effects of BQ123 treatment: moderate ARF. Technical difficulties (too low GFR) prevented us from performing acute renal clearance studies in the severe ARF model. Therefore, these studies were carried out in rats with moderate ARF. As the results in Fig. 3 show, GFR and renal blood flow (RBF) were decreased by 90 and 75% from basal values, respectively, at 24 h after 30 min ischemia. GFR increased gradually (not significantly) during the 3-h infusion of BQ123, and RBF remained unchanged (Fig. 3 *A* and *B*). In contrast, the effect of BQ123 on the excretion of electrolytes was acute and highly significant. FE_{Na^+} decreased from 7.2±0.8 to 1.5±0.4% during the 3-h infusion (Fig. 3 *C*) and K^+ excretion (absolute) increased from 1.29±0.03 to 3.65±0.1 μEq/min (not shown).

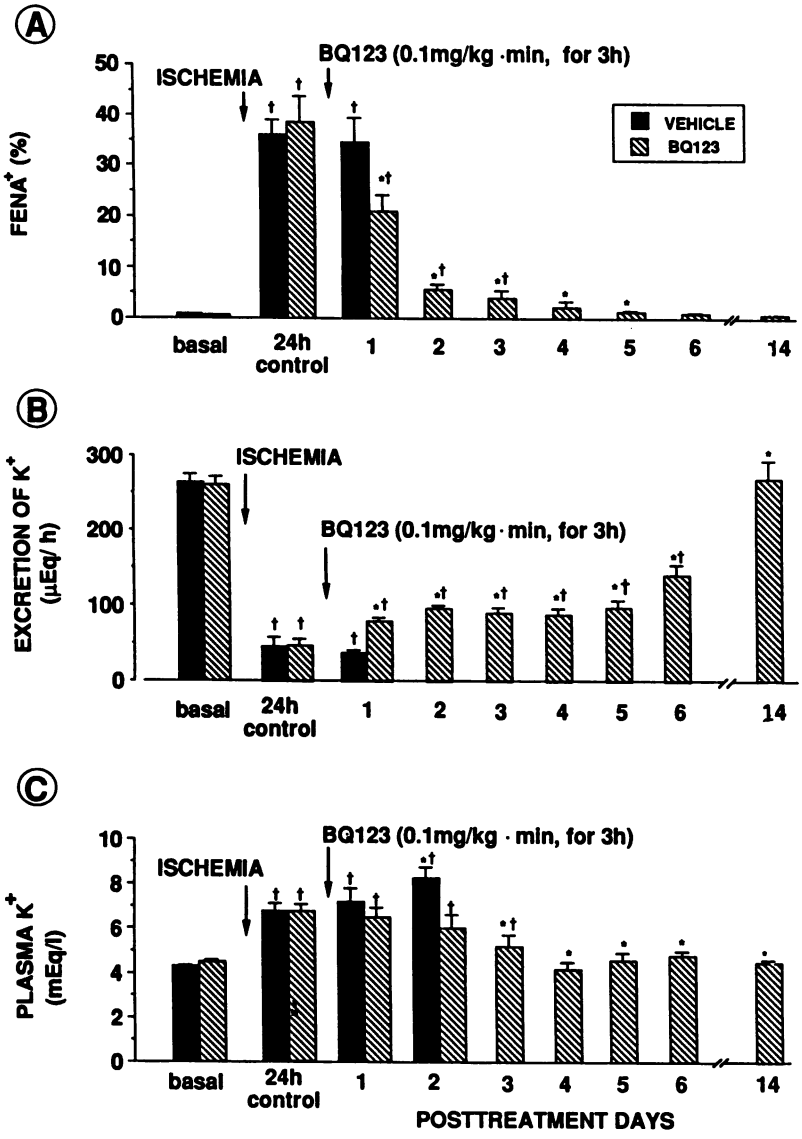

Figure 2. Effects of vehicle or BQ123 treatment on (*A*) fractional excretion of sodium (FE_{Na^+}), (*B*) urinary excretion of K^+, and (*C*) plasma K^+ in rats with postischemic ARF. *†Significant differences from 24 h control and basal values, respectively, $P < 0.05$.

Plasma K^+ level (4.5±0.2 mEq/liter) of rats treated with BQ123 was also significantly lower at 48 h after ischemia (1 d after treatment) compared to the vehicle-treated rats (5.2±0.1 mEq/liter). Vehicle treatment had no effect on any of the measured indices.

Renal effects of BQ123 in normal rats. When infused in conscious rats with intact kidneys, BQ123 infusion resulted in moderate (not significant) lowering of blood pressure from 113±4 to 106±3 mmHg, but had no effect on renal blood flow, which averaged 21.8±1.3 ml/min. As shown in Fig. 4, the ET_A receptor antagonist lowered urine flow in both the low (Fig. 4 *A*) and the high sodium groups (Fig. 4 *B*) without changing GFR. Decrease in osmolal excretion alone accounted for the drop in urine flow. As shown by the changes in the fractional excretion of electrolytes, BQ123 significantly increased Na^+ reabsorption and also decreased K^+ excretion in both groups.

Discussion

The results of these studies show that in rats with severe ARF, blockade of the ET_A receptor subtype with a selective antagonist, BQ123, initiated a regeneration process that normally occurs in moderate ARF (for review see reference 14). The data also indicate that BQ123 effected an immediate improvement in Na^+ reabsorption and K^+ excretion, which led to the reversal in the increase and eventual lowering of plasma K^+. The initial improvement in tubular function was followed by a slow but complete recovery in GFR and urinary concentrating mechanism.

A role for ET in the pathogenesis of ischemia-induced ARF has been proposed and supported by various experimental evidence. For example, progressive increase in ET-1 levels in plasma (4), renal tissue (5), and cultured kidney cell lines (15) after ischemia has been observed. The measured increase in renal tissue ET-1 levels could result from increased synthesis, decreased degradation, or both. We recently reported that ET receptor affinity increases in the renal cortex during the first 24-h postischemic period parallel with functional impairment (6). Results from earlier work with ET antibodies (5) and a recent study with BQ123 (9) suggested an involvement for ET during the early phase of ARF. We also found in preliminary studies that when infused for 2 h starting at 30 min before occlusion, BQ123 attenuated postischemic ARF, but only at a

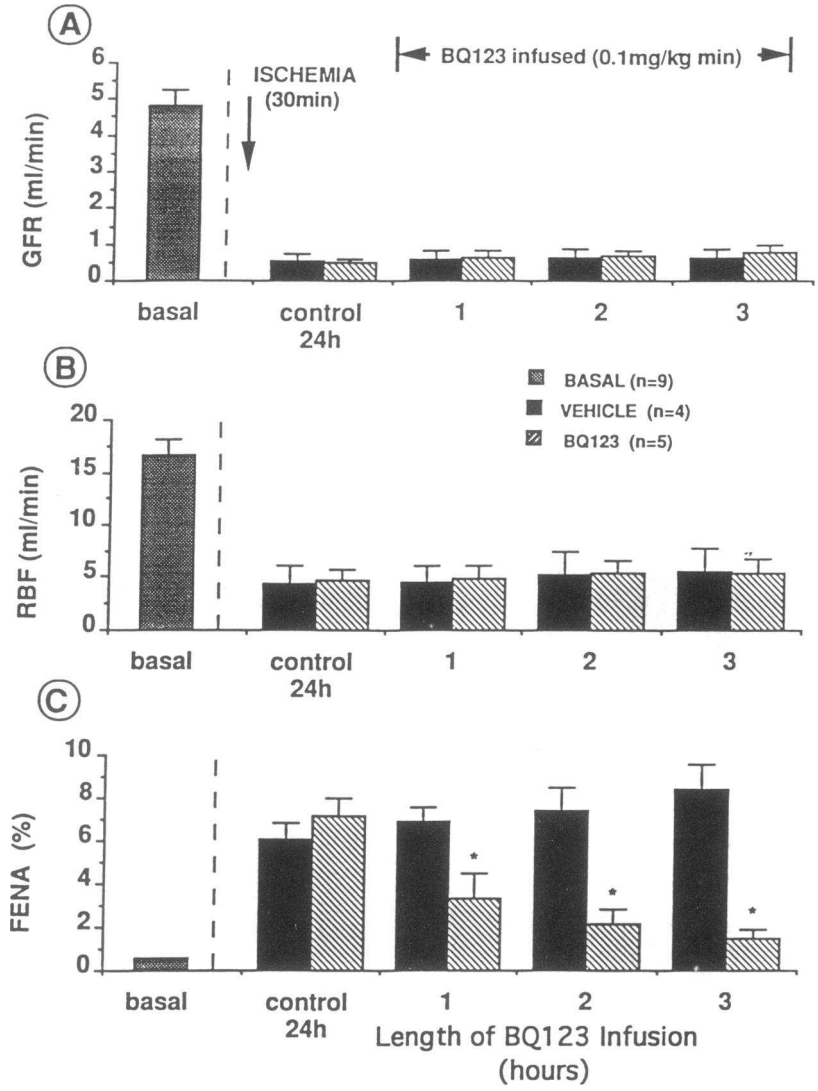

Figure 3. Effects of vehicle or BQ123 infusion on GFR (*A*), RBF (*B*), and FE_{Na+} in rats with moderate postischemic ARF. *Significantly different values from those of vehicle-treated group at same time interval, $P < 0.05$.

high dose (0.3 mg/kg · min) and only in moderate ARF. The efficacy of the blocker in reversing established severe (terminal if not treated) ARF at a lower dose leads us to conclude that ET plays a critical pathogenic role during the maintenance phase. An earlier report by Kon and colleagues (7), showing amelioration of 48-h postischemic ARF with an ET antibody, supports such a role for ET. Perhaps ET is one of the factors proposed by Mason and co-workers (16) that is responsible for cell deterioration and eventual death during the later stages of reperfusion.

The possible mechanisms mediating the initial changes in

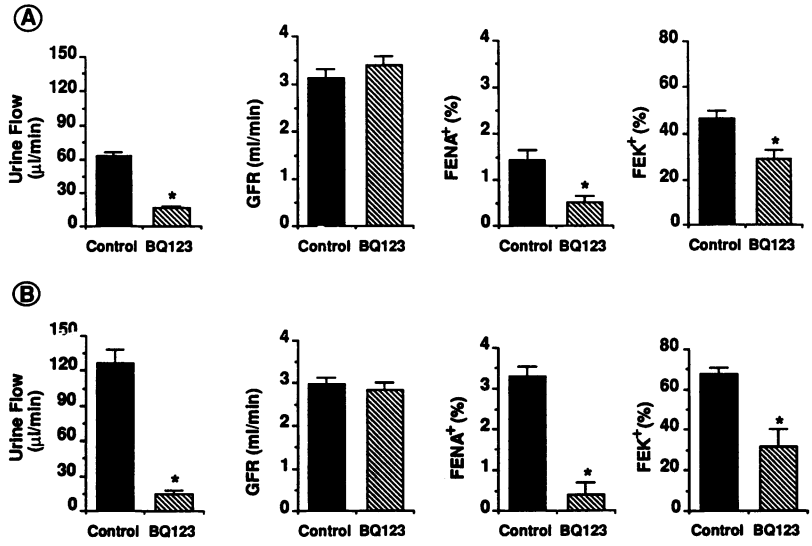

Figure 4. Effect of BQ123 in conscious, normal rats infused with total NaCl at a rate of 1 µEq/min · 100 g (*A*) or 3 µEq/min · 100 g (*B*). Bars represent average values of 2 × 30 min clearance periods during control or during infusion of 0.1 mg/kg · min BQ123. *Significantly different vs control values, $P < 0.05$.

tubular function after BQ123 administration or the cascade of events leading to full recovery cannot be inferred from our results at this time. First of all, there is scarce information on the two ET receptor subtypes' role in the kidney, and the available evidence is conflicting. On the one hand, results from radioligand binding (17), as well as mRNA measurements (18), suggest that ET_A receptors are located primarily on vascular smooth muscle cells and ET_B receptors are found on the tubular epithelial cells. In contrast, there is emerging evidence from whole animal studies with selective agonists and antagonists of ET_A and ET_B receptors, indicating that at least in the rat, renal vasoconstriction is not mediated by the ET_A receptor (19, 20). Early attempts to assess the tubular effects of endothelin mostly involved infusion of exogenous ET-1. Results of a current study by Uzuner and Banks in anesthetized rats indicate that the ET-1–induced increase in sodium excretion is mediated by an increase in blood pressure (21). We have made similar observations in the conscious rats (unpublished observations), leading us to conclude that selective antagonists to the ET receptor subtypes will have to be used to elucidate the tubular effects of endogenous ET. Our results from the clearance studies in conscious rats with intact kidneys (Fig. 4) clearly show that ET_A receptors mediate the urinary excretion of Na^+ and have no detectable role in the control of GFR, renal blood flow or resting blood pressure. The observation that blockade of ET_A receptors in these rats resulted in a reduction of Na^+ excretion to the same level, regardless of its infusion rate, would suggest that ET may serve as a "natriuretic hormone" in the rat. The decrease in K^+ excretion in the normal rats could be explained by the decrease in urine flow and/or Na^+ excretion, but a direct effect of ET_A receptors on the tubular handling of K^+ cannot be inferred. It is not clear at this time whether the ET_A receptor–mediated natriuresis is caused by direct inhibition of Na^+-K^+-ATPase reported by Garvin and Sanders (22) or to some secondary factors mediated by the ET_A receptor.

Secondly, the physiologic effect of ET on tubular epithelial cells may be multifaceted, resulting from interaction of a variety of signal transduction pathways activated by ET. In addition to Na^+-K^+-ATPase, ET analogues have been shown to modulate many signal transduction pathways, including activation of Ca^{2+} channel, phospholipase (A_2, C, and D), nitric oxide synthase, etc. (for review see reference 23). Most of these factors have been implicated in the pathogenesis of ARF or in the regenerative process (24). It remains to be determined how these pathways are affected after ET_A receptor blockade. Nevertheless, it is evident from our functional data that ET contributed significantly to tubular epithelial malfunction after ischemia. In the vehicle-treated rats, impaired tubular function and reduced GFR led to elevation of plasma K^+ to fatal levels within 2–3 d. Infusion of BQ123 reversed the deteriorating tubular function and initiated a recovery process by increasing Na^+ reabsorption, GFR, and urinary excretion of K^+. The data listed in Table I clearly show that the increase in K^+ excretion was caused by increased filtration; its fractional excretion actually decreased. Extrapolation from these results would suggest that increased Na^+-K^+-ATPase activity after ET_A receptor blockade may well be the triggering mechanism of increased Na^+ reabsorption. Improved potassium excretion may contribute to the gradual decrease in extracellular K^+ level, one of the putative stimulants of growth factor release by the tubular epithelial cells (25, 26). However, other factors may be more important in bringing on improvement in the internal K^+ balance; e.g., decrease in cell death or moderation of metabolic acidosis. Further studies are needed to characterize these, as well as the specific morphologic changes and biochemical pathways during the regenerative process, leading to eventual restoration of GFR and urinary concentrating mechanism.

From the observations presented here, we conclude that ET is an important factor in the kidney during the prolonged reperfusion phase after ischemia and contributes significantly to the deterioration of the tubular epithelial cell function. This effect is mediated by the ET_A receptor subtype, whose major role under normal physiologic conditions in the rat kidney appears to be controlling sodium reabsorption. Severe ischemia-induced ARF is reversed by the selective ET_A receptor antagonist BQ123 by significantly improving the tubular reabsorption of Na^+, and moderately increasing GFR and K^+ excretion. A possible role for the ET_B receptor subtype cannot be addressed at this time, because of the lack of ET_B-selective antagonist. These findings may be of clinical importance, providing a possible therapeutic approach in the treatment of postischemic ARF during the maintenance phase.

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