ENDOTHELIN-INDUCED NATRIURESIS AND DIURESIS ARE PRESSURE DEPENDENT EVENTS IN THE RAT

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by

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ABSTRACT

Doses of endothelin-1 (ET) that do not markedly affect the glomerular filtration rate (GFR) cause a natriuresis and diuresis. The goal of the current study was to determine the mechanism by which endothelin induces this renal action. ET was infused into pentobarbital anesthetized female rats, i.v., at 50 ng kg^{-1} min^{-1} for 30 min. In Group I (controls, n=6; n=5 in all other groups), ET increased mean arterial blood pressure (MAP) from 95±2 (mean±SE) to 131±2 mmHg, Na excretion ($U_{Na}V$) from 0.34±0.07 to 1.83±0.2 μEq/min and urine flow rate (V) from 13±1 to 24±3 μl/min (all $p<.01$ vs baseline). At 15 min during infusion of ET, the GFR was not affected (2.1±0.1 to 2.2±0.1 ml/min) but modestly decreased to 1.8±0.1 ml/min at 30 min ($p<.05$ vs baseline). To determine to what extent an intact renal capsule is required for ET-induced natriuresis and diuresis, the capsule was removed from both kidneys during surgery (Group II) and to determine to what extent increases in renal arterial pressure contribute to ET-induced natriuresis and diuresis, this variable was fixed at the baseline value by means of an adjustable clamp positioned on the aorta above the exit of the right renal artery (Group III); in both of these experiments the ET-induced increases in $U_{Na}V$ and V were abolished. To determine to what extent prostaglandins are involved in ET-induced natriuresis and diuresis, experiments (Group IV) with meclofenamate (5 mg bolus injection) were performed; meclofenamate did not alter the ET-induced increase in MAP, V or $U_{Na}V$. To determine the intrarenal site of action of ET, experiments were conducted with ET plus amiloride (0.3 mg/kg bolus followed by 0.04 mg kg^{-1} min^{-1}) (Group V) or with a combination of amiloride + furosemide (0.8 mg/kg bolus followed by 0.1 mg kg^{-1} min^{-1}) (Group VI); there was a larger ET-induced diuresis and natriuresis in amiloride-treated rats and an even larger response with amiloride + furosemide compared to controls. Finally, as a control for the decapsulation procedure, amiloride alone (0.2 mg bolus followed by 0.02 mg/min for 45 min) resulted in

similar increases in V and $U_{Na}V$ in intact rats (Group VII) and in rats without renal capsules (Group VIII). Our data indicate that ET-induced natriuresis and diuresis are arterial blood pressure-related phenomena (pressure natriuresis and diuresis) resulting from an inhibition of sodium reabsorption proximal to the thick ascending limb, and that changes in prostaglandin synthesis are not involved.

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I. Introduction and Specific Aims

The goal of this thesis is to elucidate the mechanism of endothelin-1 (ET-1) induced increases in urinary sodium excretion and urine flow rate.

Although increased renal perfusion pressure (RPP) results in a natriuresis without significant changes in renal blood flow or glomerular filtration rate (GFR), a phenomenon commonly referred to a pressure natriuresis (14,31,34,35,92,110,115,125), the mechanism(s) of pressure natriuresis still remains to be fully elucidated. Interest in the pressure natriuresis mechanism is largely due to the fact that pressure natriuresis has been attributed to a central component of a feedback system for long term control of extracellular fluid volume and arterial pressure (35,36,37,41,42).

Endothelin (ET) is a 21 amino acid, endothelium derived peptide with powerful biological effects (19,46,68,71,74,75,80,136,141,143). The administration of pharmacologic doses of this peptide to experimental animals elicits a marked and sustained increase in arterial pressure and systemic vascular resistance, a decrease in cardiac output, a profound reduction in renal plasma flow (RPF) and glomerular filtration rate (GFR) and a fall in sodium excretion as a result of the increased vascular resistance and contraction of mesangial cells (3,6,59,68,79,82). Release of prostanoids and endothelium-derived relaxing factor (EDRF)/nitric oxide (NO) have been suggested to be the endogenous modulators of vascular responses to the endothelins (17,104,118). A competitive analog of L-arginine, that prevents the formation of nitric oxide, blocks the hypotensive effects of ET-1 (106), suggesting that ET-1 induces the release of EDRF. Moreover, ET-1 induces the release of vasodilatory prostaglandins (11,102,109). ET-1 reduces oubain sensitive Na-K ATPase activity at picomolar concentrations in inner medullary collecting duct cells (145). The inhibition of Na-K ATPase appears to be a prostaglandin dependent process. In addition, in vivo and in vitro studies show that ET also modulates the synthesis and release of several endogenous substances such as renin. ET-1 infusion is associated with increased plasma renin activity when ET is

administered at doses associated with a marked systemic and renal vasoconstrictor response (82). However, the effect of ET-1 on isolated juxtaglomerular cells or glomeruli is inhibition of renin release (105,131,132).

There are number of interactions between endothelin and other hormone systems. Endothelin stimulates aldosterone biosynthesis in isolated zona glomerulosa cells (15), and stimulates release of aldosterone in vivo (15,27,82). ET-1 inhibits vasopressin induced cAMP accumulation in the distal nephron, thereby exerting a blockade of the antidiuretic effect of vasopressin (124,134,135). Co-infusion of pharmacological doses of atrial natriuretic peptide (ANP) blunts the systemic and renal effects of endothelin (53). Although ET-1 is a potent secretagogue of ANP (24,87), it is unclear whether ANP exerts a counterregulatory influence in vivo (27,82,89,90). Water deprivation leads to release of ET from hypothalamic neurons (144). It has been suggested that the potent effect of endothelin on microvascular tone (21,60,81,136) and the rapid clearance of circulating ET-1 in the pulmonary vascular bed indicate that locally derived ET-1 may be an important regional, or paracrine, cardiovascular control mechanism (17,74). The available evidence summarized above indicates that ET-1 could have a primary physiological role in many aspects of the modulation of extracellular fluid volume and blood pressure homeostasis.

It is evident that this novel peptide affects factors that influence the regulation of extracellular fluid volume and composition in a dose-dependent manner because there are some reports that infusions of relatively low doses of ET into rats are associated with significant increases in MAP and a natriuresis and diuresis (27,43,60,130). Although changes in the plasma concentration of atrial natriuretic peptide, lipoxygenase pathway products and reductions in the Na-K ATPase activity in inner medullary duct cells have been suggested to mediate the endothelin induced natriuresis and diuresis, the relationship between endothelin-induced increases in renal perfusion pressure and a potential resultant pressure-induced natriuresis and diuresis have not been fully

evaluated (87,102,145). Therefore, the goals of the current study were a) to determine whether endothelin-1 induced natriuresis and diuresis are pressure-related phenomena, b) to determine the intrarenal site of action of endothelin and c) since prostaglandins have been proposed to mediate pressure-dependent natriuresis and diuresis (33,35,92,100,113,114), to determine to what extent prostaglandins mediate the natriuretic and diuretic responses to the peptide

Experiments designed to test the preceding objectives had the following four specific aims:

1. **Constant renal perfusion pressure**

Determine to what extent prevention of the renal arterial pressure rise during ET-1 infusion alters the endothelin-induced natriuresis and diuresis.

2. **Acute bilateral renal decapsulation**

Determine to what extent removing the renal capsule affects endothelin-induced changes in renal function.

3. **Inhibition of prostaglandin synthesis**

Determine to what extent the inhibition of endogenous prostaglandin synthesis with meclofenamate alters ET-1 induced natriuresis and diuresis.

4. **Distal blockade of sodium reabsorption**

Differentiate between proximal and distal tubular sites of endothelin action by utilizing the technique of distal blockade in which sodium reabsorption in the distal nephron is inhibited with amiloride or with a combination of amiloride plus furosemide.

Since neither the mechanism of pressure-induced natriuresis and diuresis nor the mechanism of ET-induced natriuresis and diuresis are currently clear, the data generated from these experiments should provide information with regard to the possible modulation of extracellular fluid volume and composition by the novel peptide, endothelin.

II. Literature Review

A. Pressure natriuresis and Diuresis

Although acute changes in renal perfusion pressure (RPP) are known to alter tubular reabsorption of water and electrolytes (125), the mechanisms responsible for the change in sodium reabsorption have not been fully defined (14,31,34,42,116). The pressure natriuresis and diuresis hypothesis was originally proposed by Guyton and associates (35) to explain this phenomenon; some of these factors are summarized in Figs. 1 and 2. They argued that whenever arterial pressure is elevated, the pressure diuresis mechanism would increase the loss of sodium and water to reduce the blood volume and, thereby, produce a fall in arterial pressure. If the pressure natriuresis phenomenon occurs via a non-adaptive mechanism, this process would be sustained until the level of arterial pressure returned to the control value. Thus, chronic hypertension could develop only when renal function was reset to higher values so that the ability of the kidney to carry out pressure natriuresis and diuresis was compromised (34,36,37,41,42).

It is believed that pressure natriuresis and diuresis is an intrinsic property of the kidney. Thus, it can be demonstrated in perfused isolated kidneys (52,93) suggesting that it is independent of neural or circulating humoral control (35). Furthermore, studies in rats (39,91,92,111,112) have shown that pressure natriuretic and diuretic responses can occur without significant changes in glomerular filtration rate (GFR) or renal blood flow (RBF) indicating an unchanged filtered load of sodium. Thus intrarenal events might involve decreases in renal tubular sodium reabsorption during changes in renal perfusion pressure (RPP).

The mechanism of pressure natriuresis and the nephron segments involved are not clear. Thus, the proximal tubule (5,20,22,67), the limb of the loop of Henle (39,125)

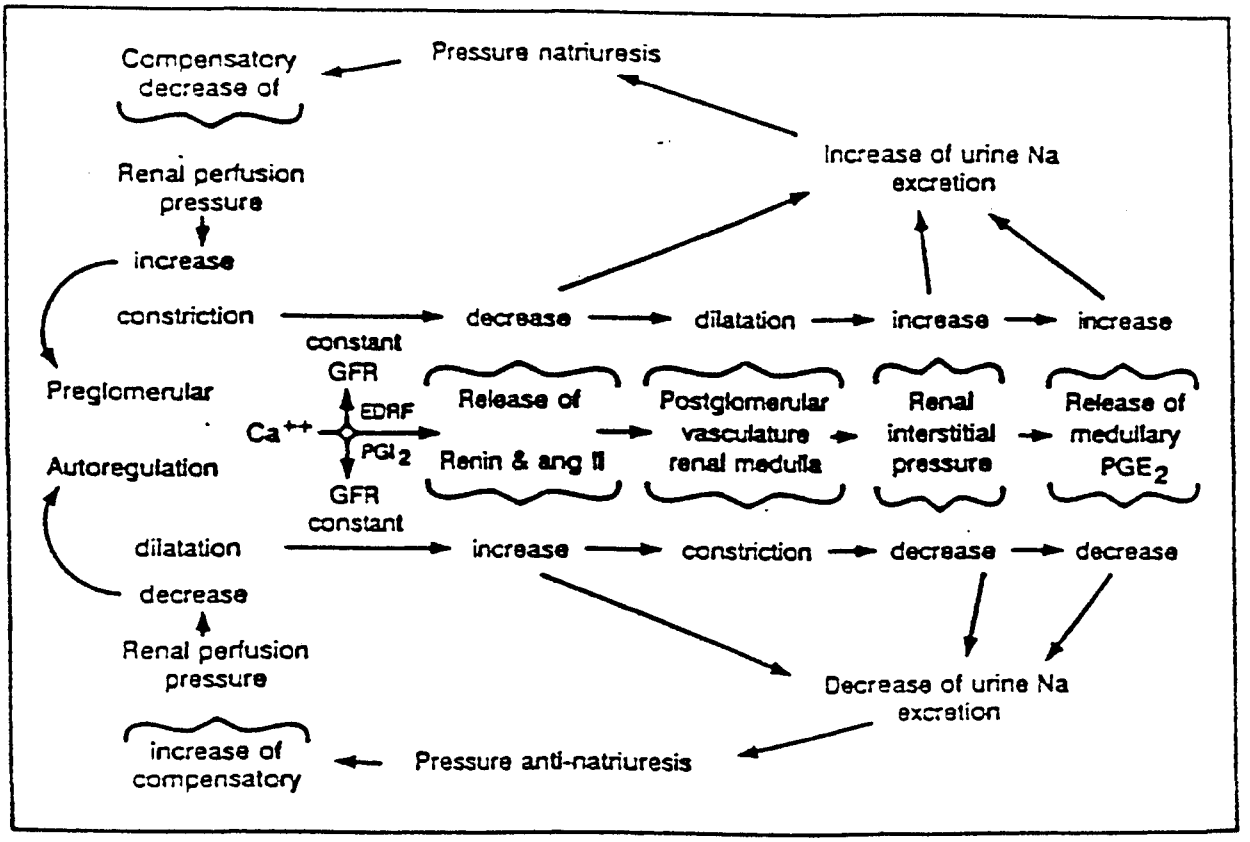

Fig. 1. Possible intrarenal sequence of events induced by increments or decrements in renal perfusion pressure that are involved in the regulation of pressure-related natriuresis. ang II= angiotensin II; Ca^{++}= calcium ions; EDRF= endothelium derived relaxing factor; GFR= glomerular filtration rate; Na= sodium; PGE_2 and PGI_2= prostaglandins E_2 and I_2. (Reproduced from; ROMERO, J.C., M. D. BENTLEY, S. C. TEXTOR AND F.G KNOX. Alterations in blood pressure by derangement of the mechanisms that regulate sodium excretion. Mayo Clin. Proc. 64: 1425-1435, 1989.).

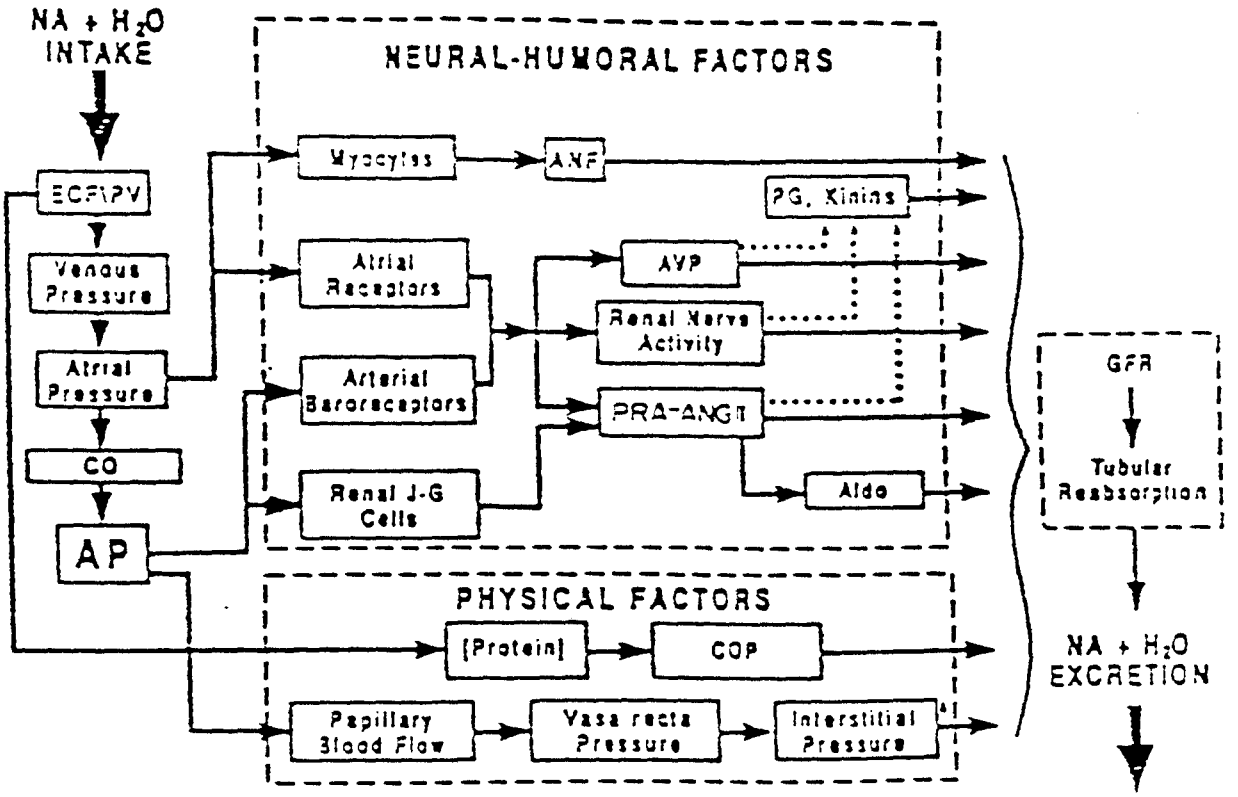

Fig. 2. Schematic representation of mechanisms whereby changes in blood volume are sensed and signals are transduced via neural-humoral and physical factors to control sodium and water retention. Aldo= aldosterone; ANF= atrial natriuretic factor; ANG II= angiotensin II; AP= arterial pressure; AVP= arginine vasopressine; CO= cardiac output; COP= colloid osmotic pressure; ECF= extracellular fluid; GFR= glomerular filtration rate; J-G= juxtaglomerular; PG= prostaglandins; PRA= plasma renin activity; PV= pressure-volume. (Reproduced from; COWLEY, A. W. JR. Long-term control of arterial blood pressure. Physiol. Rev. 72(1): 231-300, 1992).

and collecting duct (66,91,129) have all been suggested as the site where perfusion pressure alters tubular reabsorption. An inhibition of sodium reabsorption in the proximal tubule and/or thin descending limb of the loop of Henle of deep nephrons has been reported (14). It is also associated with changes in renal interstitial hydrostatic pressure (RIHP) and in the pressure and flow of the vasa recta circulation (111,112,115,116). A current hypothesis is "In the absence of adequate autoregulation of papillary blood flow, vasa recta capillary pressure increases with elevations of renal perfusion pressure, the rise in vasa recta capillary pressure would inhibit fluid uptake from the papilla, and renal interstitial fluid pressure would increase" (Fig. 3) (14). It is proposed that elevations of interstitial fluid pressure (RIHP) reduce the net tubular reabsorption of sodium and water (31,32).

Direct measurements of renal interstitial hydrostatic pressure in the dog and rat have provided evidence that increases in renal perfusion pressure are associated with significant increases in RIHP, despite efficient autoregulation of whole kidney renal blood flow and glomerular filtration rate (33,56). Renal vasodilation induced by intrarenal infusion of acetylcholine increases both RIHP and sodium excretion (38,44). Preventing RIHP from increasing during acetylcholine induced vasodilation abolishes the increase in sodium excretion. In addition, Hartupee and co-workers (44) found a positive correlation between changes in renal interstitial pressure and the fractional excretion of sodium. Similar findings have been reported with other renal vasodilators (33). Garcia-Estan and Roman (25) reported that renal interstitial hydrostatic pressure, sodium excretion and urine flow all increased during controlled increases in renal arterial perfusion pressure. Moreover, these relationships were all significantly attenuated by decapsulation of the kidney (55,57), suggesting that an intact capsule is required to keep the RIHP rises throughout the kidney during increases in renal perfusion pressure.

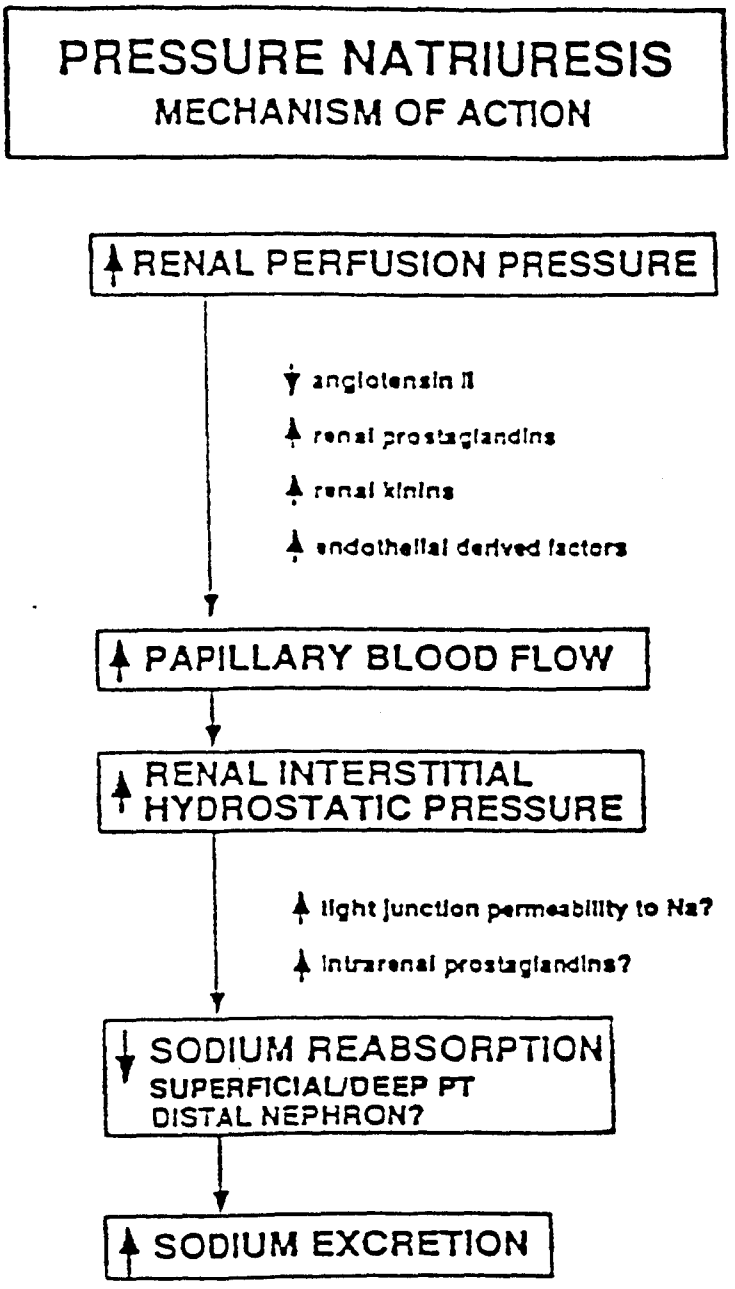

Fig.3. Suggested mechanism of pressure-natriuresis sequence whereby increases in renal perfusion pressure increase sodium excretion. ↑ increase; ↓ decrease; PT= proximal tubule. (reproduced from; GRANGER, J. P. Pressure natriuresis: Role of renal interstitial hydrostatic pressure. Hypertension 19(suppl I): I9-I17, 1992).

Baer and Navar (4) previously demonstrated that the effects of renal perfusion pressure on sodium excretion are markedly enhanced during the renal vasodilation prompted by prostaglandins, suggesting that enhanced transmission of renal perfusion pressure into the renal interstitium during vasodilation could provide a mechanism whereby renal vasodilation potentiates the effect of renal perfusion pressure on sodium excretion (33). Pressor hormones, including angiotensin II, vasopressin, norepinephrine and the novel peptide endothelin stimulate prostaglandin production (14).

The exact mechanism whereby renal interstitial hydrostatic pressure influences tubular reabsorption is unknown. RIHP could influence tubular sodium reabsorption directly (54,56,64) or indirectly through the release of medullary humoral factors, such as prostaglandins (28,62,100,114) and endothelium-derived relaxing factor (122).

Roman et al. (113) have suggested that the renal prostaglandin system plays an important role in both sodium excretion and renal hemodynamics. There are some reports that prostaglandin synthesis blockade blunts the pressure natriuresis response. Specifically, Carmines et al. (10) found in anesthetized dogs that controlled decreases in renal arterial pressure were accompanied by decreases in sodium excretion and prostaglandin E_2 excretion; indomethacin reduced significantly the influence of renal perfusion pressure upon sodium excretion. Pawloska et al. (100) evaluated the effects of renal interstitial volume expansion on fractional excretion of sodium and on the release of PGE_2. Expansion of renal interstitial volume by injecting 50 micro-liter of 2.5% albumin into the renal interstitum of the rats via a chronically implanted polyethlene matrix capsule produced a marked increase in RIHP which was followed by an increment in the fractional sodium excretion without significant changes in GFR, peritubular capillary pressure, tubular pressure or arterial blood pressure. Inhibition of PG synthesis with meclofenamate or indomethacin prevented the response during similar increments in interstitial volume expansion and RIHP. In another study, pressure natriuresis was blunted but not abolished by prostaglandin synthesis blockade (28); there

was no change in RIHP in the presence or absence of indomethacin treatment but significant decreases in both effective RBF and GFR during PG synthesis blockade in pentobarbital anesthetized dogs. In the later study, matrix capsules were implanted chronically at the corticomedullary junction and RIHP was measured before and after PG synthesis inhibition in the same animal; sodium excretion was measured simultaneously with RIHP. The results of the later study by Gonzalez-Campoy et al. (28) do not indicate a significant contribution of prostaglandin synthesis in the pressure-natriuresis since the blunted pressure natriuresis may be the resultant of the reduction of filtered sodium load in the presence of PG synthesis blocker.

The participation of NO in pressure natriuresis was suggested by Salom et al. (116,122) who showed that the intrarenal infusion of L-NAME (N^G-monomethyl-L-arginine) into dogs abolished the increase in urine sodium excretion produced by increasing renal perfusion pressure from 100 to 165 mmHg. This inhibitory effect of L-NAME was prevented by an infusion of L-Arginine. However, in another study, the intravenous infusion of NO-synthesis inhibitors alone caused pressure induced-natriuresis and diuresis; the natriuresis was prevented when renal perfusion pressure was maintained at a constant value (50). Overall, more studies are needed to define the involvement of EDRF and/or PGs in pressure-natriuresis and diuresis.

Studies by Roman and colleagues (112,113) have provided evidence that renal medullary hemodynamics are closely linked to changes in RIHP. They propose that the increases in renal perfusion pressure result in increases in vasa recta flow and vasa recta hydrostatic pressure which in turn lead to a reduction in fluid uptake across the vasa recta capillary wall; the net result is an increase in medullary interstitial hydrostatic pressure. The increase in medullary interstitial hydrostatic pressure is then thought to be transmitted throughout the kidney. Studies by Roman et al. (112,113) also indicate that papillary blood flow is not autoregulated as efficiently as cortical blood flow in response to increases in renal perfusion pressure. Thus, increases in papillary blood flow are

associated with increases in vasa recta hydrostatic pressure. The mechanism by which papillary plasma flow increases in response to increases in renal perfusion pressure is not known. It is possible that endothelial or renal interstitial factors in the medulla are released in response to increases in renal perfusion pressure. Once released, these intrarenal factors could then alter vascular resistance and papillary blood flow. Changes in these medullary hemodynamic factors may play an important role in mediating increases in renal interstitial hydrostatic pressure during pressure natriuresis. On the base of these assumptions, the endothelium derived mediators may have a major role in pressure natriuresis and diuresis.

Simultaneous measurement of cortical and medullary interstitial hydrostatic pressures illustrates that when renal arterial pressure is increased, interstitial hydrostatic pressure changes uniformly throughout the kidney when the renal capsule is intact (25). Thus, renal decapsulation has been used to quantitate the role of RIHP in mediating increases in sodium excretion in response to volume expansion, renal vasodilation and increases in renal perfusion pressure (25,55). Garcia-Estan and Roman (112) reported that decapsulation of kidneys in volume expanded rats markedly attenuates the increased RIHP in response to an increase in renal perfusion pressure. Associated with the blunted increase in RIHP was a significantly attenuated (by 40%) pressure natriuretic response. Similar findings were reported by Kharibi and Knox (44) in spontaneously hypertensive and Wistar-Kyoto rats. Recently, Haas et al. (40) showed that lithium clearance was significantly lower in rats with bilateral renal decapsulation both in control and volume expansion conditions. This reduced lithium clearance occured without changes in the GFR indicating that the renal capsule plays an important role in proximal sodium reabsorption. Attenuation of the sodium excretory response to increases in renal perfusion pressure in kidneys that were decapsulated provides further evidence that renal interstitial hydrostatic pressure may be an important mediator of the pressure-natriuretic

response. The fact that renal decapsulation blunts only 40-50% of the response suggests that mechanisms other than RIHP may also contribute to pressure natriuresis.

Renal interstitial hydrostatic pressure could theoretically decrease tubular reabsorption of sodium and water in the proximal tubule by inhibiting passive transport, active transport, or both. The paracellular back-leak hypothesis suggests that increases in renal interstitial volume, pressure or both, increase the permeability of the tight junctional complexes of the proximal tubule (30). This effect in turn results in an increase in the back leak of sodium from the interstitium into tubule lumen. The net effect of this mechanism would be a reduction of sodium and water reabsorption across the proximal tubule (30).

In addition to altering the passive transport of sodium and water, RIHP might also alter the active transport of sodium across renal tubules by activating renal autocoids such as prostaglandins. Along these lines, PGs may be involved in the inhibition of Na-K ATPase in inner medullary collecting duct by endothelin (145). Alternately, the novel peptide endothelin could modulate and control the sensitivity of the pressure diuresis and natriuresis responses by altering intrarenal hemodynamics.

B. Endothelin

1. Structure of Endothelin and Related Peptides

Endothelin is a 21-amino acid potent vasoconstrictor peptide. It was originally discovered in the supernatant of cultured bovine aortic endothelial cells, and subsequently isolated from cultured porcine aortic endotelial cells (46,141). Numerous studies indicate that ET-1 affects a wide variety of biological systems (19,68,71,74,77,80,136,143) (Table I). However, the fact that it is some 10-fold more potent than the vasoconstrictor angiotensin II and is an extremely long-lasting agent prompts questions whether ET-1 may be locally secreted to fill some crucial physiological role such as in either the short term or long term regulation of arterial blood pressure, or whether its actions are purely pathological in nature (Table II).

The primary sequence of human endothelin has been deduced from a human placental cDNA library and found to be identical to that of porcine endothelin. It is referred to as endothelin-1 (ET-1). Two other related peptides designated endothelin-2 (ET-2) and endothelin-3 (ET-3), differ from ET-1 by 2 and 6 amino acid residues, respectively (48,49,126). All three peptides appear to be coded for by three separate genes in the human, rat and porcine genomes. Two endothelin-related genes were identified by cloning and sequence analysis of the mouse genome (119). One encoded the peptide ET-1, whereas the other encoded a new peptide differing by three amino acid residues. The gene for this novel peptide is only expressed in the intestine and has been referred to as "vasoactive intestinal contractor" (VIC) (119). The structures of these peptides, ET-1, ET-2 and ET-3 and the related peptide, VIC, are shown in Fig. 4.

Interestingly, the endothelins exhibit remarkable structural similarities to a group of toxins, the sarafatoxins S6 isolated from the venom of the snake *Atractaspis engaddensis*, suggesting that the expression of this peptide has been highly conserved during the course of vertebrate evolution and may perform similar homeostatic functions in a variety of mammalian and nonmammalian species. Although circulating levels of ET-1 increase in several pathological states (see Table II), it is yet unclear whether ET-1 functions primarily as a paracrine substance or as a hormone. However, since the plasma concentration of ET-1 is very low, it is a common belief that ET-1 may act locally on cells which surround the ET-1 producing cell rather than as a circulating hormone (78,80,81).

2. Biosynthesis and Tissue Distribution of ET-1

ET-1 is derived from a 203 amino acid peptide precursor known as preproendothelin, which is cleaved after translation by an endopeptidase to form a 38 (human) or 39 (porcine) amino acid peptide, proendothelin or big endothelin (Figs. 5 and 6) (137,143). The biological significance of differences in the amino acid sequence

Table I. Some Biological Actions of Endothelin (Adapted from references 16 and 70)

Tissue/Organ	Effect
Vascular smooth muscle	Long-lasting constriction of isolated vascular smooth muscle. Mitogenic actions in cultured smooth muscle and endothelial cells. Prostanoid (PGI_2, PGE_2, TXA_2) release. Release of endothelium-derived relaxing factor. Coronary arterial vasoconstriction, increased perfusion pressure. Lymphatic vessel constriction.
Nonvascular smooth muscle	Constriction of intestinal, muscle and heart tracheal and uterine smooth muscle. Increased contractility. Increased heart rate. Stimulation of ANP release.
Nervous tissue and pituitary	Enhanced neurotransmitter release (substance P) Increase in release of luteining (LH) and follicle stimulating (FSH) hormones, inhibition of prolactin release.
Kidney	Inhibition of renin release in-vitro. Stimulation of renin release in-vivo. Decreases in RBF, GFR, K_f (ultrafiltration coefficient) and urinary Na-K excretion, vasoconstriction of afferent and efferent renal arteriole. Diuresis and natriuresis, decrease in oubain sensitive Na-K ATPase activity in rabbit inner medullary collecting duct cells. Mitosis and contraction of mesengial cells.
Adrenal Glands	Stimulation of aldosterone biosynthesis and release of catecholamines.

Table II. Possible Pathological and Physiological Implications Ascribed to ET-1

(Adapted from references 16 and 70).

Pathological:

Cardiovascular diseases	Myocardial ischemia. Congestive heart failure. Arrhythmia. Unstable angina. Hypertension.
Bronchoconstriction	Pulmonary hypertension. Asthma.
Neuronal action	Cerebral vasospasm. Subarachnoid hemorrhage.
Endocrine	Pre-eclampsia.
Renal disease	Acute/chronic renal failure.
Vascular disorders	Atherosclerosis. Complications in diabetes.
Cancer	Pulmonary carcinoma.
Gastric mucosal damage	Gastrointestinal disorders.
Other	Endotoxic shock, septicemia, surgical operations.

Physiogical:

Physiological regulation of blood pressure.
Neuroendocrine regulation.
Closure of umbilical vessels.
Wound healing.
Control of menstruation.
Penile erection

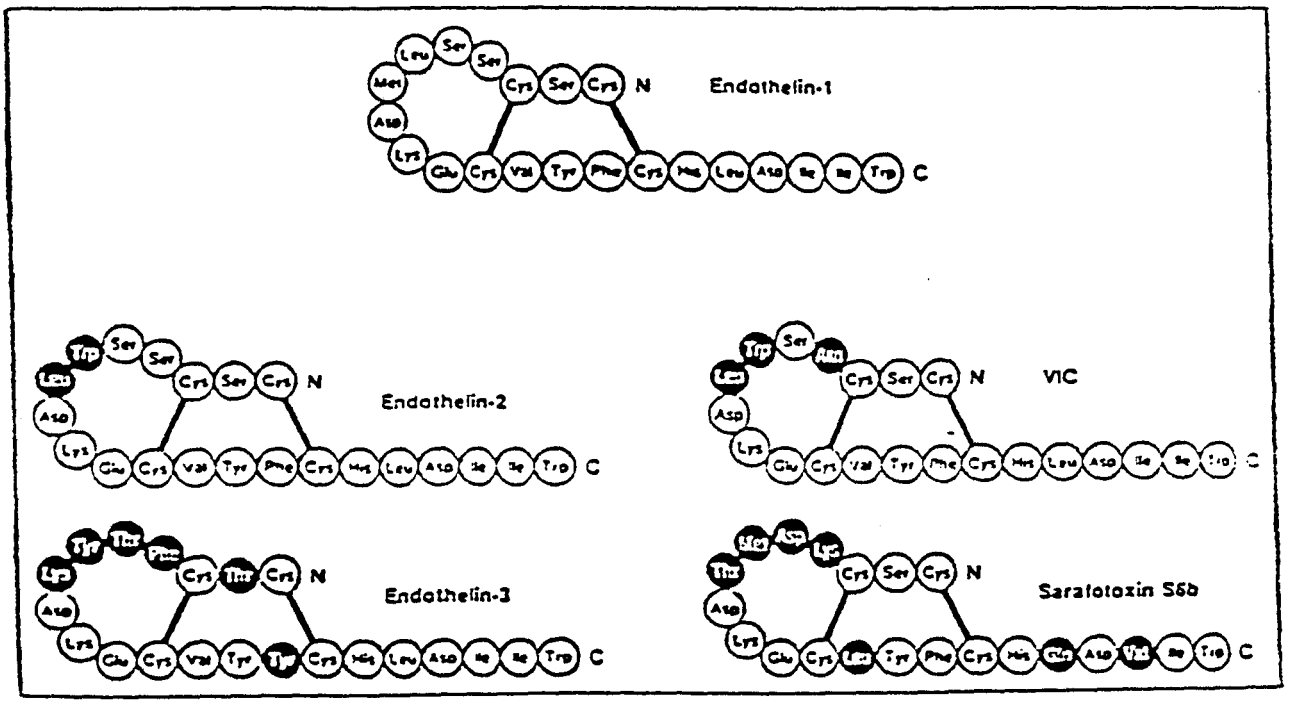

Fig. 4. Amino acid sequences of the endothelin family: ET-1, ET-2, ET-3, VIC (vasoactive intestinal contractor-mouse) and sarafatoxin-6b. Filled circles represent amino acid residues different from those of ET-1. (Reproduced from; CHABRIER, P. E. AND P. BRAQUET. ENDOTHELIN. Horm. Res. 34: 169-174, 1990.)

between the prepropeptides are presently unclear. The identity of the specific endopeptidase is not currently known.

Big ET is converted to active ET by a putative endothelin converting enzyme (ECE) (97,141,143). The physiological importance of cleavage of ET (1-39) is indicated by the reported 140-fold increase in vasoconstrictor activity upon cleavage to ET-1 (58).

There has been some speculation that the biosynthetic pathway may be tissue and possibly species specific (19).

ET-1 is synthetized by endothelial cells; the precise sites of synthesis of ET-2, ET-3 and VIC are uncertain although ET-3 appears to be expressed by neural tissue (126) and VIC by the intestine (119). The expression of the preproendothelin gene in cultured endothelial cells is stimulated by thrombin, TGF-*beta*, epinephrine, vasopressin, phorbol esters and the calcium ionophore, A23187 (142,143). Shear stress, hypoxia, oxyhemoglobin, elevated glucose concentrations and endogenous digitalis-like factor are other factors that stimulate the release of ET (80,81,140). In the intact circulation, thrombin and A23187 have been demonstrated to enhance ET-1 release, while endothelium derived relaxing factor inhibits its production (7). Since there is a lack of secretory granules in endothelial cells (19,79), endothelin is thought to be constitutively released from these cells after intracellular processing. Therefore, both the production and secretion of ET-1 may be regulated at the level of messenger RNA transcription (19,79). ET-1 mRNA is widely expressed in rat, porcine, guinea pig and human tissues (61,94). The distribution of the propeptide, big ET and immunoreactive (ir)ET-1, have been compared in porcine tissues (94). The concentration of ir big ET was highest in the aortic intima and lung, while the highest concentration of ir ET-1 was in the kidney inner medulla (13).

3. Mechanism(s) of ET-1 induced constriction

At least, two distinct ET-receptor subtypes termed ET_A (selective for ET-1) and ET_B (nonselective with respect to isopeptides of the ET family) have been cloned and

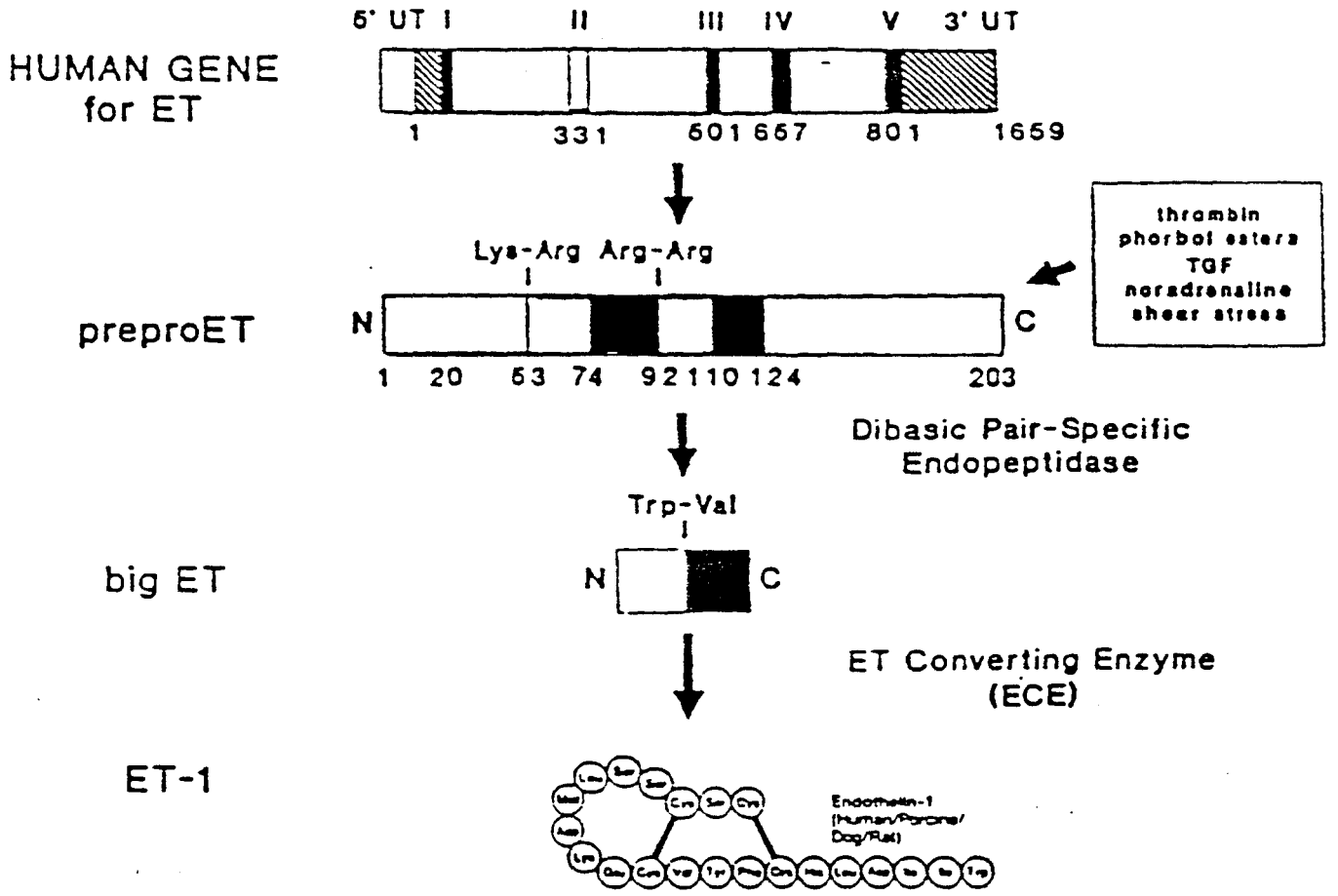

Fig. 5. Biosynthesis of endothelin (Reproduced from; DOHERTY, A. M. Endothelin: A new challenge, Medicinal Chemistry 35(9): 1493-1508, 1992).

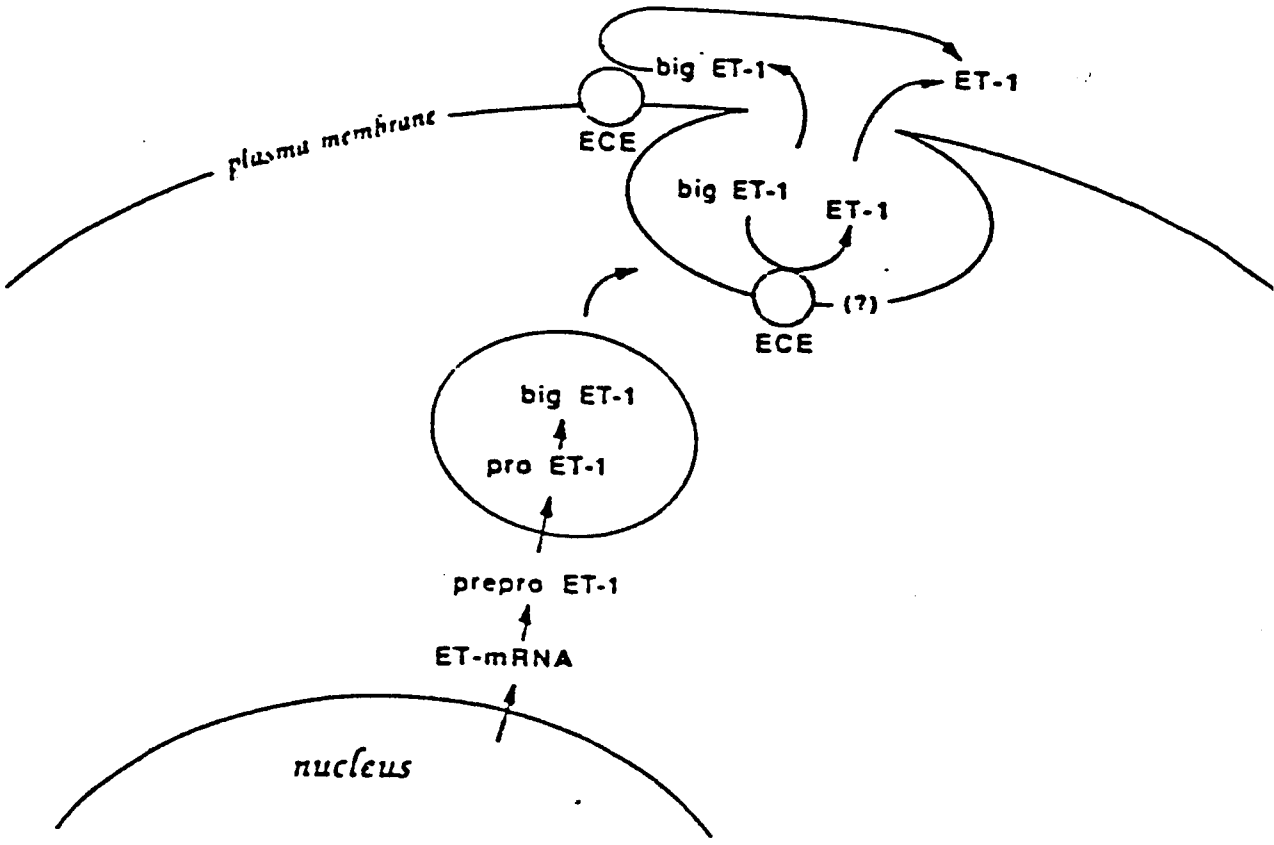

Fig. 6. Processing and releasing mechanism of endothelin in endothelial cell. ECE: endothelin converting enzyme. (Reproduced from; MASAKI, T, AND M. YANAGISAWA. Physiology and pharmacology of endothelins. Medicinal Research Reviews 12(4): 391-421, 1992).

expressed (1,121). Both are G-protein-coupled, belong to the rhodopsin family and contain seven transmembrane domains. ET_A receptors are abundant in cardiovascular tissue and the central nervous system (CNS) and are highly specific for ET-1. The ET_B receptor is a "non-selective" subtype that binds ET-1, 2 and 3 with similar affinities (121). These receptors are abundant in noncardiovascular tissues including kidney, adrenal gland and the central nervous system (121).

Expression of ET receptor on vascular smooth muscle is regulated by angiotensin II, Arginine-vasopressin as well as by ET itself (47,117).

A direct involvement of endothelin with the slow calcium channel was originally postulated by Yanagisawa et al. (141) and the activation of dihydropyridine-sensitive, voltage dependent calcium channels (L-type calcium channels) by ET-1 has been confirmed with other investigators in coronary artery (29,79), subsequent studies have demonstrated that the vasoconstrictor response to endothelin can be observed in calcium free conditions (95,108). Thus, the mechanisms of vasoconstriction and pressor responses induced by ET still remain unclear. Depending upon the smooth muscle cell employed, ET-1 appears to activate opening of various calcium channels (receptor operated and/or voltage sensitive calcium channel) as well as the release of calcium from intracellular stores (75,78,79,80,142).

Although the exact mechanism whereby ET-1 causes biological effects after binding to cell surface receptors has yet to be sufficiently defined, the current view (illustrated in Fig. 7) is that ET-1 binds to its G-protein-coupled receptor to activate phospholipase C (PLC), resulting in increased formation of inositol tris- and bisphosphatases (IP) and 1,2-O-diacyl-glycerol (DAG), with subsequent stimulation of protein kinase C (PKC) (127,128).

These events have been implicated in the initial rise in intracellular calcium and phosphorylation of myosin light chains leading to the vascular contractile responses of ET-1. Further work is needed to define the pathways of calcium influx activated by

ET-1. PKC appears to inhibit ET-induced Ca^{++} signalling, thereby serving as a negative feedback signal (16). There is some evidence that ET-1 also activates phospholipase A_2 (PLA_2) in cultured smooth muscle and mesangial cells, causing stimulation of the arachidonic acid cascade (108,109). It is not known whether ET-1 activates PLA_2 directly via a G protein or indirectly by increasing intracellular Ca^{+2}. During bolus injections of ET-1, an initial transient vasodilator action has been attributed to the release of a prostaglandin PG_2 (Prostaglandin I_2) and/or EDRF (74,125,138). The vasodilator and vasoconstrictor effects of ET-1 may be mediated via different receptors.

4. Cardiovascular and endocrine actions of ET-1

Synthetic endothelins have been used to study the biologic actions of these peptides. The most striking property of ET-1 is its long lasting hypertensive action. In many species, intravenous injections of ET-1 produce intense vasoconstriction and increases in mean arterial blood pressure (107). In contrast to this prolonged hypertensive activity, rapid elimination of endothelins from the blood stream has been observed in rats with high uptake in the lung, kidney, and liver (17,123). In vivo intravenous bolus injections of ET-1 cause an initial transient depressor response followed by a prolonged systemic hypertension both in anesthetized and chemically denervated rats and in conscious rats (60,71). A low dose of ET-1 produces hypotension rather than a pressor response, as well as vasodilation (43,72,83,139).

Endogenous vasoactive substances including endothelium-derived relaxing factor, prostaglandins and atrial natriuretic peptide have been implicated in the hypotension observed at the initial step after bolus injection of ET-1. EDRF has been considered the most probable candidate in this response (17). In conscious rats, the hypotensive response to ET-1 was attenuated in the presence of N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthesis (138). On the other hand, in rat and dog renal arteries, ET-1 has been shown to cause a release of vasodilating prostanoids (109).

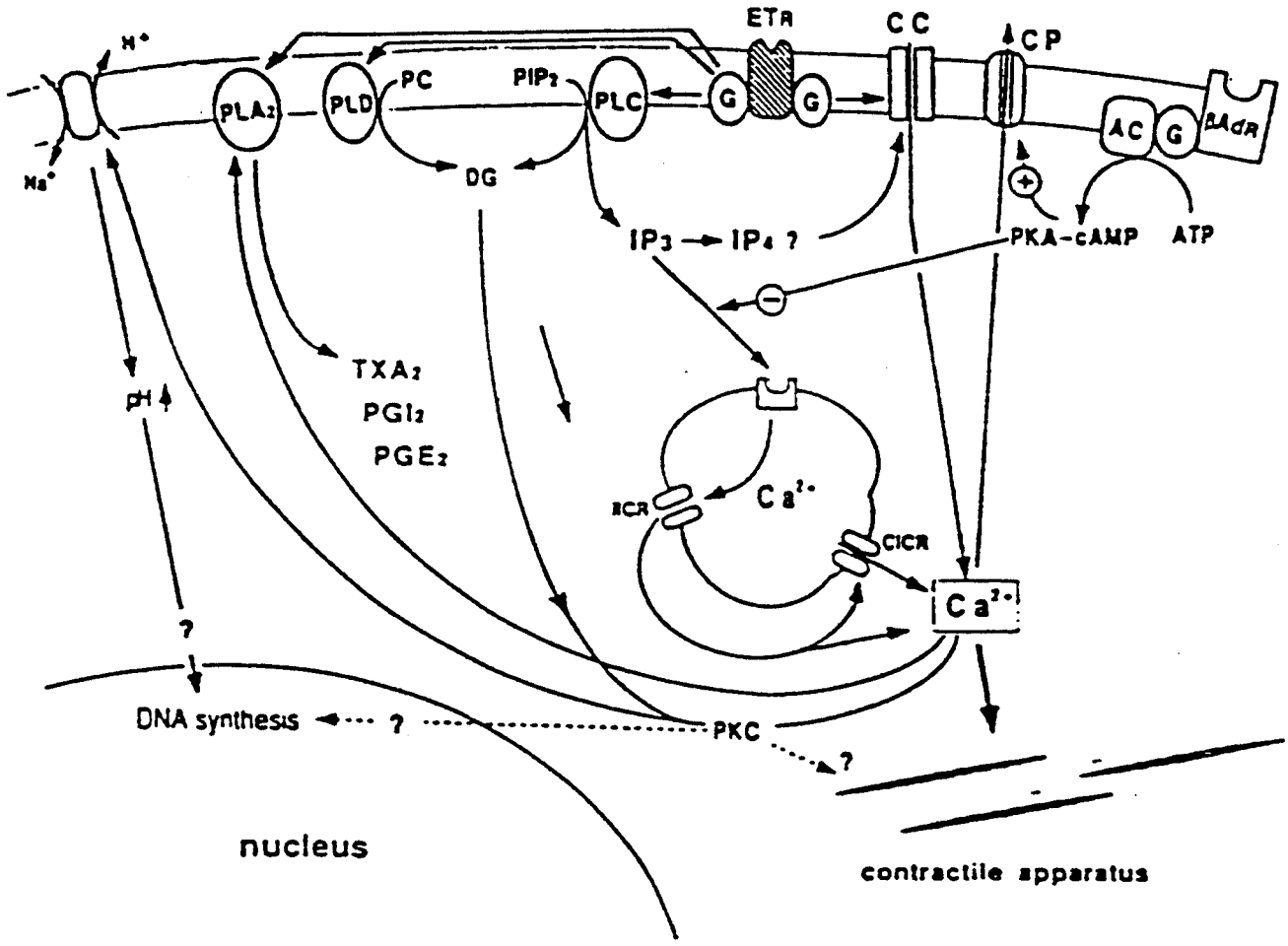

Fig. 7. Suggested signal transduction pathways stimulated by endothelin in vascular smooth muscle. ET_R= endothelin receptor; PLC= phospholipase C; CP= calcium pump; AC= adenylate cyclase; G= G-protein; ßAdR= ß-adrenergic receptor; PKA= protein kinase A; PLA_2= phospholipase A_2; PLD= phospholipase D; PC= phosphatidylcholine; DG= diacylglycerol; PIP_2= phosphoinositol 4,5-diphosphate;IP_3= inositoltriphosphate; IP_4= inositol 1,3,4,5-tetrakiphosphate; PKC= protein kinase C; CICR= calcium-induced calcium release mechanism; IICR= inositoltrisphosphate-induced calcium release mechanism. (Reproduced from; MASAKI, T, AND M. YANAGISAWA. Physiology and pharmacology of endothelins. Medicinal Research Reviews. 12(4): 391-421, 1992).

In general, ET does not appear to affect the baroreflex control of sympathetic nerve activity or heart rate (141,142).

Although it is questionable whether ET-1 has a circulatory hormonal effect, ET-1 does elevate the plasma levels of renin, aldosterone, atrial natriuretic peptide (ANP) and vasopressin (9,27,82,89,90). ET-1 stimulates ANP release in vivo (9,27,82) in conscious and anesthetized dogs, and in cultured rat atrial myocytes (24). ET-1 stimulates the biosynthesis of aldosterone in dispersed zona glomerulosa cells from the adrenal cortex (15,86). However, ET-1 directly inhibits the release of renin from juxtaglomerular cells in vitro (131). ET-1 concentrations as low as 10^{-11} to 10^{-10} M have been shown to inhibit the release of renin from dispersed rat juxtaglomerular cells (132). In vivo studies, however, have shown a significant increase in plasma renin activity after intravenous infusions of pharmacological doses of endothelin (27,82). It is possible that the increase in plasma renin concentrations in vivo are mediated by ET-1 induced intrarenal vasoconstriction which would be expected to cause a decreased distal delivery of sodium and a resultant stimulation of renin release from juxtaglomerular cells. Thus, it remains uncertain whether the ability of ET-1 to increase the level of circulating hormones is a direct effect or is secondary to the hemodynamic actions of the peptide.

The threshold pressor dose of ET-1 in intact rats or dogs is between 10 and 100 ng kg^{-1} min^{-1}. The pressor response of ET-1 appears to reflect a direct vasoconstrictor action of the peptide on peripheral vessels. The calcium channel blockers verapamil and manganase blocked the ET-induced pressor response in anesthetized rats (8), but not the renal response. These results are similar to those obtained with isolated vascular strips (75). Cardiac output and heart rate were significantly decreased only by the highest infusion rate of ET (75).

5. Effect of ET-1 on renal function

Several lines of evidence implicate that, in addition to being a target of ET-1 (3,6,11,89), the kidney might be an important source of this peptide. ET-1 production

and release have been demonstrated in glomerular endothelial cells, in mesangial cells and in variety of renal epithelial cell lines (MDCK, LLC-PK1) (65,75,76,77,120).

The kidney is especially sensitive to ET action. Thus, numerous studies have shown that ET-1 has an important impact on the kidney. The administration of pharmacological doses of this peptide to experimental animals elicits marked and sustained increases in arterial pressure and systemic vascular resistance, a decrease in cardiac output, a profound reduction in renal plasma flow (RPF) and glomerular filtration rate (GFR) and a fall in sodium excretion as a result of the increased vascular resistance and contraction of mesangial cells in a dose and time dependent manner (6,8,9,53,68,79,90). By contrast, relatively low doses of endothelin do not change renal blood flow or renal vascular resistance and are associated with diuretic and natriuretic responses (43).

In the kidney, mRNA for ET has been detected in the cortical and medullary regions. Autoradiographic studies using ^{125}I-ET-1 in rats have localized ET receptors in the renal artery and vein, in the glomerulus, in the arcuate artery, in the interlobular artery, in vascular bundles and in the renal papilla (59). The vasoconstrictor effects of ET-1 on renal hemodynamics are significantly modified by its ability to enhance production of vasodilators, including prostacyclin and EDRF (13,138). Taken together, the evidence for glomerular and renal ET-1 binding sites, raises the possibility of a local role of ET-1 in the modulation of renal hemodynamics.

Endothelin contracts rat and rabbit renal afferent and efferent arterioles in vitro (21,73). These studies report a slightly greater sensitivity of efferent compared to afferent arterioles to the contractile action of ET.

There are several conflicting reports regarding the effects of endothelin on urinary excretion of sodium and water (79). Renal artery pressure, GFR, renal blood flow and tubular functions are the major factors that determine electrolyte and water excretion. Endothelin-1 increases blood pressure which may cause pressure natriuresis

and diuresis by increasing renal perfusion pressure even in the presence of modest decreases in GFR. King et al. (60) reported that endothelin-induced natriuresis was abolished when renal perfusion pressure was controlled by an aortic snare. A high dose of ET-1 administered intravenously causes marked reductions in renal blood flow and glomerular filtration rate in anesthetized rats and dogs (69,71,74). In general these changes are accompanied by a sustained reduction in sodium excretion, an increase in plasma renin activity and a sustained increase in renal vascular resistance. Thus, if the glomerular filtration rate decreases markedly, urinary sodium and water excretion usually decrease because of the lowered filtered load. On the other hand, when a low dose of ET (1-10 ng kg^{-1} min^{-1}) is administered, renal vascular resistance decreases and renal plasma flow increases; GFR remains unchanged (43). Moreover, lower doses of ET-1 generally cause a diuresis and natriuresis with a concomitant decrease of proximal sodium reabsorption based on the observation that ET-1 increased lithium clearance. It is also of interest to note that even in the presence of a marked reduction in renal blood flow and GFR during ET infusion into isolated-perfused rat kidneys, an increase in urinary sodium excretion has been reported (93). Along these lines, Munger et al. (87) investigated whether a secondary release of atrial natriuretic peptide (ANP) might be responsible for ET-induced natriuresis. Non-pressor doses of ET-1 cause a significant increase in plasma ANP levels and a natriuresis. Munger et al. (87) reported that pretreatment with a rabbit anti-rat ANP antibody attenuated the ET-induced natriuresis and diuresis. On the other hand, Perico et al. (101) reported that bolus i.v. infusion of 150 pmol ET into rats caused a natriuresis and diuresis with a concomitant fall in the GFR and RPF of 33% and 36%, respectively. Estimation of the change in tubular handling of sodium by lithium clearance during ET infusion indicated a reduction in absolute and fractional proximal sodium reabsorption (101). In the latter study, ET infusion was not associated with significant changes in plasma ANP levels; a 5-

lipooxygenase inhibitor administered orally did prevent the diuretic and natriuretic response to the 150 pmol ET infusion.

The possibility that endothelin inhibits the action of vasopressin has been suggested (88,96,124,134,135). Tomita et al. (134) have demonstrated that endothelin can block cAMP accumulation induced by vasopressin in the cortical collecting duct and subsequently reported that it also inhibits vasopressin-stimulated fluid absorption in this segment.

There are no reports concerning the direct inhibition of renal tubular sodium transport by endothelin. An inhibition of Na-K-ATPase by endothelin has been proposed in rabbit inner medullary collecting duct cells (IMCD cells) (145). This conclusion is based on the fact that oxygen consumption by IMCD cells is reduced by endothelin. These investigators also showed that this effect was blocked by cyclooxygenase inhibition and was reproduced by exogenous PGE_2, indicating an involvement of ET-stimulated prostaglandin synthesis.

Taken together, there is only a small body of data (much of it conflicting) concerning natriuretic and diuretic actions of endothelin. Clearly more studies are needed to define the role of endothelin in volume and blood pressure regulation.

MATERIALS AND METHODS

Surgical Procedures and Experimental Design

Forty one female Spraque-Dawley rats (200 - 250 g) were maintained on standard rat chow and water *ad libitum*. Rats were deprived of water overnight before the renal clearance experiments. At the time of each experiment, rats were anesthetized with sodium pentobarbital (60 mg/kg) and rectal temperatures were maintained at 37 % 0.5 ° C with a radiant heat lamp connected to a temperature controller. The left femoral vein and artery were cannulated with PE-50 tubing. Mean arterial blood pressure was monitored with a pressure transducer and displayed on a chart recorder. Immediately

after catheterization of the femoral vein, a solution containing 3% creatinine in saline was infused at a rate of 25 ml/min and maintained throughout the experiment. Finally, the bladder was cannulated with PE-100 tubing via an abdominal incision. The animal was positioned on its side and urine allowed to flow, by gravity, into collection vials located below the level of the rat.

Following surgery and a 60-min stabilization period, 3X15 min baseline clearance periods (C_1, C_2 and C_3) were performed with a 0.2 ml arterial blood sample obtained between C_1 and C_2; reported baseline values represent the average of C_1, C_2 and C_3. In Groups I-VI and IX-XI an intravenous infusion of endothelin was then initiated for 30 min at a rate of 50 ng kg^{-1} min^{-1} . During the infusion of endothelin, 2X15 min clearance periods (E_1 and E_2) were performed and a 0.2 ml arterial blood sample collected following E_2. In Groups VII and VIII, amiloride instead of endothelin was infused for 45 min; a priming dose of 0.2 mg of the diuretic was followed by a constant infusion of 0.02 mg/min. During the infusion of amiloride, 3X15 min clearances were collected (A_1, A_2 and A_3) and a 0.2 ml arterial blood sample collected following A_3.

Experiments in Group I (n=6) represents control rats and only received endothelin. Experiments in Group II (n=5) were designed to determine to what extent removing the renal capsule affected endothelin-induced changes in renal function; the renal capsule has been reported to be an essential component of pressure-induced natriuresis and diuresis (32,56,111). During surgery, both the right and left renal capsules were removed. Experiments in Group III (n=5) were designed to determine to what extent endothelin-induced changes in mean arterial blood pressure contributed to the natriuretic and diuretic response to low doses of endothelin. During surgery, a segment of 000 surgical thread was positioned around the aorta above the exit of the right renal artery and the ends of the thread passed through a PE 100 section of tubing and connected to an adjustable clamp. By exerting a variable degree of tension on the aorta with this unit, renal arterial pressure (RAP) could be maintained at baseline values

during infusion of endothelin. Experiments in Group IV (n=5) were designed to determine to what extent changes in the synthesis of prostaglandins affected ET-induced natriuresis and diuresis; changes in prostaglandin synthesis have been reported to mediate pressure-induced natriuresis and diuresis (62,100,113). Following surgery, 5 mg/kg meclofenamate, a dose that has been shown to be sufficient to inhibit the renal synthesis of prostaglandins (100), was administered. Experiments in Group V (n=5) and Group VI (n=5) were designed to differentiate between proximal and distal tubular sites of action of endothelin. In Group V, following surgery, sodium reabsorption in the cortical collecting duct was blocked with amiloride (a priming dose of 0.3 mg/kg was followed by a constant infusion of 0.04 mg kg^{-1} min^{-1} throughout the experiment); as in other protocols, after 60 min, control clearances were collected and endothelin infused for 30 min. A similar protocol was used in Group VI, except that sodium reabsorption in the distal nephron was inhibited with a combination of amiloride (0.3 mg/kg bolus followed by a constant infusion of 0.04 mg kg^{-1} min^{-1}) plus furosemide (0.8 mg/kg followed by a constant infusion of 0.1 mg kg^{-1} min^{-1} infused throughout the experiment); we have previously utilized this technique of distal blockade to evaluate the intrarenal site of action of atrial natriuretic factor (63,103). In these experiments, urinary volume and electrolyte losses were closely matched with an intravenous infusion of a replacement solution containing NaCl 135 mmol/l, KCl 5 mmol/l and $NaHCO_3$ 5 mmol/l (103). To determine whether removing the renal capsules affects the natriuretic and diuretic of an agent that is known to exert its action via changes in tubular sodium reabsorption, following the 60 min stabilization period and collection of the control clearances, amiloride instead of endothelin was infused for 45 min (0.2 mg bolus followed by a constant infusion of 0.02 mg/min; this dose caused a diuretic response that was similar to endothelin) into intact rats (Groups VII) and into rats with both renal capsules removed (Group VIII).

Other Endothelin Related Eperimental Studies

Experiments were further extented to determine to what extent changes in the synthesis of endogenous EDRF/NO and changes in the sympathetic nerve activity affect ET-induced natriuresis and diuresis; also, a comparison of the cardiorenal actions of high versus low doses of endothelin was evaluated in another series of experiments

Experiments in Group IX (n=8) were designed to determine to what extent the cardiorenal actions of a high dose of endothelin are different from a low dose of endothelin. The experimental protocol used in these series of experiments was the same as that used in the control group (Group I) except that ET was infused at a higher dose (110 ng kg^{-1} min^{-1}) instead of a lower dose (50 ng kg^{-1} min^{-1}) for 30 min. Experiments in Group X were designed to determine to what extent ET-induced changes in renal nerve activity participates in endothelin-induced natriuresis and diuresis. In Group X, following surgery, an autonomic ganglion blocker, hexamethonium bromide (Sigma), was administered as a bolus injection of 20 mg/kg followed by a 5 mg kg^{-1} min^{-1} maintenance dose throughout the entire experiment (during the 60 min stabilization period, the 45 min control clearances and during the 30 min of endothelin infusion at 50 ng kg^{-1} min^{-1}). Finally, experiments in Group XI were designed to investigate to what extent the inhibition of endogenous EDRF/NO biosynthesis with Nω-L-Arginine (LNNA) is involved in the cardiorenal actions of a low dose of endothelin. In Group XI, following surgery and a one hour stabilization period, 3X15 min control clearances were collected (C_1, C_2 and C_3). LNNA infusion was then initiated at a rate of 50 μg kg^{-1} min^{-1} through the rest of the experiment. After one and a half hour infusion of LNNA, 3X15 min clearance periods (L_1, L_2 and L_3) followed by an ET-1 infusion at a rate of 50 ng kg^{-1} min^{-1}; 2X15 min clearances (E_1 and E_2) were collected during the infusion of endothelin. 0.2 ml arterial blood samples were collected following C_1, L_1 and E_2.

Upon completion of each experiment, rats were sacrificed with a lethal injection of sodium pentobarbital and the kidneys were removed, cleaned of excess tissue, blotted dry, and weighed.

Analytical Procedures

Urine volumes were determined gravimetrically. Urine and plasma sodium and potassium concentrations were determined by atomic emission spectrophotometry. Creatinine concentrations in urine and plasma were determined by the method of Folin and Wu (23). The clearance of creatinine and the excretion rates of sodium ($U_{Na}V$) and potassium (U_KV) were calculated using standard formulas. The clearance of creatinine was equated with the GFR (45). ET-1 from Peninsula Laboratories was dissolved in 0.5 N acetic acid, divided into aliquots and stored at -80 °C until the experiment.

Statistical Analysis

Differences within each group were determined by using one-way analysis of variance for repeated measurements and Duncan's new multiple-range test. Differences between groups were evaluated using Student's t test for pooled data. A p value of less than 0.05 was considered significant. Mean values ± SE are reported.

RESULTS

ET-induced Natriuresis and Diuresis: Effects of Renal Decapsulation and of Renal Arterial Pressure

Table III summarizes the systemic and renal responses to an intravenous infusion of ET (50 ng kg^{-1} min^{-1}) in Group 1 (controls), Group II (renal decapsulation) and Group III (constant RAP); Fig 8 graphically illustrates the mean arterial blood pressure and GFR data and Fig 9 illustrates the sodium and urine flow rate data. In Group I, endothelin significantly ($p<0.01$) increased mean arterial blood pressure, sodium excretion rate and urine flow rate during both clearance periods (MAP increased 23 ± 3% and 38 ± 3 % at 15 an 30 min, respectively, during infusion of endothelin). During

the first 15 min of ET infusion into control rats, the GFR was not affected whereas there was a modest decrease (11 ± 3%, $p<0.05$) in this renal variable at 30 min during infusion of the peptide.

As is illustrated in Fig. 10, there was a highly significant correlation between mean arterial blood pressure and $U_{Na}V$ ($r = 0.806$, $p<0.001$) and between mean arterial blood pressure and V ($r = 0.497$, $p=0.022$) in Group I rats. The data in Fig. 10 represent baseline MAP, $U_{Na}V$ and V values as well the values at 15 and 30 min during infusion of endothelin.

In Group II rats, mean arterial blood pressure also increased significantly during the infusion of endothelin; the increase in mean arterial blood pressure prompted by endothelin in Group II rats was not significantly different from the peptide-induced increase in Group I. However, in Group II rats there was no significant change in either sodium excretion rate or urine flow rate during infusion of endothelin. As was the case in Group I rats, the GFR in Group II rats was unaffected by endothelin during the first 15 min of infusion of the peptide, but it did modestly decrease (21 ± 5%, $p<0.01$) during the second clearance period.

Infusion of endothelin into rats that had renal arterial blood pressure values maintained at corresponding baseline levels (Group III) had no effect on either sodium excretion rate or urine flow rate. As in Group I rats, the GFR in Group III was also unaffected by endothelin during the first 15 min of infusion but was modestly decreased (20 ± 6%, $p<0.01$) at 30 min during infusion of the peptide.

Meclofenamate Experiments

Results from experiments in Group IV in which the effects of meclofenamate on the renal and systemic response to endothelin in intact rats were evaluated are also summarized in Table III. Meclofenamate did not alter the endothelin-induced increases in mean arterial blood pressure, sodium excretion rate or urine flow rate. There were also no significant changes in the GFR during either E_1 or E_2 in Group IV rats.

Table III. Effects of endothelin (50 ng kg^{-1} min^{-1}) on selected systemic and renal function variables are summarized for Group I (intact, n=6), Group II (renal decapsulation, n=5), Group III (constant renal arterial pressure, n=5) and Group IV (meclofenamate treated, n=5) rats. Values are means ± SE.

	Basal	ET-1 (15 min)	ET-1 (30 min)
GFR (ml/min)			
I	2.1 ± 0.09	2.2 ± 0.14	1.8 ± 0.1*
II	2.2 ± 0.07	2.1 ± 0.07	1.7 ± 0.08**
III	2.0 ± 0.1	1.8 ± 0.13	1.6 ± 0.12**
IV	2.2 ± 0.2	2.2 ± 0.2	1.9 ± 0.1
MAP (mmHg)			
I	95 ± 2	116 ± 3**	131 ± 2**
II	104 ± 5	122 ± 3**	134 ± 3**
III	100 ± 4	100 ± 4	100 ± 3
IV	92 ± 1	119 ± 3**	134 ± 4**
V (μl/min)			
I	12 ± 1	19 ± 1.4**	24 ± 3**
II	13 ± 0.8	13 ± 0.8	11 ± 0.6
III	14 ± 0.8	14 ± 0.05	12 ± 1.2*
IV	14 ± 3	23 ± 6*	27 ± 7*
UNaV(μeq/min)			
I	0.34 ± 0.07	1.22 ± 0.15**	1.83 ± 0.2**
II	0.36 ± 0.08	0.41 ± 0.09	0.33 ± 0.04
III	0.30 ± 0.07	0.34 ± 0.1	0.21 ± 0.08
IV	0.43 ± 0.13	1.30 ± 0.3*	2.00 ± 0.6**
FE_{Na} (%)			
I	0.18 ± 0.03	0.44 ± 0.08**	0.72 ± 0.12**
II	0.15 ± 0.03	0.20 ± 0.04	0.14 ± 0.02
III	0.11 ± 0.02	0.13 ± 0.03	0.10 ± 0.03
IV	0.24 ± 0.1	0.84 ± 0.30*	0.89 ± 0.3*
U_KV (μeq/min)			
I	1.4 ± 0.12	1.9 ± 0.13*	1.63 ± 0.25
II	1.4 ± 0.13	1.8 ± 0.17	1.22 ± 0.17
III	1.6 ± 0.18	1.5 ± 0.2	1.00 ± 0.22
*IV	3.3 ± 0.4	4.0 ± 0.7	3.64 ± 0.3
FE_K (%)			
I	18.1 ± 1.4	25.9 ± 1.9**	24.3 ± 1.6**
II	15.4 ± 1.4	19.4 ± 1.8	17.6 ± 1.7
III	18.6 ± 2.1	20.0 ± 1.8	14.3 ± 2.7
IV	18.1 ± 1.0	21.7 ± 1.7	23.6 ± 2.0

* = $p < 0.05$ and ** = $p < 0.01$ compared to corresponding baseline value.

Statistical evaluations of differences between groups are shown in Figs. 8 and 9.

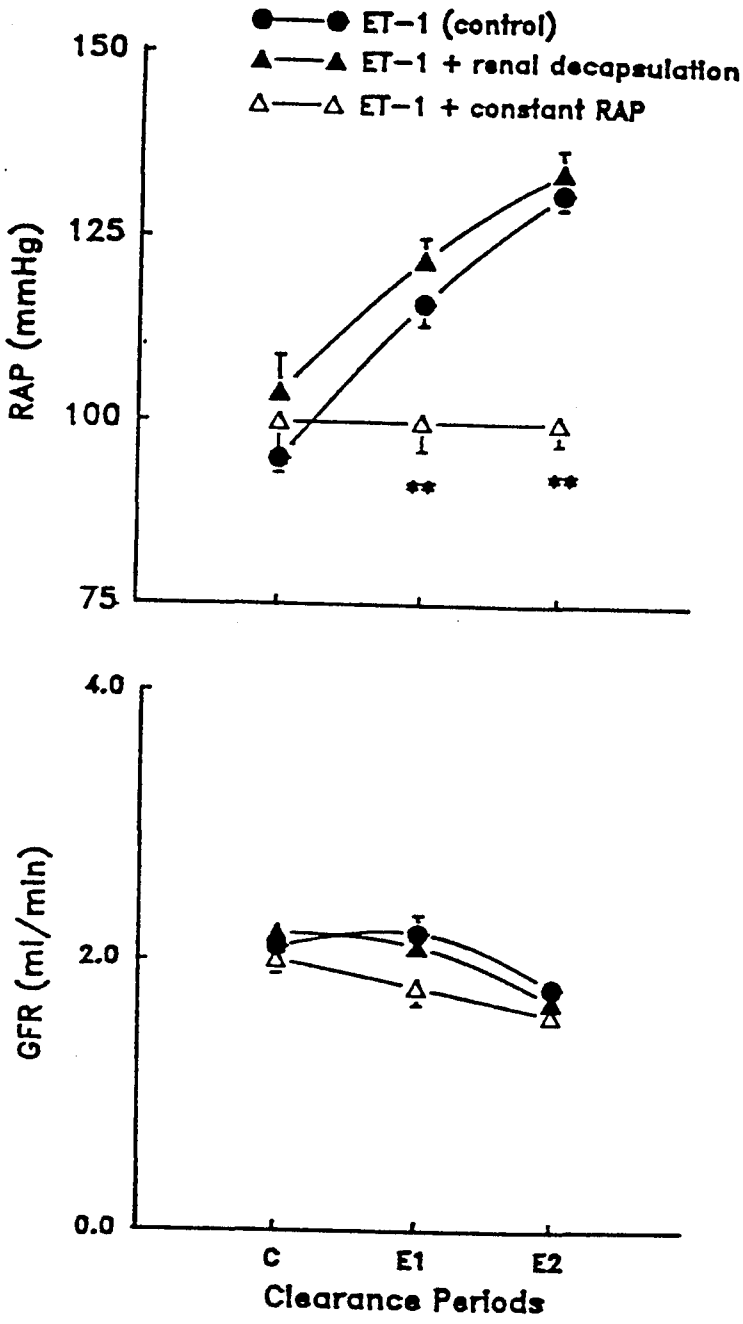

Fig. 8. Renal arterial pressure (RAP, upper panel) and glomerular filtration rate (GFR, lower panel) values are shown before and during intravenous infusion of endothelin-1 (50 ng kg^{-1} min^{-1}) into intact rats (Group I), into rats with bilateral renal decapsulation (Group II) and into rats with constant renal arterial pressure (Group III). ** = $p<0.01$ compared to Group I. Statistical evaluation of values relative to baseline are reported in Table III.

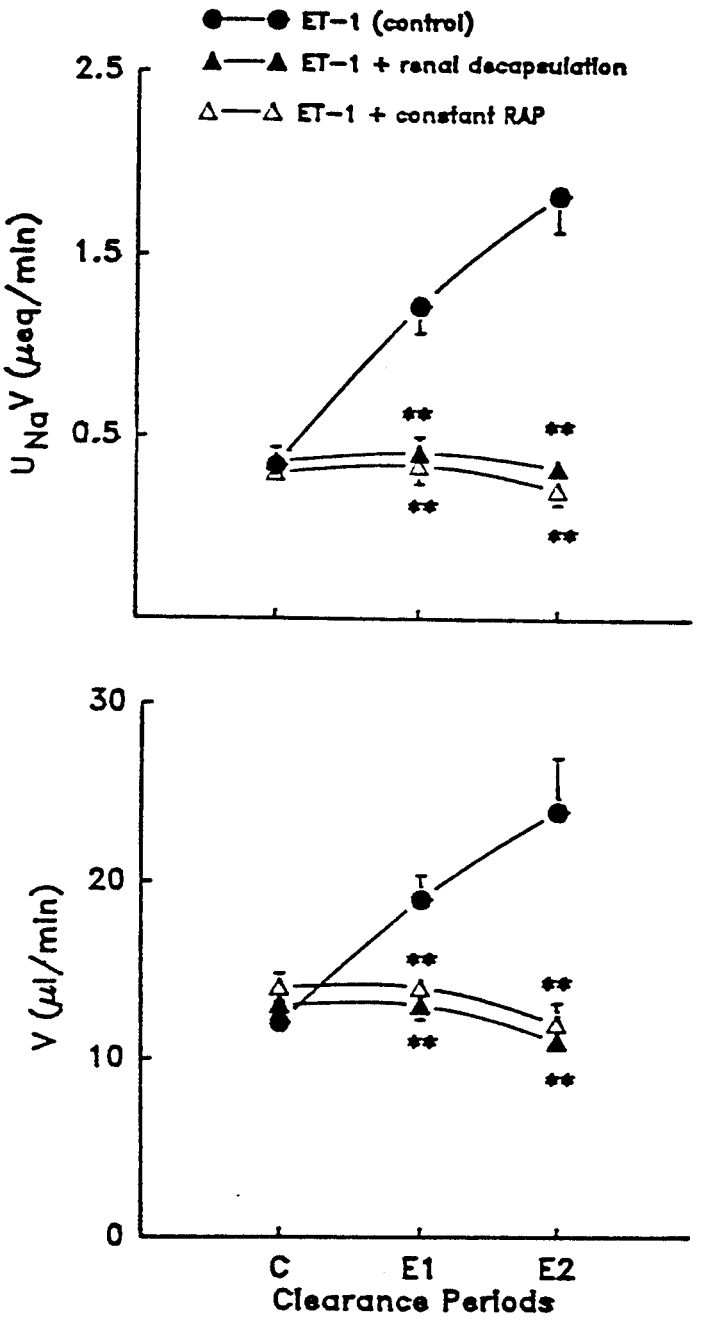

Fig. 9. Sodium excretion rate ($U_{Na}V$, upper panel) and urine flow rate (V, lower panel) values are shown before and during intravenous infusion of endothelin-1 (50 ng kg^{-1} min^{-1}) into intact rats (Group I), into rats with bilateral renal decapsulation (Group II) and into rats with constant renal arterial pressure (Group III). ** = $p<0.01$ compared to Group I. Statistical evaluation of values relative to baseline are reported in Table III.

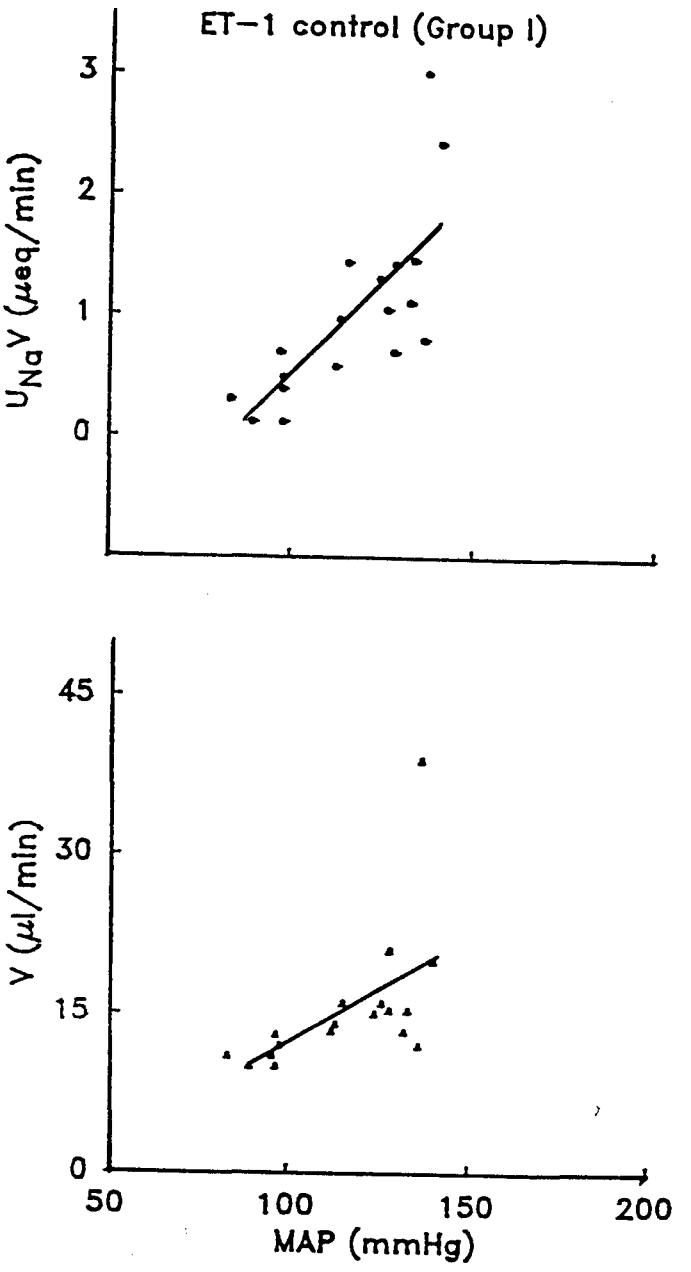

Fig. 10. Sodium excretion ($U_{Na}V$, upper panel) and urine flow rate (V, lower panel) values are plotted as a function of mean arterial blood pressure (MAP) before and during intravenous infusion of endothelin-1 (50 ng kg^{-1} min^{-1}) into control rats (Group I); baseline values and values from E_1 and E_2 are plotted separately. Linear regression lines are indicated ($U_{Na}V$ = 0.03 MAP - 2.66, r=0.748, p=0.001 and V = 0.21 MAP - 8.84, r=0.563, p=0.015).

Distal Blockade Experiments

The effects of amiloride (Group V) and of a combination of amiloride plus furosemide (Group VI) on endothelin-induced natriuresis and diuresis are summarized in Table IV. Endothelin caused a significant increase in mean arterial pressure at 15 min (31 ± 6 and 36 ± 3% in Groups V and VI, respectively) and 30 min (49 ± 6 and 50 ± 4 %, respectively) during infusion of the peptide; the increases in MAP at 30 min were significantly greater ($p<0.05$) in Groups V and VI compared to Group I controls. There was also a significant endothelin-induced natriuresis and diuresis during the first 15 min of infusion of ET-1 in both amiloride and amiloride + furosemide treated rats, a response that was not sustained at 30 min. As may also be seen in Table IV, there was a relatively large decrease in the GFR at 30 min during infusion of endothelin into both groups of rats.

Figs 11 illustrates the changes in sodium excretion ($\Delta U_{Na}V$) and urine flow rate (ΔV), respectively, at 15 and 30 min induced by endothelin in Groups I (control rats), V and VI. During the first 15 min of the endothelin, the peptide caused a significantly greater natriuresis and diuresis in amiloride-treated compared to controls (Group I), an effect which was even greater in amiloride + furosemide-treated rats; the enhanced natriuresis and diuresis was not observed in either group during the second 15 min of endothelin infusion. It is of interest to note that at 30 min during infusion of endothelin, the GFR decreased significantly ($p<0.01$) by 42 ± 7 and 25 ± 3% in Groups V and VI, respectively; the decreases in the GFR in both groups were significantly greater than in Group I controls ($p<0.05$).

As is illustrated in Fig 12, there were significant correlations between MAP and $U_{Na}V$ in the amiloride-treated rats ($r=0.805$, $p=0.005$) and in amiloride + furosemide-treated rats ($r=0.754$, $p=0.005$); there was a significant correlation between MAP and V in amiloride+furosemide-treated rats ($r=0.755$, $p=0.006$), but the

Table IV. Effects of amiloride (0.3 mg bolus followed by a constant infusion of 0.04 mg min^{-1} kg^{-1}, Group V, n=5) and amiloride plus furosemide (0.8 mg bolus followed by a constant dose of 0.11 mg min^{-1} kg^{-1}, Group VI, n=5) on selected systemic and renal function variables are summarized before and during ET infusion (50 ng min^{-1} kg^{-1}) into rats. Values are means ± SE.

	Basal	**ET-1 (15 min)**	**ET-1 (45 min)**
GFR (ml/min)			
Amiloride	2.3 ± 0.2	2.0 ± 0.1**	1.4 ± 0.2**
Amiloride + Furosemide	2.4 ± 0.2	2.2 ± 0.2	1.8 ± 0.1**
MAP (mmHg)			
Amiloride	100 ± 4	130 ± 4**	147 ± 4**
Amiloride + Furosemide	99 ± 3	134 ± 4**	149 ± 3**
V (μl/min)			
Amiloride	80 ± 6	120 ± 30*	110 ± 20
Amiloride + Furosemide	500 ± 70	640 ± 70**	520 ± 70
$U_{Na}V$ (μeq/min)			
Amiloride	10.5 ± 1.0	17.0 ± 1.7**	12.5 ± 2.7
Amiloride + Furosemide	51.7 ± 4.8	80.2 ± 9.6**	59.0 ± 8.2
FE_{Na} (%)			
Amiloride	3.7 ± 0.35	6.7 ± 0.8**	7.2 ± 1.0**
Amiloride + Furosemide	16.3 ± 2.4	26.8 ± 4.9**	22.3 ± 3.0*
U_KV (μeq/min)			
Amiloride	0.04 ± 0.01	0.17 ± 0.05	0.26 ± 0.09**
Amiloride + Furosemide	1.74 ± 0.2	2.62 ± 0.3**	1.91 ± 0.3
FE_K (%)			
Amiloride	0.47 ± 0.11	2.3 ± 0.7	5.5 ± 1.8**
Amiloride + Furosemide	22.6 ± 3.6	30.8 ± 5.2*	26.3 ± 8.3

* = $p < 0.05$ compared to corresponding baseline value
** = $p < 0.01$ compared to corresponding baseline value

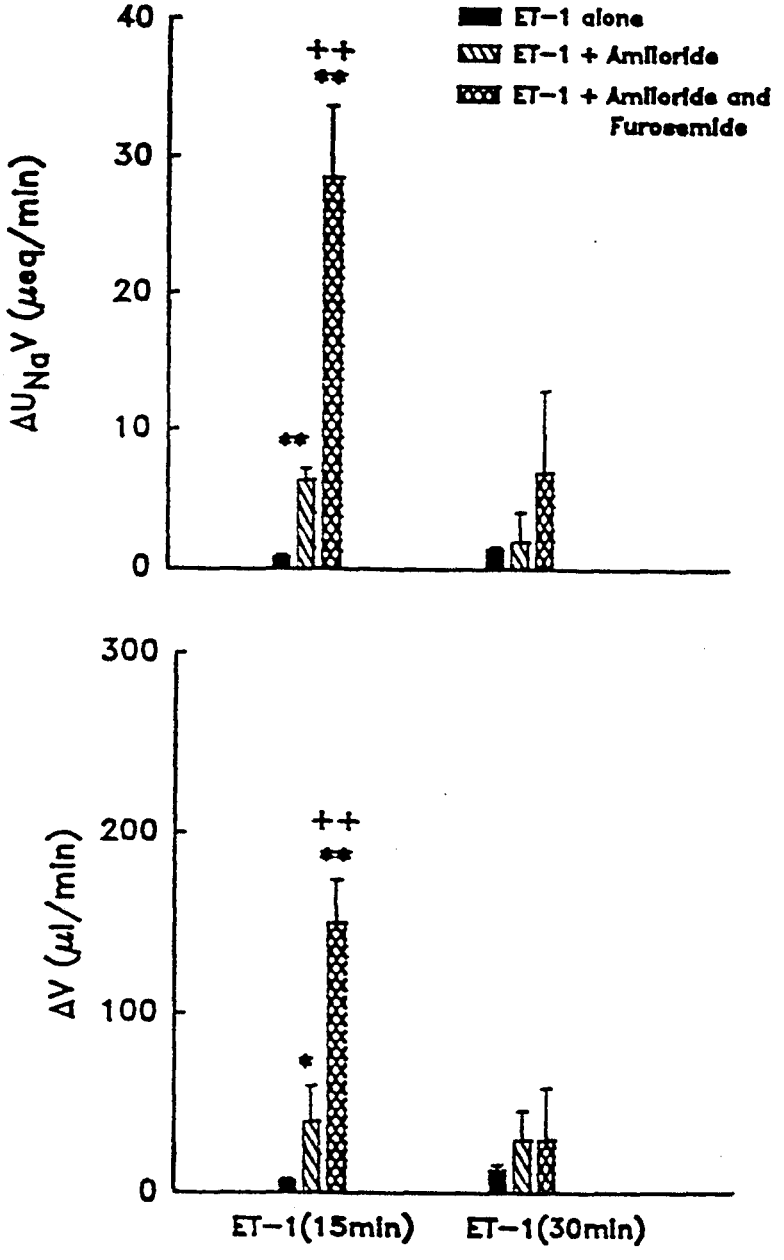

Fig. 11. Endothelin-induced increases (values at 15 and 30 min during ET infusion minus baseline) in sodium excretion rate ($\Delta U_{Na}V$, upper panel) and urine flow rate (ΔV, lower panel) are shown for control rats (Group I), for amiloride treated rats (Group V) and for amiloride plus furosemide treated rats (Group VI). ** = $p < 0.01$ compared to Group I and ++ = $p < 0.01$ compared to Group V. Statistical evaluation of values relative to baseline are reported in Table III and IV.

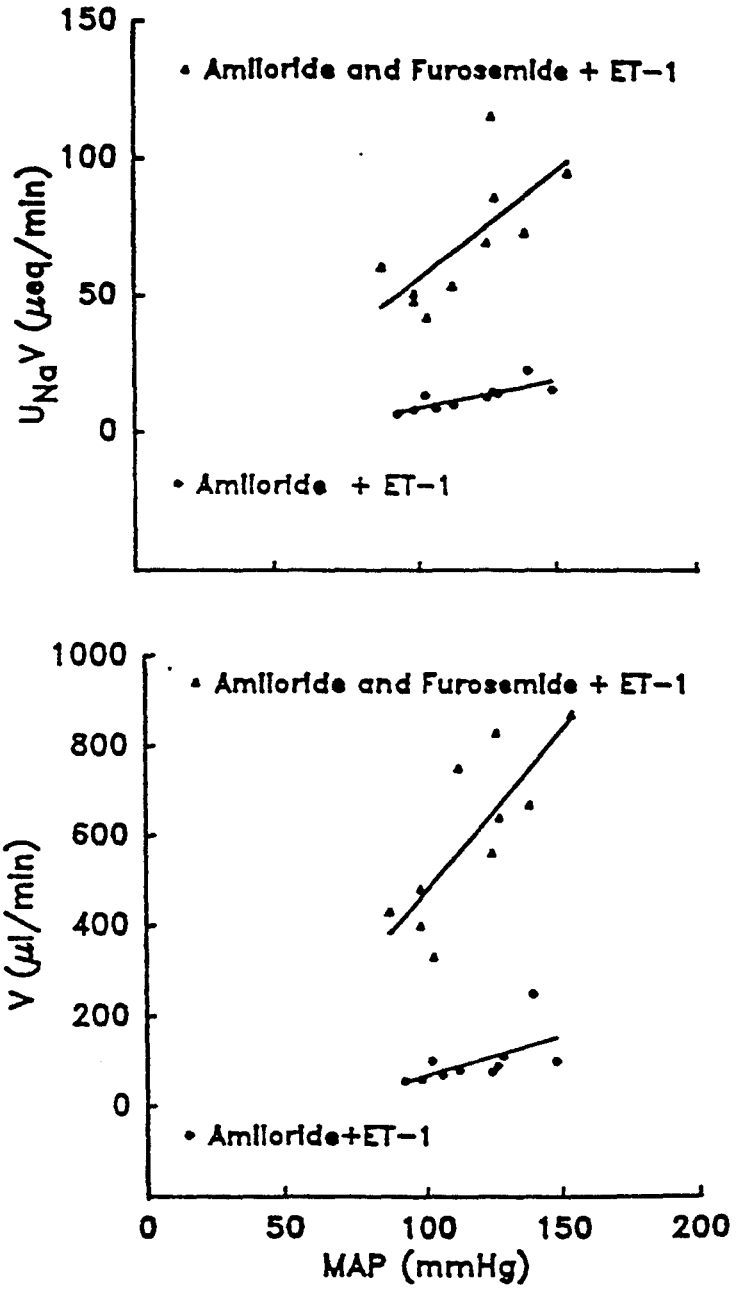

Fig. 12. Sodium excretion ($U_{Na}V$, upper panel) and urine flow rate (V, lower panel) are plotted as a function of mean arterial blood pressure (MAP) before and during intravenous infusion of endothelin-1 (50 ng kg^{-1} min^{-1}) into amiloride-treated rats (Group VII) and into amiloride + furosemide-treated rats (Group VIII); baseline values and values from E_1 are plotted. Linear regression lines are indicated: Group V ($U_{Na}V$ = 0.2 MAP - 10.5, r=0.805, p=0.005 and V = 1.67 MAP - 98.8, r=0.56, p=0.096) and Group VI (($U_{Na}V$ = 0.80 MAP - 23.1, r=0.705, p<0.02 and V = 7.2 MAP - 3.09, r=0.755, p<0.004).

correlation coefficient (0.555) between MAP and V in amiloride-treated rats was of boarderline significance (p=0.096). The data in Fig 12 represent baseline (diuretic only) MAP, $U_{Na}V$ and V values as well the values at 15 and 30 min during infusion of endothelin.

Finally, it is important to note that since urinary volume losses were continuously replaced, the rats in Groups V and VI neither lost nor gained weight during the duration of the experiment.

Amiloride-Treated Rats/Renal Decapsulation

As summarized in Table V, after 30 and 45 min of infusing amiloride instead of endothelin (Group VII rats), both sodium excretion rate and urine flow rate had increased significantly ($p<0.01$). The increases in urine flow rate were not statistically different from that prompted by endothelin in Group I, whereas the increase in sodium excretion rate was significantly greater with amiloride. Mean arterial blood pressure was not affected by the diuretic.

Table V also summarizes the results of experiments in which amiloride was infused into rats with decapsulated kidneys (Group VIII). Removing the renal capsules had no effect on the amiloride-induced increase in either urine flow rate or sodium excretion; the increases in these physiological variables in Group VIII were not significantly different from those observed in Group VII. In addition, amiloride had no effect on mean arterial blood pressure in Group VIII rats.

Other Endothelin Related Studies

High versus low doses of endothelin infusion

Table VI summarizes the systemic and renal responses to an intravenous infusion of a high dose of ET (110 ng kg^{-1} min^{-1})in Group IX; Fig 13 illustrates the changes in the mean arterial pressure and GFR data and Fig 14 illustrates the sodium and urine flow rate data from two group of rats, Group I (low dose ET, 50 ng kg^{-1} min^{-1}) and Group IX (high dose ET). The high dose of endothelin caused

Table V. Effects of amiloride (0.2 mg bolus followed by a constant infusion of 0.02 mg/min) on selected systemic and renal function variables are summarized for Group VII (intact, n=5) and Group VIII (renal decapsulation, n=5). Values are means ± SE.

	Basal	**Amiloride (30 min)**	**Amiloride (45 min)**
GFR (ml/min)			
VII	2.2 ± 0.2	2.4 ± 0.2	2.2 ± 0.3
VIII	2.2 ± 0.1	2.2 ± 0.1	2.3 ± 0.1
MAP (mmHg)			
VII	91 ± 4	85 ± 3	88 ± 2
VIII	98 ± 3	93 ± 3	93 ± 3
V (μl/min)			
VII	11 ± 1	17 ± 1**	19 ± 1**
VIII	11 ± 1	17 ± 2**	18 ± 2**
$U_{Na}V$ (μeq/min)			
VII	0.38 ± 0.1	4.60 ± 0.3**	4.80 ± 0.4**
VIII	0.22 ± 0.3	3.50 ± 0.4**	3.50 ± 0.5**
FE_{Na} (%)			
VII	0.13 ± 0.02	1.54 ± 0.16**	1.74 ± 0.18**
VIII	0.10 ± 0.02	1.27 ± 0.13**	1.26 ± 0.19**
U_KV (μeq/min)			
VII	1.2 ± 0.05	0.03 ± 0.004**	0.03 ± 0.004**
VIII	1.0 ± 0.14	0.03 ± 0.002**	0.03 ± 0.005**
FE_K (%)			
VII	14.7 ± 0.5	0.3 ± 0.06**	0.3 ± 0.03**
VIII	18.1 ± 3.6	0.3 ± 0.04**	0.3 ± 0.07**

** = $p < 0.01$ compared to corresponding baseline value

a profound decrease in GFR which was not observed with the lower dose of endothelin. Similarly, there were significant decreases in V, $U_{Na}V$ and fractional excretion of sodium (FE_{Na}) with 110 ng kg^{-1} min^{-1} ET infusion but significant increases in these variables in the 50 ng kg^{-1} min^{-1} ET group. In Group IX rats, the MAP increase was significantly higher ($p<0.01$) than in Group I during the first 15 min of infusion of the peptide; however, there was no significant difference in MAP between the two groups at 30 min.

Autonomic nerve blockade

As shown in Table VII and Figs 15 and 16, there were no significant differences in V, $U_{Na}V$ and fractional excretion of sodium (FE_{Na}) or in the increase in MAP or decrease in the GFR between the Group I (Control) rats and the rats treated with hexamethonium.

EDRF/NO-synthesis blockade

As shown in Table VIII, under the current experimental conditions, LNNA infusion into anesthetized rats produced a marked natriuresis ($p<0.01$) and diuresis ($p<0.01$) that was associated with a substantial increase in MAP ($p<0.01$). LNNA treatment further enhanced both the systemic and renal vasoconstriction actions of ET-1 during the infusion of the peptide compared to control group (Group XI) (Figs 17 and 18). The magnitute of the natriuretic and diuretic response observed with LNNA treatment period decreased during ET-1 infusion. During the infusion of ET, there was a substantial decrease in the GFR in this group of rats.

Table VI. Endothelin-1 induced changes in selected physiological variables in two groups of rats: Group I-ET50 (50 ng kg^{-1} min^{-1}) and Group IX-ET110 (110 ng kg^{-1} min^{-1}). Values are means ± SE.

GFR (ml/min)	**Basal**	**ET-1 (15 min)**	**ET-1 (30 min)**
I ET50 (n=6)	2.10 ± 0.09	2.20 ± 0.14	1.8 ± 0.19*
IX ET110 (n=8)	2.40 ± 0.12	1.27 ± 0.1**	0.21 ±0.04**
MAP (mmHg)			
I ET50 (n=6)	95 ± 2	116 ± 3**	131 ± 2**
IX ET110 (n=8)	89 ± 3	128 ± 3**	134 ± 2**
V (μl/min)			
I ET50 (n=6)	12 ± 1.0	19 ± 1.4**	24 ± 3**
IX ET110 (n=8)	11 ± 1.8	11 ± 1.4	3 ± 0.7**
$U_{Na}V$ (μeq/min)			
I ET50 (n=6)	0.34 ± 0.07	1.22 ± 0.15**	1.83 ± 0.2**
IX ET110 (n=5)	0.19 ± 0.04	0.22 ± 0.05	0.04 ± 0.014**
FE_{Na} (%)			
I ET50 (n=6)	0.18 ± 0.03	0.44 ± 0.08**	0.72 ± 0.12**
IX ET110 (n=5)	0.05 ± 0.016	0.16 ± 0.07	0.17 ± 0.07
U_KV (μeq/min)			
I ET50 (n=6)	1.40 ± 0.12	1.90 ± 0.13*	1.63 ± 0.25
IX ET110 (n=5)	0.98 ± 0.19	0.96 ± 0.14	0.16 ± 0.06**
FE_K (%)			
I ET50 (n=6)	18.1 ± 1.4	25.9 ± 1.9**	24.3 ± 1.6**
IX ET110 (n=5)	10.4 ± 1.9	17.7 ± 3.7	16.7 ± 5.2

*** = $p < 0.05$ compared to corresponding baseline value**
**** = $p < 0.01$ compared to corresponding baseline value**

Statistical evaluations of differences between groups are shown in Figs. 13 and 14.

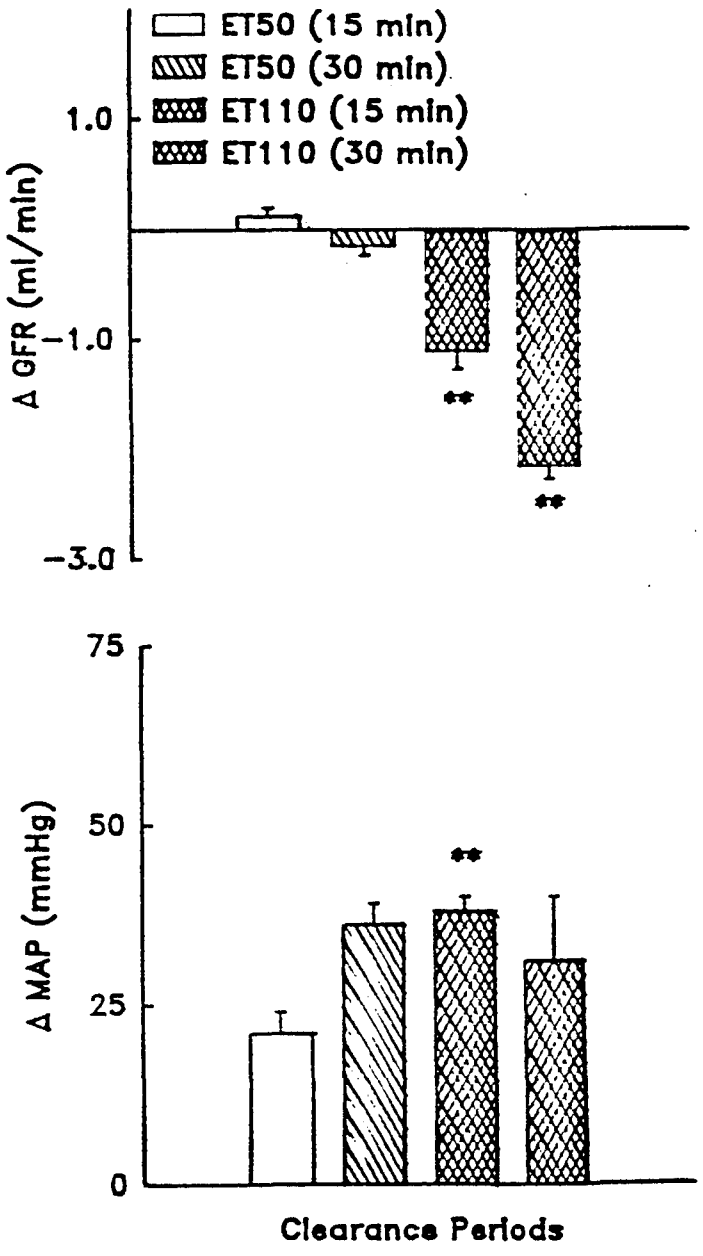

Fig. 13. Dose dependent changes in mean arterial pressure (ΔMAP, lower panel) and glomerular filtration rate (ΔGFR, upper panel) are shown during low (50 ng kg^{-1} min^{-1}) and high (110 ng kg^{-1} min^{-1}) doses of endothelin infusion into rats (values at 15 and 30 min during ET infusion minus baseline). * = $p< 0.05$ and ** = $p< 0.01$ compared to the corresponding time matched values between the groups. Statistical evaluation of values relative to baseline are reported in Table VI.

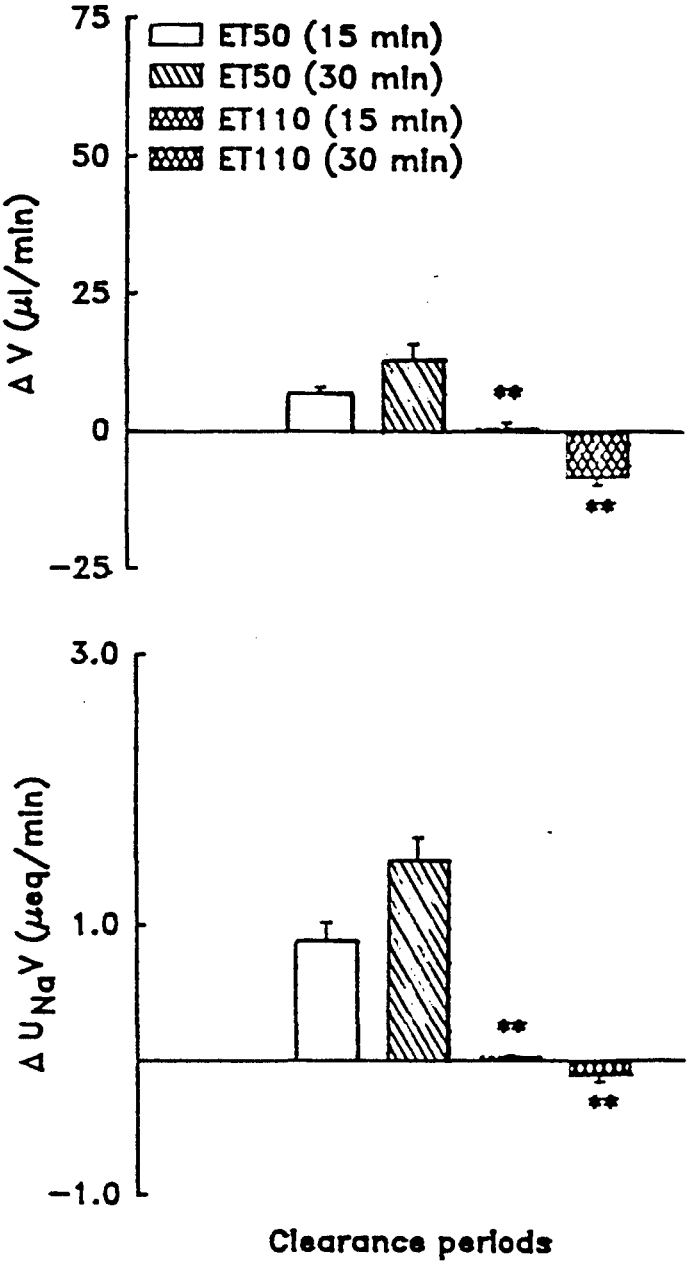

Fig. 14. Dose dependent changes in sodium excretion ($\Delta U_{Na}V$, lower panel) and urine flow rate (ΔV, upper panel) are shown during low (50 ng kg^{-1} min^{-1}) and high (110 ng $kg^{-1}.min^{-1}$) doses of endothelin infusion into rats (values at 15 and 30 min during ET infusion minus baseline). * = $p< 0.05$ and ** = $p< 0.01$ compared to the corresponding time matched values between the groups. Statistical evaluation of values relative to baseline are reported in Table VI.

Table VII. Endothelin-1 induced changes in selected physiological variables in two groups of rats. Group I-ET50 (50 ng $kg^{-1}min^{-1}$) and Group X-ET50 (50 ng $kg^{-1}min^{-1}$) plus hexamethonium (Hx) (20 mg/kg bolus injection followed by 5 mg $kg^{-1}min^{-1}$ constant infusion). Values are means ± SE.

GFR (ml/min)	**Basal**	**ET-1(15 min)**	**ET1(30min)**
I ET50 (n=6)	2.10 ± 0.09	2.20 ± 0.14	1.8 ±0.19*
X ET50 + Hx (n=6)	2.14 ± 0.06	2.27 ± 0.24	2.0 ±0.16
MAP (mmHg)			
I ET50 (n=6)	95 ± 2	116 ± 3**	131 ± 2**
X ET50 + Hx (n=6)	80 ± 5	114 ± 4**	130 ± 7**
V (μl/min)			
I ET50 (n=6)	12 ± 1.0	19 ± 1.4**	24 ± 3**
X ET50 + Hx (n=6)	12 ± 0.6	19 ± 5	24 ± 5*
$U_{Na}V$(μeq/min)			
I ET50(n=6)	0.34 ± 0.07	1.22 ± 0.15**	1.83 ±0.2**
X ET50+Hx (n=5)	0.27 ± 0.05	0.82 ± 0.08	1.77 ± 0.31**
FE_{Na} (%)			
I ET50 (n=6)	0.18 ± 0.03	0.44 ± 0.08**	0.72 ± 0.12**
X ET50 + Hx (n=5)	0.12 ± 0.04	0.60 ± 0.25	1.04 ± 0.33**
U_KV (μeq/min)			
I ET50 (n=6)	1.40 ± 0.12	1.90 ± 0.13*	1.63 ± 0.25
X ET50 + Hx (n=5)	2.24 ± 0.15	2.98 ± 0.4	2.50 ± 0.38
FE_K (%)			
I ET50 (n=6)	18.1 ± 1.4	25.9 ± 1.9**	24.3 ± 1.6**
X ET50 + Hx (n=5)	25.4 ± 2.4	29.2 ± 3.6	33.4 ± 5.0

*** = $p < 0.05$ compared to corresponding baseline value**
**** = $p < 0.01$ compared to corresponding baseline value**
Statistical evaluations of differences between groups are shown in Figs. 15 and 16.

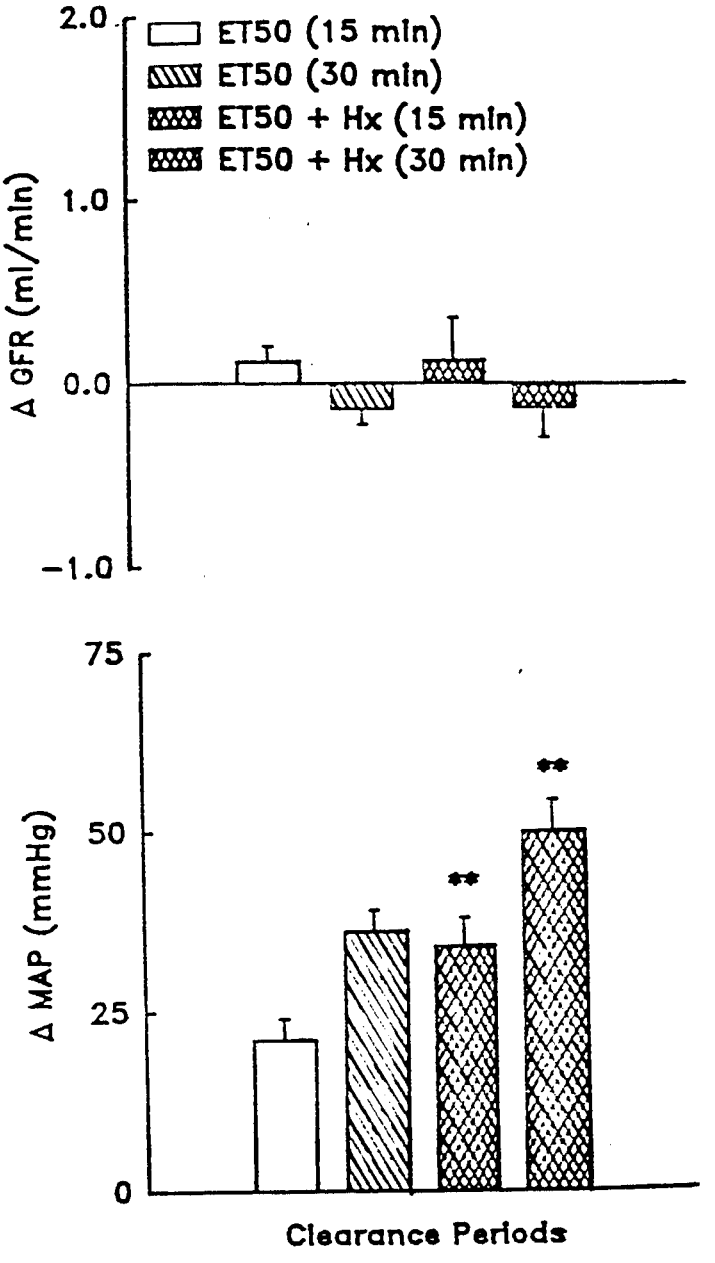

Fig. 15. Endothelin-induced changes (values at 15 and 30 min during ET infusion minus baseline) in mean arterial pressure (ΔMAP, lower panel) and glomerular filtration rate (ΔGFR, upper panel) are shown for control rats (Group I) and hexamethonium treated rats (Group X). * = $p< 0.05$ and ** = $p< 0.01$ compared to the corresponding 15 and 30 min values of the groups. Statistical evaluation of values relative to baseline are reported in Table VII.

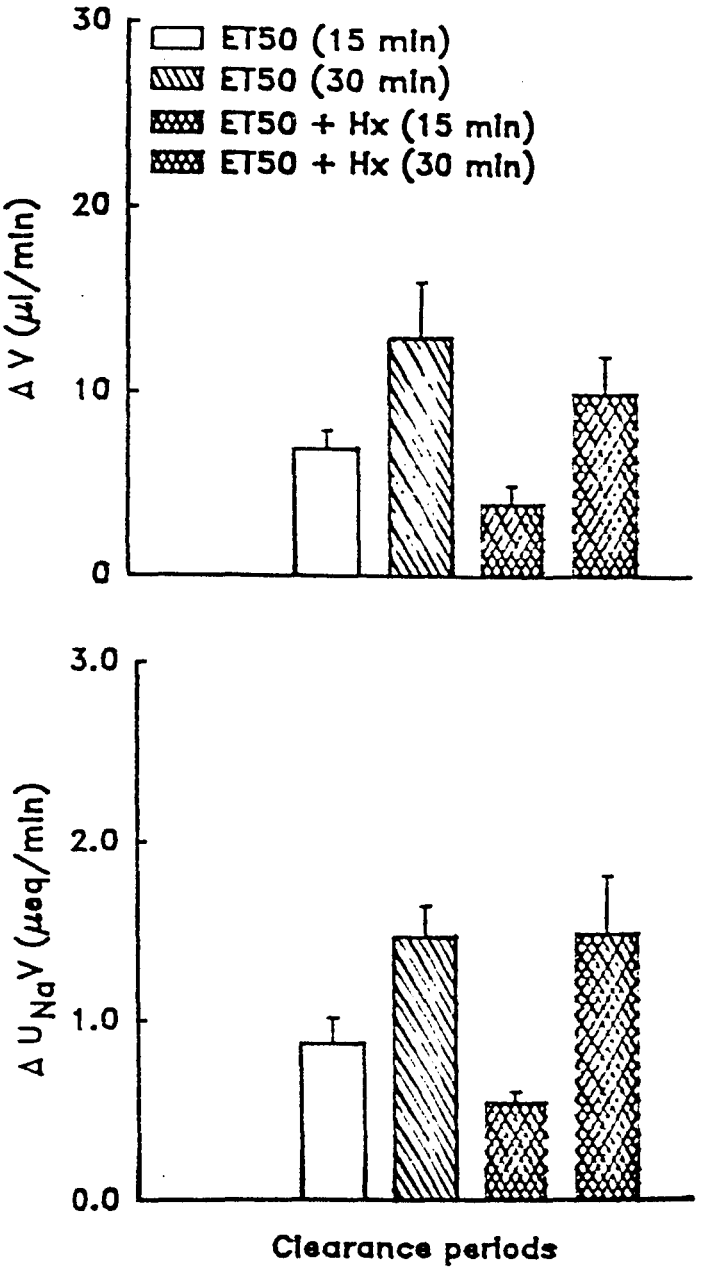

Fig. 16. Endothelin-induced changes (relative to baseline) in sodium excretion rate ($\Delta U_{Na}V$, lower panel) and urine flow rate (ΔV, upper panel) are shown for control rats (Group I) and hexamethonium treated rats (Group X). * = $p < 0.05$ and ** = $p < 0.01$ compared to the corresponding 15 and 30 min values minus of the groups. Statistical evaluation of values relative to baseline are reported in Table VII.

Table VIII. Effects of L-NNA (Nitro-L-Arginine; 50 $\mu g\ kg^{-1}\ min^{-1}$) infusion on selected systemic and renal function variables are summarized before and after endothelin (50 $ng\ kg^{-1}\ min^{-1}$) infusion (n=6). Values are meansa ± SE.

	Basal	L-NNA	ET-1(15 min)	ET1(30 min)
GFR (ml/min)	2.3 ± 0.08	1.9 ± 0.08*	1.13 ± 0.15**	0.68 ± 0.1**
MAP (mmHg)	87 ± 3	122 ± 4**	153 ± 3**	146 ± 2**
UV (μl/min)	18 ± 3	48 ± 7**	26 ± 9	16 ± 3
$U_{Na}V$ (μeq/min)	0.17 ± 0.03	3.9 ± 0.2**	2.7 ± 0.5**	1.2 ± 0.6
FE_{Na} (%)	0.07 ± 0.01	1.98 ± 0.3**	1.68 ± 0.2**	1.14 ± 0.4**
U_KV (μeq/min)	1.1 ± 0.2	1.34 ± 0.13	0.87 ± 0.06	0.5 ± 0.1**
FE_K (%)	16 ± 3	20.1 ± 1.7	19.7 ± 2	17 ± 2.3

* = $p < 0.05$ compared to corresponding baseline value

** = $p < 0.01$ compared to corresponding baseline value
Statistical evaluations of differences between groups are shown in Figs. 17 and 18

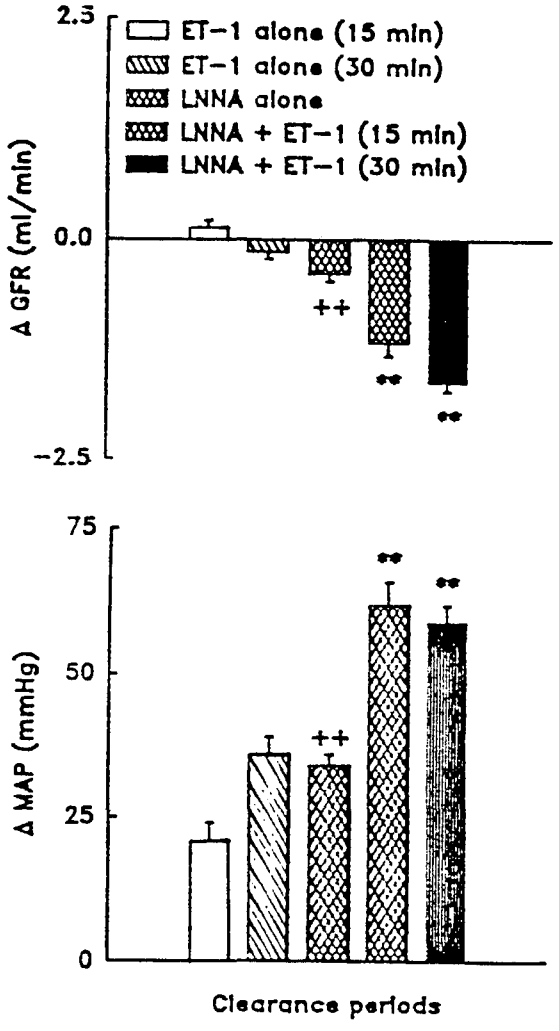

Fig. 17. Endothelin-induced changes (values represent the differences between the experimental values and the corresponding baseline values for each group) in mean arterial pressure (ΔMAP, lower panel) and glomerular filtration rate (ΔGFR, upper panel) are shown for control rats (Group I) and for LNNA treated (50 μg kg^{-1} min^{-1}) rats before and during intravenous infusion of endothelin-1 (50 ng kg^{-1} min^{-1}). * = $p< 0.05$ and ** = $p< 0.01$ compared to the time matched values of Group I (control) and LNNA treated rats at 15 and 30 min during ET infusion; ++ = $p< 0.01$ and xx = $p< 0.01$ represent LNNA control values compared with Group I values at 15 and 30 min ET infusion, respectively. Statistical evaluation of values relative to baseline are reported in Table VIII.

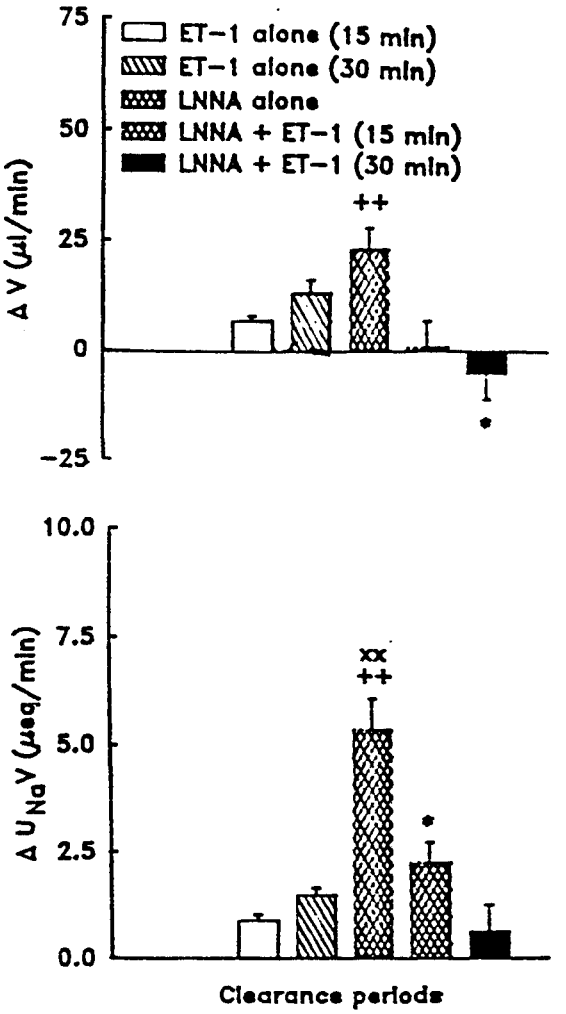

Fig. 18. Endothelin-induced changes (values represent the differences between the experimental values and the corresponding baseline values for each group) in sodium excretion ($\Delta U_{Na}V$ lower panel) and urine flow rate (ΔV, upper panel) are shown for control rats (Group I) and for LNNA treated (50 μg kg^{-1} min^{-1}) rats before and during intravenous infusion of endothelin-1 (50 ng kg^{-1} min^{-1}). * = $p< 0.05$ and ** = $p< 0.01$ compared to the time matched values of Group I (control) and LNNA treated rats at 15 and 30 min during ET infusion; ++ = $p< 0.01$ and xx = $p< 0.01$ represent LNNA control values compared with Group I values at 15 and 30 min ET infusion, respectively. Statistical evaluation of values relative to baseline are reported in Table VIII.

DISCUSSION

The primary goal of the current study was to examine the mechanism by which endothelin induces a natriuresis and diuresis. The results of these experiments demonstrate that the endothelin-induced natriuresis and diuresis are enhanced in amiloride and in amiloride + furosemide treated rats, that either removing the renal capsules or maintaining a constant renal arterial pressure during intravenous infusions of endothelin abolish the endothelin-induced natriuresis and diuresis and that renal decapsulation has no effect on the natriuretic and diuretic response to amiloride, a diuretic agent known to exert its actions via a direct inhibition of sodium reabsorption at the tubular level. These data indicate that endothelin inhibits renal sodium and water excretion proximal to the thick ascending limb and that this inhibition occurs in a fashion that is dependent on an elevation of systemic arterial blood pressure, i.e., a mechanism consistent with the interpretation that endothelin-induced natriuresis and diuresis represent phenomena that are generally referred to as pressure natriuresis and diuresis (31,35,36,92,125). Moreover, it would appear that the direct inhibition by endothelin of sodium reabsorption at the tubular level (145) does not significantly contribute to the natriuretic and diuretic actions of the peptide under the current experimental conditions since decapsulating the kidneys or maintaining a constant renal arterial pressure during infusion of the peptide would not be expected to markedly affect the endothelin-induced natriuresis and diuresis if a direct tubular action did predominate.

Although, a number of studies from numerous laboratories have indicated that endothelin causes an increase in renal vascular resistance and a resultant large decrease in the glomerular filtration rate, urine flow rate and urinary excretion of sodium (6,8,9,53,68,79,90), the data shown in Table VI illustrate that there are marked differences between low and high doses of endothelin-1. The data suggest that a relatively low dose of endothelin are which does not cause marked reductions in GFR, is

associated with a natriuresis and diuresis and a marked increases in mean arterial blood pressure.

The results obtained in Group III rats (constant renal arterial pressure) confirm and extend those of King et al. (60). They reported that the natriuresis and diuresis associated with intravenous bolus injections of endothelin in the rat were also abolished when renal arterial pressure was maintained at baseline values. Results of the current study demonstrate that under steady-state conditions utilizing a constant infusion of the peptide, that there is also a primary dependence of the endothelin-induced natriuresis and diuresis on increases in renal arterial pressure. Indeed, there was a very high correlation between mean arterial blood pressure and sodium excretion rate (Fig 10), a phenomenon also recently described by Takabatake et al. (130).

The fact that removing the renal capsule also abolishes endothelin-induced natriuresis and diuresis provides additional evidence that these are pressure-related phenomena. Several laboratories have demonstrated that an intact renal capsule is a critical component of pressure natriuresis and diuresis (25,31,55). Moreover, in contrast to the endothelin experiments, removing the renal capsule had no effect on the amiloride-induced natriuresis and diuresis (results of Group VII and VIII). These data indicate that non-specific factors such as surgical trauma associated with renal decapsulation do not account for the inhibition by this procedure of the renal actions of endothelin.

A number of studies have demonstrated that the integrity of the renal capsule is an absolute requirement for the increases in renal arterial pressure to be transmitted into increases in sodium excretion rate (32,56,111). The latter studies have documented that a close correlation exists between increases in renal arterial pressure and increases in renal interstitial fluid pressure. The mechanism that accounts for this correlation has not been fully defined. One possibility is that despite a very efficient autoregulation of blood flow to the renal cortex, changes in renal arterial pressure are followed by similar

changes in medullary blood flow (111). It has been suggested that pressure-induced increases in papillary plasma flow and a resultant decrease in fluid reabsorption across the vasa recta capillary might be responsible for the increase in medullary interstitial fluid pressure (32,56,111).

The exact mechanism by which changes in renal interstitial fluid pressure affect the tubular reabsorption of sodium is unknown. It has been proposed that increases in interstitial fluid pressure could theoretically decrease tubular reabsorption of sodium and water both in the presence or absence of changes in the release rate of humoral factors (60,70,100,113,122). Prostaglandins and other potentially important products of arachidonic acid metabolism as well as the renin-angiotensin, the kallikrein-kinin system and endothelium derived relaxing factor have all been reported to transduce to the nephron the changes in renal arterial and renal interstitial pressure (60,70,100,113,122). Along these lines it is of importance to note that in the current study, meclofenamate had no effect on the endothelin-induced natriuresis or diuresis, a finding which does not support a role for prostaglandins in these biological actions of endothelin. The latter results are consistent with our previous report (9) with higher doses of endothelin which demonstrated that neither meclofenamate nor indomethacin affect the cardiorenal actions of the peptide.

Harris et al. (43) reported that low doses of endothelin (10 ng kg^{-1} min^{-1}) cause a renal vasodilation and a natriuresis and diuresis. Indeed, previous data from our laboratory (6) demonstrated that intrarenal infusions of endothelin (1 ng kg^{-1} min^{-1} into the renal artery of the dog) cause a transient renal vasodilation and a natriuresis and diuresis during that time interval. Moreover, Harris et al. (43) and Perico et al. (101)demonstrated an increase in lithium clearance during the infusion of low doses of endothelin, indicating that the peptide inhibits proximal sodium reabsorption. In the current study the endothelin-induced increases in sodium and urine flow rate were significantly enhanced in amiloride and in amiloride + furosemide treated rats (Fig 11),

clearly supporting the interpretation that the major natriuretic and diuretic site of endothelin is proximal to the thick ascending limb.

Recent studies related to the inhibition of endogenous EDRF/NO biosynthesis with competitive L-arginine analogues such as L-N^{ω}-Nitro-arginine (LNNA) or N^{G}-monomethyl-L-arginine (L-NAME) have indicated that EDRF/NO contributes to the control of basal systemic and regional vascular resistances (2,12,26,70,84,85,99,106,133). The elevation of blood pressure during NO synthesis inhibition with L-NAME (75 mol/kg i.v.) into anesthetized rats is associated with a natriuresis (51). When the renal perfusion pressure was maintained at a constant level in the later study, the natriuresis was prevented, suggesting that NO inhibition causes a pressure natriuresis. On the other hand, Salom et al. (122) have reported that the intrarenal administration of L-NAME at a dose of 1 $\mu g\ kg^{-1}\ min^{-1}$ into pentobarbital-anesthetized dogs did not cause significant changes in renal blood flow or GFR but blunted the pressure natriuretic and diuretic response increases in MAP from 100 to 150 mmHg. In another study (71), the inhibition of endogenous NO production with N^{G}-monomethyl-L-arginine (L-NMMA) at a rate of 50 $\mu g\ kg^{-1}\ min^{-1}$ i.v. potentiated the systemic response to ET-1. However, the authors did not report values for sodium excretion or urine flow rate. The results of the current study support the findings of that the basal release of EDRF/NO may be involved in the homeostasis of intravascular volume and pressure regulation. Furthermore, the vasoconstrictor actions of ET-1 appear to be modified by endogenous EDRF/NO biosynthesis. However, the data from the current experiments are not conclusive with regard to the potential role of EDRF/NO biosynthesis in ET-induced natriuresis and diuresis. This is because the decrease in the natriuresis and diuresis during ET-1 infusion in LNNA treated rats might be result of a decreased filtered load of sodium. Thus, the involvement of EDRF/NO synthesis in endothelin-induced natriuresis and diuresis requires further investigation.

Renal sympathetic nerve activity may alter urinary sodium and water excretion (14,18,98) by stimulating renal tubular sodium reabsorption directly at the tubular level and/or by releasing renin from the juxta-glomerular apparatus. The results obtained in Group X rats (hexamethonium treated rats) suggest that there was no significant autonomic nervous system involvement in either the natriuresis and diuresis or the systemic response to the lower dose of ET-1 infusion under current experimental conditions.

In summary, the present data demonstrate that endothelin-induced increases in sodium excretion and urine flow rate are dependent on increases in renal arterial blood pressure and that the intrarenal site of action of endothelin appears to be proximal to the thick ascending limb. Thus, the natriuretic and diuretic actions of the peptide are abolished either by maintaining a constant renal arterial pressure or by removing the kidney capsule and are enhanced in rats treated with amiloride and furosemide. In addition, neither meclofenamate nor hexamethonium treatment affects the cardio-renal actions of endothelin suggesting that changes in prostaglandin synthesis or changes in sympathetic nerve activity are not involved in these responses. On the other hand, LNNA-treatment enhances the endothelin induced increase in MAP as well as the decrease in GFR indicating that endogenous EDRF/NO counteracts the vasoconstrictive actions of ET-1.

In conclusion, under the conditions of the current study, it appears that the natriuretic and diuretic actions of endothelin are pressure-related phenomena and that direct tubular actions of the peptide do not contribute significantly to these responses.

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