This is to certify that the

dissertation entitled

The Cardiovascular Physiology of Endothelin-1
-Characterization of Endothelin-Induced Hypertension-

presented by

Luke Henry Mortensen

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Pharmacology/
Toxicology

Major professor

Date 6/16/92

THE CARDIOVASCULAR PHYSIOLOGY OF ENDOTHELIN-1

~CHARACTERIZATION OF ENDOTHELIN-INDUCED HYPERTENSION~

By

Luke Henry Mortensen

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Pharmacology and Toxicology

1992

ABSTRACT

THE CARDIOVASCULAR PHYSIOLOGY OF ENDOTHELIN-1
~CHARACTERIZATION OF ENDOTHELIN-INDUCED HYPERTENSION~

By

Luke Henry Mortensen

The etiology of essential hypertension is unknown. Experimental models of hypertension implicate a role for various endogenous vasoactive factors, such as arginine vasopressin and angiotensin II, in certain forms of essential hypertension. **Endothelin (ET-1)** also has been shown to have remarkably potent and long-lasting vasoactive properties, *exceeding those of angiotensin II and arginine vasopressin*. Therefore, the hemodynamic characteristics of exogenously administered ET-1 were assessed in conscious rats in order to elucidate the potential role for this novel peptide in the pathogenesis of essential hypertension.

Chronic intravenous infusion of ET-1 produced sustained, dose-dependent, salt-dependent and reversible increases in mean arterial pressure (MAP) associated with increases in total peripheral resistance (TPR). Mechanisms involved in the sustained increase in TPR may be direct vascular smooth muscle constriction and/or an alteration in the level of sympathetic nerve activity. In support, endothelin-immunoreactivity and receptors have been identified throughout the vasculature and the central nervous system.

Experiments were performed to elucidate a role for the renin-angiotensin system in endothelin-hypertension since other laboratories have described potent renal actions of endothelin. Chronic infusion of angiotensin I converting-enzyme

(ACE) inhibitors, captopril or enalapril, completely prevented endothelin-hypertension development. Alternatively, the angiotensin II type-1 (AT_1) receptor antagonist losartan was not able to prevent the development of endothelin-hypertension. These observations suggest an action of ACE products in endothelin-hypertension which are independent of an action of angiotensin II on AT_1 receptors.

As observed in animal models of angiotensin-hypertension, the contribution of ET-1 to the autonomic control of arterial pressure may also depend on the integrity of certain circumventricular brain regions such as the area postrema. Area postrema ablation, however, did not prevent or attenuate endothelin-hypertension. Sino-aortic denervation, however, significantly augmented endothelin-hypertension, suggesting a significant action of ET-1 on the baroreceptor reflex.

These results suggest a potential role for ET-1 in essential hypertension development and maintenance by interactions with components of the renin-angiotensin system, the baroreceptor reflex, and renal fluid and electrolyte homeostasis.

To Greg,

with appreciation, admiration, and thanks

ACKNOWLEDGEMENTS

My sincerest gratitude goes to my advisor, Dr. Gregory D. Fink, for his enduring patience, encouraging support, and his ever-present and transcending enthusiasm for scientific research. I will always be thankful for his guidance and his friendship. He, as well as Dr. Susan Barman, have constantly provided a stimulating and model research environment on which I hope to ultimately pattern my own research. Additionally, I would like to thank Drs. K.E. Moore and R.L. Stephenson for their kind services on my thesis committee.

Truly, the investigations presented in this dissertation could not have been accomplished without the incomparable technical and surgical assistance of my friends and co-workers John Fuentes, Vyvian Gorbea-Oppliger, Nancy Kanagy, Annette McLane, John Meier, Corinne Pawloski and Renee Petite. The contributions of other investigators to this research is also gratefully acknowledged. Specifically, I would like to thank Dr. Michael Mangiapane for his invaluable surgical instruction on area postrema ablation and Dr. Eric Schultze for histological assessment of area postrema ablation.

My thanks also go to the staff of the Department of Pharmacology and Toxicology and the Department of Physiology for their continued assistance throughout my training. I especially want to thank Nelda Carpenter and Sharon Shaft for their genuine concern and friendship.

Lastly, I would like to thank Robert L. Stinnett (Bobby) and my parents for their nurturing love, support and patience throughout the years. Thank you all.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACE ..... angiotensin I converting enzyme
Aldo ..... aldosterone
ANF ..... atrial natriuretic factor
ANP ..... atrial natriuretic peptide
$[AngII]_P$ ..... plasma angiotensin II concentration
AngII ..... angiotensin II
APX ..... area postrema ablation
AT_1 ..... angiotensin, type 1 receptor
AVP ..... arginine vasopressin
cGMP ..... cyclic guanosine monophosphate
CO ..... cardiac output
CSF ..... cerebrospinal fluid
DAG ..... diacylglycerol
DOCA ..... deoxycorticosterone acetate
EDCF ..... endothelial-derived constricting factor
EDRF ..... endothelial-derived relaxing factor
$[ET\text{-}1]_P$ ..... plasma endothelin-1 concentration
ET-1 ..... endothelin-1
ET-2 ..... endothelin-2
ET-3 ..... endothelin-3
ET_{ir} ..... endothelin immunoreactivity
GFR ..... glomerular filtration rate
HR ..... heart rate
hr ..... hour, hours
i.a.. ..... intraarterial
i.m. ..... intramuscular
i.p. ..... intraperitoneal
i.v. ..... intravenous
IP_3 ..... inositol triphosphate
IP_4 ..... inositol tetrakisphosphate
MAP ..... mean arterial pressure
min ..... minute, minutes
NF-1 ..... nuclear factor-1
NTS ..... nucleus tractus solitarius
P_K ..... plasma potassium concentration
P_{Na} ..... plasma sodium concentration
ppET-1 ..... preproendothelin-1
PRA ..... plasma renin activity
RAS ..... renin-angiotensin system
RBF ..... renal blood flow
RIA ..... radio-immunoassay
SAD ..... sino-aortic deafferentation

SEM ..... standard error of the mean
SHR ..... spontaneously hypertensive rat
STX ..... sarafotoxin
SV ..... stroke volume
TGF_{β} ..... transforming growth factor-β
TPA 12-*O*-tetradecanoylphorbol-13-acetate
TPR ..... total peripheral resistance
UO ..... urine output
U_KV ..... urinary potassium excretion
$U_{Na}V$ ..... urinary sodium excretion
WB ..... daily water balance
WI ..... daily water intake
WKY ..... Wistar-Kyoto rat

INTRODUCTION

A. Early Background

The physiologic role of endothelin (ET-1) is presently unclear. ET-1, like other peptide hormones, is nearly ubiquitous throughout the body and may also play a role in the regulation of homeostasis. Serving in an endocrine, paracrine or even neuroendocrine fashion, peptide hormones can control many physiological processes such as cardiovascular hemodynamics; neurotransmission and neuromodulation; skeletal, cardiac or smooth muscle contractility; as well as cell secretion and proliferation. In addition, peptide hormones also have been shown to play major pathophysiological roles in certain clinical diseases such as atherosclerosis, hypertension and oncogenesis (Sporn *et al.*, 1988). It is, therefore, not surprinsing that the recent discovery of endothelin has attracted so much investigational interest (Yanagisawa *et al.*, 1988).

ET-1 is a 21-amino acid peptide, released from vascular endothelial cells, which has been shown to produce a potent and sustained vasoconstriction, both *in vitro* and *in vivo*, in a variety of species including dog (Goetz *et al.*, 1988), pig (Kimura *et al.*, 1988), rat (Hirata *et al.*, 1988), cat (Lippton *et al.*, 1988), rabbit (Thiemermann *et al.*, 1988), and guinea pig (Uchida *et al.*, 1988). The contractility of vascular smooth muscle is controlled by various neural and hormonal signals

together with the local control mechanisms intrinsic to the blood vessel wall and it is well recognized that the vascular endothelium plays an important role in the control of vascular tonus primarily through the production of both vasorelaxant and vasoconstrictor substances. In 1980, Furchgott and Zawadski showed that acetylcholine-induced vasodilatation is dependent on the presence of endothelial cells, which release a dilating substance now identified as endothelium-derived relaxing factor (EDRF). Substantial data exist which demonstrate that, in addition to mediating vasodilation, the endothelium can also facilitate contractile responses of the vascular smooth muscle (Rubanyi, 1988). Various chemical and mechanical factors, including noradrenaline, thrombin, neuropeptide Y, calcium ionophore A23187, arachidonic acid, hypoxia, stretch and an increased transmural pressure have all been shown to produce a vasoconstriction which is dependent on an intact endothelium. Similarly, in 1984, investigations by O'Brien *et al.* demonstrated the presence of a protease-sensitive, vasoconstrictive substance produced by the vascular smooth muscle endothelium which they named endothelium-derived contracting factor (EDCF). Since this earlier discovery, several "EDCF's" have been reported and the recently isolated ET-1 has been shown to have many of the cardiovascular properties of the EDCFs described by O'Brien and Gillespie (1985).

Since its isolation and characterization, many investigations have focused on the ability of ET-1 to produce a potent and sustained vasoconstriction. Several studies have also shown that ET-1 produces a bi-phasic blood pressure response, an initial brief depressor response followed by a sustained pressor response

(Wright *et al.* 1988), which further complicates any proposed physiologic role for ET-1.

B. Molecular Identification of Endothelin

Experimental evidence suggests the presence of a mammalian "endothelin family" predicted by three endothelin-related genes located on three separate chromosomes (Inoue *et al.*, 1989). Three distinct endothelin-related genes were cloned by screening a human genomic DNA library. Additional genomic southern-blot analysis demonstrated that the three corresponding genes existed not only in the human genome but also in rat and porcine genomes. The amino acid sequences of the 21-residue peptides predicted by the three human genes were similar to but distinct from each other at several amino acid residues. These peptides were named ET-1 (after the originally discovered porcine/human endothelin), ET-2 (two amino acid substitutions) and ET-3 (six amino acid substitutions) (Table 1, page 7). Synthetic human ET-2 and ET-3, like ET-1, produce a strong vasoconstriction *in vitro* further characterized by a transient depressor response followed by a sustained pressor response *in vivo* (Takasaki *et al.*, 1988). The potencies of the *in vitro* constrictor activity and the *in vivo* pressor activity were defined as: ET-2>ET-1>ET-3. In contrast, the initial transient *depressor* response *in vivo* was most profound upon ET-3 administration.

ET-1 also elicits markedly different *regional* hemodynamic response patterns (Le Monnier de Gouville *et al.*, 1990). An intravenous (i.v.) bolus injection of ET-1 induces vasodilation predominantly in the hindquarters, but produces a biphasic

blood pressure response in rat mesenteric artery characterized by a transient vasodilation with a subsequent longer-lived vasoconstriction. These depressor and pressor responses may depend on which blood vessel is employed as well as the concentration of the peptide that is administered where a low dose of ET-1 usually elicits a prompt vasodilation in almost all vascular beds. The vasodilation and vasoconstriction appear to be mediated by different mechanisms; the endothelin-induced vasodilation has been controversially reported to involve contributions by EDRF or prostacyclin release by ET-1 itself from endothelial cells (Wanner *et al.* 1989).

N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of nitric oxide (EDRF) synthesis, has no effect on hypotensive responses to *in vivo* administration of ET-1. Alternatively, vasodilation in rat mesenteric artery, induced by a low dose of ET-3, was inhibited by L-NMMA treatment, uncovering a hidden vasoconstrictor action of ET-3 at this previously "vasodilating" dose (Fukuda *et al.*, 1990). In addition, initial ET-3-induced vasodilation of rat renal artery was not affected by pretreatment with indomethacin, suggesting that the initial depressor response is not induced by cyclooxygenase products. In contrast, ET-1 has been demonstrated to release certain vasoconstricting cyclooxygenase products (Gardiner *et al.*, 1990). Indomethacin, in this instance, was able to reduce the contractile effects of ET-1 on rat aorta. These results strongly suggest that endothelin-induced responses are dependent on the vessel employed, the isoform administered, and the dose of the isoform applied.

Examination of the endothelins' structure-activity relationships suggest that these differences in biological activities arise primarily from sequence heterogeneity at the peptides' amino-termini, particularly at the region between the second and the seventh residue (Nakajima *et al.*, 1989). These studies also demonstrate that replacement of the amino-terminal residue affects the biological potency of endothelins or sarafotoxins. As well, residues in the region of the amino acid sequence from Thr[2] to Lys[7] appear to be important in binding of the isoforms to the receptor(s). Hydrolysis of either of the two disulfide bonds in the endothelins' superstructure prompts marked decreases in functional activity. Two synthesized analogs of ET-1, having two disulfide bonds other than the native 1-15 and 3-11 bonds, also demonstrate far less receptor-binding activity than the native peptide (Hirata *et al.*, 1989). Additionally, the residues near the amino terminus appear to be important in terms of the endothelins' vasoconstrictor action. Replacement of Phe[14] with Ala results in complete loss of vasoconstrictor activity; further examination suggests that Phe[14] may be important in the binding of ET-1 to its receptor. Information from similar residue-substitution studies also shows that the integrity of the charged residues, Asp[8] and Glu[10], appears to be essential in the production of the endothelins' vasoconstrictor action. These results suggest the importance of the configuration of the amino-terminus as well as the inner ring structure of the endothelin peptides in terms of their biological activity.

The carboxyl-terminal end of the ET-1 molecule is described as "strongly hydrophobic" and "constructing a nonflexible, β-pleated sheet" (Kimura *et al.*,

1988). Not surprisingly, this carboxyl-terminal domain also appears essential in the exertion of the endothelins' biological activities. Removal of the carboxyl-terminal Trp[21] residue attenuates the vasoconstrictor property of ET-1 to one-one thousandth of its original activity, probably through a decrease in the peptide's effective receptor-binding properties. Further truncation of amino acid residues from the carboxyl-terminus produces larger decreases in biological activity; a synthetic, 18-amino acid isopeptide, ET[1-18], shows no activity.

Table 1. The amino acid structures of the endothelins (ETs) and sarafotoxins (STXs). One-letter chemical notation is used. All peptides shown have two intramolecular disulfide bonds and are designated by the half-cysteine residues. Letters in italics indicate amino acid substitutions as compared to the structure of ET-1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
ET-1	**C**	**S**	**C**	**S**	**S**	**L**	**M**	**D**	**K**	**E**	**C**	**V**	**Y**	**F**	**C**	**H**	**L**	**D**	**I**	**I**	**W**
ET-2	**C**	**S**	**C**	**S**	**S**	**W**	**L**	**D**	**K**	**E**	**C**	**V**	**Y**	**F**	**C**	**H**	**L**	**D**	**I**	**I**	**W**
ET-3	**C**	*T*	**C**	*F*	*T*	*Y*	*L*	**D**	**K**	**E**	**C**	**V**	**Y**	*Y*	**C**	**H**	**L**	**D**	**I**	**I**	**W**
STXa	**C**	**S**	**C**	*K*	*D*	*M*	*T*	**D**	**K**	**E**	**C**	*L*	*N*	**F**	**C**	**H**	*Q*	**D**	*V*	**I**	**W**
STXb	**C**	**S**	**C**	*K*	*D*	*M*	*T*	**D**	**K**	**E**	**C**	*L*	**Y**	**F**	**C**	**H**	*Q*	**D**	*V*	**I**	**W**
STXc	**C**	*T*	**C**	*N*	*D*	*M*	*T*	**D**	*E*	**E**	**C**	*L*	*N*	**F**	**C**	**H**	*Q*	**D**	*V*	**I**	**W**

C. Multiple Endothelin Receptor Subtypes

The diverse effects of the endothelin isoforms mentioned above suggest the existence of multiple endothelin receptor subtypes, perhaps distributed in various tissues at different ratios. Indeed, endothelin receptors have been shown to be widely distributed, not only throughout the vasculature, but also in such tissues as kidneys, lungs, adrenal glands and neurons (Koseki *et al.*, 1989). Endothelin receptors on these tissues may vary in their subtypes; for instance, the six amino acid isopeptide, ET[16-21], is able to distinguish between the receptors of guinea pig bronchus and the rat aorta (Maggi *et al.*, 1989), suggesting the existence of at least two distinct subtypes of endothelin receptor (as well as a possible species differentiation in endothelin receptors).

Numerous studies of endothelin receptors utilizing scatchard analysis of ^{125}I-labeled ET-1 and SDS-polyacrylamide gel electrophoresis of the membrane fraction of such tissues as chick heart, rat lung, rat mesangial cells, human placenta, bovine cerebellum, etc. (Schvartz *et al.*, 1990), also demonstrated the existence of three different endothelin-binding proteins. Vascular smooth muscle appears to have only one endothelin-binding protein with a molecular weight of ~73,000 (Martin *et al.*, 1990). A large 44 kDa receptor in rat lung has been shown to have a higher affinity for ET-1 or ET-2 than for ET-3, while a smaller 32 kDa receptor bound selectively to ET-3. Existence of a receptor specific to ET-3 was also demonstrated in cultured rat anterior pituitary cells and in a rat PC12 pheochromocytoma cell line.

Three cDNA clones of endothelin-receptor have been isolated (Arai *et al.*, 1990). The predicted mature endothelin-receptors deduced from the nucleotide sequence of the cDNAs from bovine lung, rat lung and A10 cells consist of 417, 441 and 426 amino acids, respectively. The differences in their molecular sizes and the previously reported values are primarily accounted for by polypeptide *N*-glycosylation; they have relatively long amino terminals preceding the first transmembrane sequence. The encoded polypeptides each contain seven spanning regions containing 20-27 hydrophobic amino acid residues, revealing a probable seven-looped, G-protein coupled receptor which would place it in the rhodopsin receptor super-family. The first type of receptor has an order of affinity for the endothelins described as: ET-1≥ET-2>>ET-3. The second type of receptor shows equipotent affinity for the three peptide isoforms. Ligand affinity for the third type of receptor is very similar to that of the first type. These results are compatible with the previous pharmacological and ligand-binding experimental results in terms of the relative orders of potency. Therefore, endothelin-receptors might be divided into three distinct prototypes, designated as ET_A, ET_B and ET_C. Where ET_A demonstrates high affinity for ET-1 and ET-2 but not for ET-3, ET_B has equal affinity for the three peptides, and ET_C has high affinity for ET-3. ET_A has been identified as the receptor subtype existing on the smooth muscle cell and is most likely responsible for the ET-1's vasoconstrictor action. This subtype of receptor has also been identified on central neurons as well as skeletal, cardiac, and bronchial smooth muscle. ET_B has been identified on the membranes of endothelial cells and may be responsible for production of

EDRF. ET_C has been identified on gastrointestinal smooth muscle cells and central and spinal neurons.

The expression of ET-1 receptors on vascular smooth muscle has been shown to be regulated by angiotensin II (AngII) as well as by ET-1, itself (Hirata *et al.*, 1988, Roubert *et al.*, 1989). Arginine vasopressin (AVP) and phorbol 12,13-dibutyrate were also shown to induce significant decreases in the total number of endothelin-receptors *in vitro*, but at relatively high doses. The mechanisms underlying this receptor regulation are currently unknown, however, it was shown that ET-1-mediated endothelin receptor down-regulation is not mediated through activation of protein kinase C since this activity could not be blocked by pretreatment with protein kinase C inhibitors.

D. Endothelin-Induced Vasoconstriction (Cellular Mechanism of Action)

ET-1 has been shown to have many vascular functions. Among these, endothelin-induced vasoconstriction and vasodilation appear to be important in the homeostatic regulation of vascular tonus. Despite numerous reports, the mechanisms underlying these vasopressor and vasorelaxor responses remain unclear. The initial studies demonstrate that the contractions produced by ET-1 are resistant to the following receptor antagonists and enzyme inhibitors: α-adrenergic (phentolamine), H_1-histaminergic (diphenhydramine), serotonergic (methysergide), cyclooxygenase (indomethacin) and lipoxygenase (nordihydroguiaretic acid) (Yanagisawa *et al.*, 1988). Later studies showed that endothelin-induced vasoconstriction may be mediated through two distinct

intracellular signal transduction systems, that is, opening of receptor-operated calcium channels (Goto *et al.*, 1989) and phospholipase C activation (Takuwa *et al.*, 1990); both pathways require the activation of G-proteins. In addition, phospholipase A_2 has been shown to be activated by ET-1 to produce various prostanoids including prostacyclin, prostaglandin E_2 and thromboxane A_2; these prostanoids may be involved in the modulation of endothelin-induced vasoconstriction and vasorelaxation (Resnik *et al.*, 1989). Numerous studies demonstrate that ET-1 elicits the mobilization of intracellular free calcium ions. Following the administration of ET-1 to smooth muscle or endothelial cells, intracellular free calcium ion concentration increases promptly and then falls to a level significantly higher than the initial base line concentration. The initial transient increase has been attributed to calcium ions from a caffeine-sensitive calcium store, which is stimulated by inositol triphosphate (Kai *et al.*, 1989). Each of the two distinct endothelin receptors elicit phosphoinositide breakdown to produce inositol triphosphate (IP_3) and 1,2-diacylglycerol (DAG) and an increase in intracellular free calcium ions (Arai *et al.*, 1990). It was also demonstrated, in A10 cells, that this process most likely occurs through stimulation of G-proteins. The latter sustained phase in endothelin-induced increases in intracellular free calcium ion level is ascribed to the influx of calcium ions from the extracellular milieu (Takuwa *et al.*, 1990). Application of nickel ion, a calcium channel antagonist, was able to abolish the sustained increase in intracellular calcium ion as well as the constriction induced by ET-1 in porcine coronary artery and rat aorta (Blackburn *et al.*, 1990). Indeed, in porcine coronary artery, ET-1 clearly

activates the L-type calcium channel (Goto *et al.*, 1989) where it induces the influx of extracellular calcium ion in the absence of membrane depolarization. Much controversy exists, however, in terms of ascribing only one cellular mechanism for ET-1's bioactivity and, indeed, alternate pathways may exist. For example, Miasiro *et al* (1988) and others have demonstrated that ET-1 stimulates the efflux of calcium ion from primary cultured rat vascular smooth muscle but not calcium ion influx, and many investigators have demonstrated that application of dihydropyridine calcium channel antagonists or removal of extracellular calcium ion does not always abolish endothelin-induced vasoconstriction (Ohlstein *et al.*, 1989). These discrepancies may be partly due to differences in the type of smooth muscle cell or the species of animal used.

The elevation of intracellular free calcium ion levels in the sustained phase may be mediated in several ways. ET-1 activates both the L-type calcium channel and the receptor-operated, ATP-sensitive cation channel (Benham *et al.*, 1987); the latter channel is insensitive to nifedipine. It was also demonstrated in guinea pig portal vein that ET-1 stimulates at least two types of calcium channels (Inoue *et al.*, 1990). In rat uterus or human bronchus, the contractile response occurs in two steps (Kozuka *et al.*, 1989, Advenier *et al.*, 1990). In the first step, constriction is induced by a lower concentration of ET-1 requiring extracellular calcium influx since it is inhibited by dihydropyridine-calcium channel antagonists. The second step is characterized by a higher efficacy but a lower potency than those observed in the first step and does not involve an activation of dihydropyridine-sensitive calcium channels. The activation of dihydropyridine-sensitive, voltage-dependent

calcium channels by ET-1 in porcine coronary artery smooth muscle is also mediated by a G-protein, insensitive to application of pertussis toxin. Further details remain unclear as to molecular steps involved in the activation mechanism of L-type calcium channel by ET-1. One possibility is that the α-subunit of the corresponding G-protein stimulates the L-type channel (Brown *et al.*, 1988). Another possibility is that IP_3 and inositol 1,3,4,5-tetrakisphosphate (IP_4) mediate this calcium channel stimulation (Reiser *et al.*, 1989).

E. Endothelin Production and Release

Various factors, including thrombin, phorbol esters, ionomycin, and transforming growth factor-β (TGF-β) induce the production of ET-1 mRNA in the endothelium (Masaki *et al.*, 1990). In addition, the administration of TGF-β, TPA (12-*O*-tetradecanoylphorbol-13-acetate), ionomycin, AngII, or AVP into the incubation medium of cultured endothelial cells elicits release of ET-1 (Masaki *et al.*, 1990). Further complication exists in describing a physiological basis for these stimulatory actions of endogenous agents on ET-1 release. Thrombin similarly stimulates the release of EDRF as well as the production of ET-1. Alternatively, the increase in production of ET-1 due to thrombin is shown to be inhibited by EDRF since this process was potentiated by the decrease in EDRF by application of L-NMMA and methylene blue, and was reduced by the increase in EDRF due to the presence of superoxide dismutase and 8-bromo cGMP (Boulanger *et al.*, 1990). Additionally, Stewart *et al* (1990) demonstrated that production of ET-1 in the endothelium was markedly reduced in co-culture with smooth muscle cells

or fibroblasts, further suggesting the existence of some feedback regulatory mechanism in the production of ET-1.

As mentioned earlier, TPA and calcium ionophore elicit cellular phosphoinositide breakdown, producing diacylglycerol (DAG) and inositol triphosphate (IP_3), as well as increasing cytosolic free calcium ion which, in turn, activates protein kinase C. The activation of protein kinase C has been shown to induce the production of ET-1 mRNA (Inoue *et al.*, 1989). This is compatible with the existence of TPA-responsive elements in the 5'-flanking region of the human ET-gene (Yanagisawa *et al.*, 1988). These *cis*-acting nucleotide sequences have been shown to be the binding sites for proto-oncogene products such as c-*jun* and c-*fos*, suggesting that activation of protein kinase C by TPA is functionally coupled to activation of these transcriptional factors, thereby affecting the prepro-ET gene. The human prepro ET-1 (ppET-1) gene also contains a consensus motif for the binding site of nuclear factor-1 (NF-1) and other hexanucleotide sequences for the acute phase reactant regulatory elements which may mediate the induction of ppET-1 by TGF-β and may be involved in the induction of ET-1 under acute physiological stress *in vivo* (Yanagisawa *et al.*, 1989). The latter sequence also may be responsible for the increase in plasma level of ET-1 following surgery or acute myocardial infarction (Miyauchi *et al.*, 1989).

The mechanism for the release of ET-1 from endothelial cells is also unclear. After the addition of thrombin, the maximal induction of the total amount of prepro ET-1 in cultured endothelial cells requires 30-min or more (Yanagisawa *et al.*, 1988). However, a more rapid release of ET-1 into human plasma (reported

as an increase in total ET-1 concentration), was reported to occur after cold exposure; a seven-fold increase in the plasma ET-1 was observed within 2-min (Fyhrquist *et al.*, 1990). Since endothelin secretory granules have not been detected in endothelial cells, ET-1 may be synthesized and immediately released or may not be stored in vesicles for sustained periods of time. *In situ*, ET-1 may be transiently stored in vesicles and released by yet unknown systemic mechanisms, however, once the tissue is excised, ET-1 in the vesicle may be immediately released and depleted.

Circulating ET-1 is stable in the bloodstream but is eliminated quickly from the circulation with a half-life of ~7-min (Shiba *et al.*, 1989). In contrast, blood pressure following exogenous administration of ET-1 is sustained at elevated levels for long periods of time suggesting that ET-1 remains bound to its receptor on smooth muscle in a manner supporting a slow kinetic hydrolysis or cellular internalization. Circulating ET-1 is quickly eliminated through the lungs and kidneys. Though these two tissues demonstrate a high density of high-affinity binding sites for ET-1, they also exhibited high neutral endopeptidase (enkephalinase) activity, which may play an important role in the ET-1 degradation (Vijayaraghavan *et al.*, 1990).

F. Endothelin Biosynthesis

ET-1 is most likely produced, through precursor maturation, in the cytoplasm of the cell. Analyses of the sequences of the 203 amino acid-porcine and the 212-amino acid, human prepro- forms of ET-1 have revealed the existence of a

signal peptide sequence. Porcine preproET-1 (ppET-1) has twelve successive hydrophobic amino acid residues at the amino terminal side (Yanagisawa *et al.*, 1988). Human placental ppET-1 also has a very similar signal peptide (Itch *et al.*, 1988). This fact suggests that the immature form of the endothelin precursor is transported *in toto* across the internal cellular membrane prior to further processing. This signal peptide may be cleaved at Gly[17]-Ala[18] after membrane penetration. A pair of di-basic amino acid residues, Lys-Arg, is found in both porcine and human ppET-1 at the amino-terminal side of the mature ET-1 sequence, but not at the carboxyl-terminal side. Instead, a di-basic amino acid pair, Arg-Arg, was found at position 92-93 of the porcine, and Lys-Arg at position 91-92 of the human ppET-1, further suggesting the existence of an unusual processing pathway of the endothelins. In other words, the existence of a 39-amino acid residue intermediate, designated big endothelin (big ET) in porcine, and a 38-residue intermediate in human could be predicted from these ppET sequences and, indeed, in a cultured medium of porcine aortic endothelial cells, big ET-1[1-39], peptide[22-39], and peptide[23-39] as well as ET-1[1-21] could be detected (Sawamura *et al.*, 1989). The sum of the amounts of the two carboxyl-terminal peptides of big ET-1 is approximately equal to the sum of the amounts of ET-1 and its oxidized peptide remnant, suggesting the generation of ET-1 from big ET-1. Additionally, equi-molar big ET-1 and ET-1 were detected in human plasma (Miyauchi *et al.*, 1989). These data suggest that big ET-1 is probably secreted from the endothelium and converted into ET-1 in the extracellular space. In support, extracellular conversion of big ET-1 was

demonstrated in an *in vivo* experiment (D'Orléans-Juste *et al.*, 1990). Bolus i.v. administration of big ET-1 produced a slow increase in plasma ET-1 levels detectable even at 60-min after administration. Alternatively, a significant increase in ET-1 could be detected for only 5-min after similar administration of ET-1 further suggesting that big ET-1 is slowly converted into ET-1 in the plasma. Likewise, increases in blood pressure following administration of big ET-1 reached peak levels after 5-min rather than after 1-min as seen with ET-1. Because big ET-1 is as efficacious as ET-1 in eliciting a pressor response, despite a low vasoconstrictive activity *in vitro* (Kimura *et al.*, 1989), these results strongly suggest that big ET-1 can be converted to ET-1 at the endothelial cell surface.

Three fractions of endothelial cells have been demonstrated to display 'endothelin converting enzyme activity' (Sawamura *et al.*, 1990, Okada *et al.*, 1990). Two of these are cytosolic fractions and the other is a membrane fraction. The most probable candidate for the converting enzyme is the membrane-bound fraction, which is inhibited by EDTA, EGTA, o-phenanthroline and phosphoramidon (Okada *et al.*, 1990). This membrane-bound enzyme has been identified as a neutral metalloendopeptidase and is further described as being more sensitive to big ET-1 than to big ET-3. Characteristically, the enzyme is surprisingly not inhibited by thiorphan, which is a specific inhibitor of a neutral metalloproteinases. The latter proteinase cleaves a number of biologically active peptides, suggesting that endothelin converting enzyme may be a subtype of metalloendopeptidase. Phosphoramidon specifically suppresses the production of ET-1 from cultured endothelial cells. In addition, ET-1 or the big ET-1

carboxyl-terminal fragment was barely detectable in lysates of the cells treated with this inhibitor (additional evidence that big ET-1 conversion occurs on the surface of the cell membrane).

Finally, although the sequences of the three mature endothelins are conserved, the mRNA sequences of the three isopeptides of ET-1 are different, suggesting the diversity of the processing pathways of the prepro- forms of the three endothelins. However, the endothelium produces exclusively the ET-1 isoform, indicating the importance of the ET-1 processing pathway in the vascular system. In tissues other than vascular endothelial cells, the conversion mechanism may be different since in porcine nervous tissue, big ET-1 is barely detectable, suggesting that, at least in neurons, big ET-1 may be converted intracellularly and stored in neurons as a neurotransmitter (Shinmi *et al.*, 1989)

G. Proposed Role of Endothelin-1 in Hypertension

The numerous available reports on the pharmacological effects of ET-1 suggest an important role for the peptide in the maintenance of blood pressure in addition to other reported cardiovascular roles suggested by the following actions of ET-1: contraction of non-vascular smooth muscle (Koseki *et al.*, 1980); positive inotropic and chronotropic effects on the myocardium (Ishikawa *et al.*, 1988, Shah *et al.*, 1989); stimulation of atrial natriuretic factor (ANF) release (Fukuda *et al.*, 1988); renal effects (Rakugi *et al.*, 1988); proliferative effect on vascular smooth muscle cells (Komuro *et al.*, 1988, Takuwa *et al.*, 1991); central nervous system effects (Takahashi *et al.*, 1988).

Stimulation of the endothelium by various factors, including AVP and AngII, elicits the production and release of ET-1. The released ET-1 may then act, in a paracrine fashion, on smooth muscle cells underlying endothelial cells (Masaki *et al.*, 1990). Although there is no direct evidence for the direction of secretion of endogenous ET-1 from the endothelial cell, a greater amount of ET-1 or big ET-1 is likely to be secreted on the basal side of the endothelium than on the apical side since it was demonstrated that a greater amount of ET-1 and big ET-1 could be detected in the intima of porcine aorta (Kitamura *et al.*, 1990). More direct evidence for the physiological importance of ET-1 in the maintenance of blood pressure was demonstrated recently in two patients with malignant hemangioendothelioma (Yokokawa *et al.*, 1991). These patients presented with elevated levels of plasma ET-1 as well as significant increases in blood pressure. Following surgical excision of the tumors, both plasma ET-1 and blood pressure returned to normal levels. In one case, recurrence of the tumor again induced an elevation of plasma ET-1 and hypertension. Additionally, several investigators have found significantly higher plasma ET-1 levels in chronic renal failure patients with essential hypertension than in normal subjects. A hemodialyzed hypertensive group had higher plasma ET-1 than the comparable normotensive group (Saito *et al.*, 1990, Shichiri *et al.*, 1990). However, the importance of the plasma ET-1 level associated with essential hypertension and other cardiovascular diseases is still the subject of controversy.

Additional evidence supports the idea that ET-1 regulates circulatory hemodynamics and the responses to alterations in cardiorenal homeostasis in

human pathologic states. Cernacek *et al* (1989) reported increased plasma levels of ET-1 in cardiogenic shock and pulmonary hypertension suggesting that circulating ET-1 is indeed responsive or conducive to altered cardiovascular conditions. Increased plasma concentrations of ET-1 have also been noted after major abdominal surgery (Saito *et al.*, 1989) and in certain pathologic atherosclerotic states (Dubin *et al.*, 1989, Lüscher *et al.*, 1989). Lerman *et al* (1991) reported that plasma levels of ET-1 were increased after orthotopic liver transplantation and that these levels remained increased throughout a 7-day postoperative period. This increase in plasma ET-1 was also associated with a significant increase in mean arterial pressure (MAP) which is consistent with a role for ET-1 in the acute hypertensive response associated with liver transplantation surgery.

To elucidate the mechanism(s) of hypertension, animal models of experimental hypertension have proved useful for the study of other vasoactive peptides. However, the experimental results reported to date are conflicting in regard to endothelin. For example, although plasma ET-1 levels do not increase in Goldblatt II and spontaneously hypertensive rats (SHR) (Suzuki *et al.*, 1990, Tomobe *et al.*, 1988), both greater sensitivities and enhanced maximal responses to ET-1 were demonstrated in isolated blood vessels of both groups of rats. Similarly, a greater reactivity to ET-1 was observed in deoxycorticosterone acetate-salt (DOCA-salt) hypertensive rats (Catelli deCarvalho *et al.*, 1990). However, other investigators demonstrate no such difference between SHR and Wistar-Kyoto (WKY) rats in the sensitivity of aorta to ET-1 (Wright *et al.*, 1990,

Wu *et al.*, 1990), or between Goldblatt II, DOCA-salt hypertensive rats and their respective controls (Tomobe *et al.*, 1991).

H. Hypotheses

As indicated earlier, investigations describing the pressor effects of endogenous or exogenously-administered endothelins have provided little explanation as to the hemodynamic mechanism(s) by which these peptides produce increases in blood pressure. *In vitro* data (Yanagisawa *et al.*, 1989, Ishikawa *et al.*, 1988, Shah *et al.*, 1989) suggest that endothelin's potent constrictor effect on vascular smooth muscle may account for its potent pressor action *in vivo.* This suggests that the mechanism by which endothelin increases blood pressure is through an increase in total peripheral resistance (TPR). Likewise, endothelin's positive inotropic and chronotropic effects suggest that endothelin may increase arterial pressure by increasing cardiac output (CO) and heart rate (HR). This, however, does not exclude other possible mechanisms of action.

Application of ET-1, in vivo: Associated with the i.v. bolus injection of ET-1 into rats is an initial transient depressor phase followed by a sustained pressor phase (Yanagisawa *et al.*, 1988). Preliminary analysis suggests that the initial decrease in blood pressure is associated with an increase in carotid and hindquarter vascular conductance, however, this is followed by an intense vasoconstriction in other resistance vessels such as the renal and mesenteric beds resulting in the sustained vasoconstriction response. In fact, it has been shown that ET-1 produces a potent renal vasoconstriction *in vivo* (Lippton *et al.*, 1988). Renal

vasoconstriction may give rise to an increase in renin release and thus increase blood pressure; however, preliminary reports are disparate as to the actions of ET-1 on renin release in isolated rat glomeruli (Miller *et al.*, 1989, Banks *et al.*, 1990). It has been described previously that AngII, aldosterone and AVP animal models of hypertension are associated with renal sodium retention (Pawloski *et al.*, 1989, Olsen *et al.*, 1985, Cowley *et al.*, 1976). Renal sodium retention can increase blood volume and CO and thus elevate arterial pressure. It has also been shown that acute administration of endothelin can produce prominent decreases in glomerular filtration rate and renal blood flow (Katoh *et al.*, 1990). Based on these reports, it is possible that administration of ET-1 to animals will be accompanied by renal sodium retention. Likewise, any renal actions of ET-1 may be due to stimulation of the renin-angiotensin system which may contribute to the peptide's pressor effects.

In terms of central nervous system effects, it was reported that administration of ET-1 into the lateral ventricle of the brain increases blood pressure (Takahashi *et al.*, 1988), but controversy exists as to whether ET-1 affects baroreflex function (Given *et al.*, 1989, Takahashi *et al.*, 1988). Additionally, ganglion-blockade has been reported to reduce or have no effect on the pressor response to acute ET-1 administration (Given *et al.*, 1989, Hinojosa-Laborde *et al.*, 1989). These results indicate the possibility for a neural mechanism of action for ET-1's blood pressure effects. Likewise, the baroreceptor reflex is normally activated in the presence of vasoconstrictive agents, due to stretch-sensitive mechanisms, in order to attenuate their pressor effects. The maximal pressor response to exogenously administered

ET-1, like other pressor agents, should be attenuated by the baroreceptor reflex. If intact baroreflex activity is involved in reducing the apparent maximal pressor response produced during the infusion of ET-1, maximal increases in MAP will be higher and achieved more quickly in the SAD animals than in control rats receiving the same dose of ET-1. Alternatively, if ET-1 acts on some component of the baroreflex to increase sympathetic activity, then SAD animals may become less hypertensive than SHAM-treated rats during the 7-day infusion protocol.

The area postrema is a brainstem region located in close proximity to numerous medullary structures involved in the control of autonomic neural activity and blood pressure. Area postrema ablation (APX, Fink *et al.*, 1987) has been shown to prevent chronic AngII-induced hypertension despite the direct vasoconstrictive actions of AngII. This finding suggests that AngII-induced hypertension has a neurogenic component. Furthermore, the area postrema of the rat has a very high density of AngII binding sites (Saavedra *et al.*, 1986) and ET-1 binding sites (Koseki *et al.*, 1988). It has also been recently shown that i.v. injection of ET-1 in rats activates area postrema neurons while microinjection of ET-1 into the area postrema results in prompt dose-dependent decreases in MAP (Ferguson *et al.*, 1989). Thus, ET-1 may influence cardiovascular function through activation of area postrema neurons.

As previously mentioned, increases in plasma ET-1 levels have been reported in patients with essential hypertension (↑600%), cardiogenic shock (↑1400%), patients on chronic dialysis (↑400%) and in patients with pulmonary hypertension (↑600%). Again, these data suggest that circulating ET-1 concentration is

responsive to altered cardiovascular conditions. Therefore, administration of ET-1 to animals may demonstrate increased circulating plasma levels of ET-1 that are physiologically significant. These increases in ET-1 immunoreactivity (ET_{ir}) in plasma samples should demonstrate a strong correlation with increases in MAP if exogenous ET-1 administration displays a steep dose-response curve. Likewise, increased plasma ET-1 concentrations should prove to be physiologically relevant by exhibiting only slight (femtomolar) increases in ET_{ir} over control plasma levels.

In light of these data, in addition to the *in vitro* observations described above, the following hypotheses were addressed in order to further investigate the hemodynamic actions of the endothelins, particularly ET-1, in the conscious rat and the possible physiological mechanisms by which this peptide might act or interact:

1] *Acute (bolus), short-term infusion (1-hr) and chronic intravenous infusion (7-days) administration of endothelin produces a dose-dependent pressor response which is largely due to an increase in total peripheral resistance. ET-1 is more potent than ET-3 at producing this pressor response.*

2] *Endothelin-induced hypertension, produced by chronic infusion, is directly dependent on sodium intake, and is accompanied by sodium and water retention.*

3] *Endothelin and AngII are detectable in the normal rat circulation; exogenous administration of endothelin, at pressor doses, produces increased circulating plasma concentrations of both peptides which are physiologically relevant.*

4] *The baroreceptor reflex attenuates the chronic responses to exogenously administered endothelin.*

5] *The area postrema influences the magnitude of the pressor response to exogenously administered ET-1.*

6] *Activation of the renin-angiotensin (RAS) system contributes to endothelin-induced hypertension.*

MATERIALS AND METHODS

A. General Methods

1. Animals

Male Sprague-Dawley rats (300-325 grams) were purchased from Sasco-King (Madison, WI) and Harlan (Indianapolis, IN). All animals utilized in this investigation were surgically prepared and chronically maintained according to protocols approved by the Michigan State University Committee for Animal Use and Care. Standard steel metabolic cages were used for post-surgical, long-term animal housing and care in all experiments. Individual rats were tethered within each cage by use of a steel spring housing with one end of the spring attached to the cranium with dental acrylic and the other end attached to a plastic swivel mounted above the cage. This minimal-distress arrangement allowed the animal free movement within the cage and also allowed for free access to distilled water from calibrated drinking tubes and to sodium-deficient rat chow (Teklad, Madison, WI). All animals were sacrificed by i.v. bolus injection of sodium pentobarbital at the end of each experiment.

2. General Surgical Procedures

a. Catheterization: Rats were anesthetized with sodium pentobarbital (Nembutal®, Abbott Laboratories, Chicago, IL), 50 $mg \cdot kg^{-1}$, i.p., and administered

atropine sulfate, 0.2 mg, i.p. (Sigma, St. Louis, MO), to prevent bronchial congestion. Normal body temperature was maintained during anesthesia via a water-heated dermal pad (Gorman-Rupp, Inc., New York, NY). The animals were shaved at the top of the head, back of the neck and the inner left leg. Cannulae were constructed of polyvinyl chloride (Tygon® Microbore,) tubing with 4.5 cm silicone rubber tips (Silastic®, Dow Corning, Midland, MI). The silicone tips were inserted through a 3.0 cm incision made on the inner left leg for cannulation of the internal iliac vein and femoral artery; the arterial catheter was sutured to the leg in a manner preventing occlusion of the catheter during normal rat movement. Excess catheter was directed subcutaneously and externalized through a 2.5 cm incision made in the dermis overlying bregma. The cranial incision was made with a scalpel after which three 0.19" jeweler's screws were attached to the cranium in a 1.0 cm^2 area which encompasses bregma. After externalization, the catheters were threaded through a metal spring tether (Small Parts, Inc., Miami, FL). The spring tethers were then permanently attached to the skull with dental acrylic using the previously attached machine screws as cranial anchors. The animal were then administered penicillin G, 20,000 U, i.m., allowed to regain consciousness on the heated pad and placed in a metabolic cage. An i.v. sodium solution infusion was immediately begun and administered continuously using an infusion pump (Harvard, South Natick, MA). To maintain patency, the arterial catheter was filled with a heparinized sucrose solution and occluded when not in use. Rats were allowed a minimum of three post-surgical recovery days prior to any experimentation.

b. Pulsed-Doppler Flow Probe Implantation: Rats were anesthetized with halothane (5.0 volume percent in O_2 at 1.0 $L \cdot min^{-1}$). An endotracheal tube (4.0 mm O.D.) was introduced and halothane anesthesia was continued at 1.5 volume percent throughout the rest of the surgery. The thorax was shaved and then cleaned with Betadyne® antiseptic (Purdue Frederick Co., Norwalk, CT). A midline thoracotomy was then performed for implantation of an aortic directional pulsed-Doppler flow probe (Crystal Biotech, Holliston, MA). Thoracic musculature was dissected by blunt dissection and small intercostal blood vessels were cauterized. Prior to incision of the pleura parietalis, the animal was placed on a respiratory pump (Harvard, South Natick, MA) delivering a mixture of 90% O_2 and 1.5 volume percent halothane. The ascending aorta was isolated from connective tissue to accommodate the flow probe within its silicone cuff. The probe was then electronically examined for proper placement on the vessel and adjusted for maximal signal strength. The thorax was closed using nylon suture (Ethicon® Inc., Somerville, NJ). The silver/copper wire probe leads were sutured and secured to the thoracic musculature and directed subcutaneously around the thorax and placed within a subcutaneous pocket in the animal's back. The thoracic dermis was sutured and the animal was administered penicillin G, 20,000 U, i.m. The animals were allowed five days recovery from this procedure prior to any other intervention. Probe leads were externalized in ensuing catheterization surgery in the same manner as the cannulae (see *a.* above).

c. Sino-Aortic Deafferentation: Animals were anesthetized with sodium pentobarbital, 50 $mg \cdot kg^{-1}$, i.p., and administered atropine sulfate, 0.2 mg, i.p., to prevent bronchial congestion. After shaving the ventral surface of the neck, a mid-cervical incision was made in order to expose the left carotid sinus. The occipital artery, the carotid sinus and the internal and external carotid arteries were then denervated by mechanical stripping and application of 100% ethanol. The cervical sympathetic and superior laryngeal nerves were severed. The procedure was repeated at the right carotid sinus. The animal was then administered penicillin G, 20,000 U, i.m., and allowed to recover for three weeks prior to further investigational intervention. Assessment of denervation was as follows: a positive denervation was described as an animal which had three daily control MAP measurements with an average standard deviation (S.D.) significantly greater (>1.96 S.D.) than that of SHAM-operated animals (S.D. = 6.3±0.4 mmHg).

d. Area Postrema Ablation: Surgical ablation of the area postrema (APX) was performed according to instruction by Dr. M.L. Mangiapane in Groton, CT. Animals were anesthetized with sodium pentobarbital, 50 $mg \cdot kg^{-1}$, i.p. and placed in a stereotaxic device (David Kopf Instruments, Tujunga, CA). The area postrema was exposed on the surface of the medulla and impaled by a monopolar tungsten electrode (A-M Systems, Inc., Everett, WA) through which a total anodal current of 10 mA for 13 seconds (130 mC) was passed. The animals were

administered penicillin G, 20,000 U, i.m., and allowed 3 weeks recovery prior to further experimentation.

Upon completion of the experimental protocol in which this procedure was used, each animal was anesthetized with sodium pentobarbital, 40 $mg \cdot kg^{-1}$, i.v. The animal was then exsanguinated by perfusion with 0.9% normal saline followed by buffered formalin (37% formaldehyde). For assessment of the extent of APX, the brain was removed, coded and sent to the Pathology Laboratory at Michigan State University Clinical Center, East Lansing, MI, for preparation of 50 μm coronal sections of the medullary region circumscribing and including the area postrema. Animals included in the final analysis of the data exhibited at least 90% APX with little or no damage to surrounding tissue structures.

3. Blood Pressure/Heart Rate Measurements

MAP and HR were measured from the arterial catheter using a pressure transducer (Model P50, Gould, Oxnard, CA) attached to a blood pressure monitor (Model BP2, Stiemke Inc., New Orleans, LA); a hard copy was simultaneously produced on a polygraph (Model 7B, Grass Instruments, Quincy, MA). MAP and CO in sino-aortic denervated (SAD) animals were monitored for 20-min using an Apple II-Plus data acquisition system (Apple Computer Inc., Seattle, WA) and an analog signal from the Grass polygraph. The acquisition program sampled MAP and CO as analog signals every ten seconds for 20-min (120 samples). The program then calculated a mean and standard deviation (SD) of the mean for

MAP and CO during the 20-min sample period. The obtained standard deviation value for MAP was used as an indication of blood pressure lability.

4. Measurement of Other Hemodynamic Parameters

Cardiac output (CO) was estimated as blood flow velocity (in kHz) in the aortic root and recorded on the polygraph using a directional pulsed Doppler monitor (University of Iowa, Model 545C-3) attached to the implanted flow probe. The flow probe was implanted at least seven days prior to the beginning of flow measurements to ensure firm attachment of the relatively rigid probe to the aorta. Thus, aortic diameter changes under the probe were minimized and flow velocity should closely parallel absolute changes in CO (minus coronary flow). Such proportionality, between volume flow and Doppler velocities, has been shown previously (Haywood *et al.*, 1981).

Total peripheral resistance index (TPR) was calculated using the formula:

$$\textbf{TPR (mmHg·kHz}^{-1}\textbf{) = MAP/CO}$$

Stroke volume index (SV) was calculated using the formula:

$$\textbf{SV (kHz·min·beat}^{-1}\textbf{) = CO/HR}$$

5. Assay Measurement Procedures

a. Plasma ET-1 Concentration: Briefly, the system utilizes a high specific activity [^{125}I]ET-1 tracer and a highly specific and sensitive antiserum. ET-1 can be measured accurately in a range of 0.25-16.0 fmol·tube^{-1} (0.625-40 fmol·ml^{-1}) with a sensitivity of approximately 0.2 fmol·tube^{-1} (0.5 fmol·ml^{-1}, 1.2 pg·ml^{-1}). Recovery

has been determined as 49%. Inter-assay variation has been determined as 19.7%. Intra-assay variation has been determined as 3.5%. Whole blood (2.5 ml) was collected, through a femoral arterial catheter, into a syringe containing 50 µL of 0.25 M EDTA and 10 µM E-64 (a cysteine protease inhibitor, Sigma Chemicals, St. Louis, MO). The sample was then centrifuged at 15,000*g* for 10-min at 4°C to separate plasma from cells. A 1.0 ml plasma sample was collected and frozen at -70°C until extraction could be performed. ET-1 was extracted from plasma using a commercial kit (Amersham International). Prepared plasma samples were acidified, passed through methanol/water equilibrated Amersham Amprep® 500mg C2 extraction columns, collected in a methanol eluent and lyophilized in a centrifugal evaporator. Lyophilized plasma samples were reconstituted in assay buffer (as provided by Amersham) and analyzed according to the following radioimmunoassay: to compare plasma sample levels of ET-1, an ET-1 standard curve was prepared in a range from 0.25-16.0 fmol/tube; the system utilizes a high specific activity [^{125}I]-endothelin tracer (~41kBq, 1.1µCi). A rabbit antiserum, highly specific for ET-1 was also used; this antiserum has cross-reactivity with the following isoforms: ET-2, ~144%; ET-3, ~52%; Big ET-1, <0.4%; VIC, ~100%. After 24-hr incubation of the plasma samples with tracer and antiserum, separation of the antibody bound fraction from the free fraction was achieved by addition of a second antibody (donkey-anti-rabbit, containing 0.06% sodium azide). The samples were then centrifuged at 2000*g* for 10-min at 4°C, decanted and measured for radioactivity for 1-min using a Micromedic Plus® Automatic Gamma Counter.

b. Plasma AngII Concentration: Plasma AngII ($[AngII]_P$) concentrations were determined in 400 µl of EDTA-treated, ethanol-extracted plasma using a radioimmunoassay system with a sensitivity of approximately 10.0 $pg \cdot ml^{-1}$. After extraction with ethanol, plasma samples were reconstituted in 0.2 ml assay buffer (*pH7.4*) containing 0.05M tris-(hydroxymethyl)-aminomethane, 0.3% bovine serum albumin, and 0.2% neomycin sulphate. AngII assay was performed using ^{125}I-labelled AngII (New England Nuclear, Boston, MA; final concentration: 200,000 $cpm \cdot ml^{-1}$) and AngII antiserum (Arnel, New York, NY; final dilution: 1/5000). Samples were then incubated for 24-hr at 4°C. The bound fraction (supernatant) was separated by addition of dextran-coated charcoal and centrifugation at 4°C and 3000*g*. The supernatant was then counted for 1-min using the automatic gamma counter described above.

c. Plasma Na^+ and K^+ Concentration: Plasma sodium and potassium (P_{Na} and P_K) concentrations were determined from 50 µl plasma samples using a photometric electrolyte analyzer (Model 943, Instrumentation Laboratories Inc., Lexington, MA).

d. Urinary Na^+ and K^+ Concentration and Fluid Balance: All animals were provided with distilled drinking water, ad libitum, through calibrated drinking tubes for the measurement of daily water/fluid intake (WI). Any infused fluid volumes administered during the preceding 24-hr period were added to the total fluid intake volume. Likewise, daily urinary output (UO) was obtained by

collection of urine into calibrated containers. Daily water balance (WB) was determined as the difference of these two parameters (WB=WI-UO). Urine samples were collected daily for determination of urinary sodium ($U_{Na}V$) and potassium (U_KV) excretion (expressed as "$mEq \cdot day^{-1}$" using the photometric electrolyte analyzer described above.

6. Statistical Analyses All analyses were performed electronically by use of CRUNCH® statistical analysis software (Version 4.0, Crunch Software Corp., Oakland, CA) on a i486-33MHz, co-processor equipped personal computer. Most results are expressed as means±SEM. The proposed experiments were designed for analysis by a repeated measures analysis of variance (ANOVA) with each factor representing changes in a variable over time. This analysis allowed for the measurement of probable differences between- and within- treatment groups. Post-hoc comparisons of independent means were made using the Student Newman-Keuls test and/or the "protected" least significant difference test. Similar comparisons for TPR and SV (non-normally distributed variables) were performed using Friedman's test. Variance homogeneity was tested using the F-max test. A probability value of less than 0.05 ($P<0.05$) was considered statistically significant.

B. Experimental Protocols

1. Bolus and Acute Intravenous Infusion of ET-1 and ET-3

The specific aim of this set of experiments was to determine the hemodynamic component(s) involved in the sustained pressor response to the endothelins and the potency differences between exogenously administered ET-1 and ET-3 (Peninsula Laboratories, Inc., Belmont, CA). Dose-dependency of bolus and acutely infused endothelins was also assessed.

Bolus injection endothelin experiments. Human-porcine (ET-1) or rat endothelin (ET-3) [peptide content ~81.8%, sample purity ~99.1%] was dissolved in sterile 0.9% saline to a concentration of 10.0 $nmol \cdot ml^{-1}$. This dilution was used as a stock solution to produce the various concentrations of peptide for bolus injection. Rats were administered bolus injections of ET-1 at 0.1 (n=6), 0.3 (n=6) and 1.0 (n=6) $nmol \cdot kg^{-1}$, i.v. or ET-3 at 0.3 (n=4) and 1.0 (n=4) $nmol \cdot kg^{-1}$, i.v. The bolus injections were in total volumes of 0.3 ml and administered in entirety within 15-sec. Hemodynamics were monitored continuously at the two lower doses for 1-hr while those of the higher dose were monitored for 2-hr.

Acute infusion endothelin experiments. Rats receiving 1-hr (acute) infusions of endothelin were administered ET-1 at 0.003 (n=8), 0.01 (n=14) and 0.03 (n=14) $nmol \cdot kg^{-1} \cdot min^{-1}$ with a total volume flow rate of 0.0096 $ml \cdot min^{-1}$ and were monitored continuously for 1-hr, thereafter. Similarly, rats received 1-hr infusion of ET-3 at 0.01 (n=4) and 0.03 (n=4) $nmol \cdot kg^{-1} \cdot min^{-1}$.

Only one infusion or bolus dose of endothelin was administered to an individual rat per day. Although no single rat received *all* injections and

infusions, each rat received at least a full dose-range of ET-1 or ET-3 by infusion or injection. Hemodynamic parameters measured included: MAP, HR, CO, SV and TPR.

2. Chronic Intravenous Infusion of ET-1 and ET-3

a. Dose-dependency of Endothelin-Hypertension: The specific aim of this set of experiments was to determine hemodynamic dose-dependency to chronically infused endothelins. Rats placed on 7-day (chronic) i.v. infusions of endothelin received ET-1 or ET-3 at 0.0, 3.0, 5.0 and 7.5 $pmol \cdot kg^{-1} \cdot min^{-1}$. MAP, HR, CO, TPR, SV, WI, UO, $U_{Na}V$ and U_KV was monitored in all animals. Animals were monitored for three days prior to endothelin administration and for five days following endothelin administration. All animals received chronic i.v. infusions of 6.0 $mEq \cdot day^{-1}$ sodium chloride solution at a rate of 5.0 $ml \cdot day^{-1}$ for the entire 15-day protocol since it has been previously shown that an increased salt intake accelerates the development of hypertension in some animal models of hypertension (Fink etal., 1987, Pawloski et al., 1990). Blood samples were taken once during the control period, twice during the endothelin infusion period and once during the recovery period in order to determine $[ET\text{-}1]_P$, $[AngII]_P$, $[P]_{Na}$ and $[P]_K$.

b. Salt-dependency of Endothelin-Hypertension: The specific aim of this set of experiments was to determine if endothelin-hypertension is dependent on sodium-intake. Experiments utilizing the protocol in *B.2.a.* above were repeated

using an ET-1 infusion rate of 5.0 $pmol \cdot kg^{-1} \cdot min^{-1}$ and chronic i.v. infusions of 2.0 $mEq \cdot day^{-1}$ sodium chloride for the entire 15-day protocol. Hemodynamic as well as fluid/electrolyte parameters described previously were then assessed between animal groups receiving 2.0 and 6.0 $mEq \cdot day^{-1}$ sodium chloride solution infusions.

c. Plasma hormone concentrations during Endothelin-Hypertension: The specific aim of this set of experiments was to determine if ET-1 and AngII are found in the normal rat circulation as well as in increased concentrations in the ET-1-infused rat in order to elucidate ET-1's actions as a circulating hormone and its actions on other circulating vasoactive factors. In animals following the chronic ET-1 infusion protocols above, $[ET\text{-}1]_P$ and $[AngII]_P$ were determined by radioimmunoassay as described individually in *A.5.a. and A.5.b.* above.

d. Endothelin-Hypertension and renal electrolyte/fluid homeostasis: The specific aim of this set of experiments was to determine if endothelin-hypertension is accompanied by renal sodium retention. In animals following the chronic ET-1 infusion protocols above, $U_{Na}V$, U_KV, WI, UO, and WB were monitored and assessed daily throughout the 15-day protocol.

3. Endothelin-Hypertension and the Sympathetic Nervous System

a. Baroreceptor reflex afferents: The specific aim of this set of experiments was to determine if the baroreceptor reflex attenuates the pressor response to exogenously-administered ET-1. Rats underwent sino-aortic denervation (SAD) or SHAM-operation as described in *2.c.* above. Animals were then placed on the chronic infusion protocol described above. Hemodynamic differences between SAD animals and SHAM animals were assessed as described in *A.3.* above.

b. Area Postrema ablation: The specific aim of this set of experiments was to determine if ET-1's comprehensive pressor response is dependent on the integrity of the area postrema (AP). Rats underwent APX or SHAM-operation as described in *A.2.d.* above. The animals were then placed on the chronic infusion protocol described in *B.2.b.* above and assessed for differences in their hemodynamic responses to chronically infused ET-1. At the end of the experiment, rat brains were harvested and assessed for the extent of APX as described in *A.2.d.* above.

4. Endothelin-Hypertension and the Renin-Angiotensin System

The specific aim of these sets of experiments was to determine if the RAS system contributes to ET-1-induced hypertension.

a. Chronic intravenous infusion of angiotensin converting enzyme inhibitors: In order to elucidate the contributions of AngII synthesis to endothelin-hypertension, rats were chronically prepared as in *A.2.a.* above and placed on two chronic i.v. infusions consisting of a 6.0 $mEq \cdot day^{-1}$ sodium chloride solution (used as a vehicle

for the ET-1 infusion at 5.0 pmol·kg^{-1}·min^{-1}) and a 5.0% dextrose solution (used as a vehicle for continuous infusion of the angiotensin I converting enzyme (ACE) inhibitor captopril ([2*S*]-1-[3-mercapto-2-methyl-propionyl]- L-proline, Sigma, St. Louis, MO) at a dose of 0.0 (control) or 1.0 mg·kg^{-1}·hr^{-1}; these infusions were maintained throughout the experimental protocol. These experiments were also performed using the non-sulfhydryl ACE inhibitor, enalapril ((*S*)-1-[*N*-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-(*Z*)-2-butenedioate-L-proline) at a dose of 2.0 mg·kg^{-1}·day^{-1}. Animals were assessed for ACE inhibition by i.v. bolus administration of 20.0 ng of AngI.

b. Bolus administration of an AT_1 receptor antagonist: In order to elucidate the contributions of AngII at AT_1 receptors to endothelin-hypertension, rats were chronically prepared as described in *A.2.a.* above. Losartan (DuP753), a *non-peptide*, type-1, AngII receptor antagonist was administered to each rat, once during the control period, on two days during the ET-1 infusion period (DAYS 2-3 and DAYS 4-5 of the ET-1 infusion period), and once during the recovery period at a dose of 3.0 mg·kg^{-1}, i.v. MAP and HR were measured at 0-, 15-, 30-min, and 1-, 2-, 6- and 24-hr time-points after losartan was administered. Animals were assessed for AngII receptor blockade by i.v. bolus administration of 10.0 ng of AngII.

c. Chronic infusion of AT_1 receptor antagonist: In order to elucidate the long-term effects of AngII-receptor antagonism on endothelin-hypertension, rats

were chronically prepared as described in *A.2.a.* above. Losartan was administered daily as a bolus dose of 3.0 $mg \cdot kg^{-1}$, i.v. throughout the 15-day protocol. MAP, HR, UO, WI, WB, $U_{Na}V$ and U_KV were measured at 24-hr intervals. Animals were assessed for AngII receptor blockade by i.v. bolus administration of 10.0 ng of AngII.

RESULTS

1. Bolus and Acute Intravenous Infusion of ET-1 and ET-3

Human endothelin (ET-1) administered as a bolus injection, i.v. Figures 1-5 present the hemodynamic responses to bolus i.v. injections of ET-1 at 0.1 (n=6), 0.3 (n=6) and 1.0 (n=6) nmole·kg^{-1}. Each dose produced a significant rise in MAP (Figure 1) and TPR (Figure 3) which was sustained above control for at least 5-min for the lowest dose and at least 105-min for the highest dose. All values returned to control levels within 120-min after ET-1 injection. CO (Figure 4) and HR (Figure 2) were only significantly reduced by the highest dose of ET-1 and SV (Figure 5) was not consistently affected by any dose.

Human endothelin (ET-1) administered as an infusion, i.v. Figures 6-10 present the hemodynamic responses to 1-hr i.v. infusions of ET-1 at rates of 0.003 (n=8), 0.01 (n=14) and 0.03 (n=14) nmole·kg^{-1}·min^{-1}. The animals were also monitored for 1-hr following cessation of ET-1 infusion. Infusion of ET-1 at rates of 0.01 and 0.03 nmol·kg^{-1}·min^{-1} resulted in a significant and sustained rise in MAP (Figure 6) which lasted throughout the infusion period and well into the recovery period, while only the highest infusion rate resulted in a sustained increase in TPR (Figure 8) and a sustained decrease in CO (Figure 9)

and HR (Figure 7). Again, SV (Figure 10) was not significantly affected at any rate of ET-1 infusion.

Rat endothelin (ET-3) administered as a bolus injection, i.v. Figures 11-15 present the hemodynamic responses to bolus i.v. injections of ET-3 at 0.3 (n=4) and 1.0 (n=4) nmole·kg^{-1}. Both doses resulted in a significant and sustained increase in only MAP (Figure 11) and TPR (Figure 13). HR (Figure 12), CO (Figure 14) and SV (Figure 15) were not significantly affected by either bolus injection. The pressor responses to ET-3 were also noticeably shorter in duration when compared to the responses obtained from bolus injection of ET-1.

Rat endothelin (ET-3) administered as an infusion, i.v. Figures 16-20 present the hemodynamic responses to 1-hr i.v. infusions of ET-3 at rates of 0.01 and 0.03 nmole·kg^{-1}·min^{-1}. Both infusions resulted in a significant and sustained increase in MAP (Figure 16) and TPR (Figure 18) which lasted well into the 1-hr recovery following cessation of the infusion. Again, as with the bolus injections of ET-3, HR (Figure 17), CO (Figure 19) and SV (Figure 20) were not significantly affected by either infusion. The pressor responses to ET-3 were also shorter in duration as compared to the responses obtained from infusion of ET-1.

Comparison of hemodynamic effects of ET-1 and ET-3. Comparison of the maximal pressor changes induced by a bolus injection or infusion of ET-1 or ET-3 (Figure 21) reveals that there is no statistically significant difference in the *efficacies* of the two peptides. Similarly, comparison of the *times of onset* of

the maximal pressor responses (Figure 22) to either peptide shows that there is no statistically significant difference between ET-1 or ET-3 as either a bolus injection or infusion. However, comparison of the *durations* of the pressor responses induced by either peptide (Figure 23) reveals that ET-3 has a significantly shorter duration of action compared to the responses obtained by injection or infusion of ET-1. The doses of endothelin used in these experiments are comparable to those doses described in the existing literature (6, 8, 12, 14, 16). Preliminary experiments suggested that the highest doses and infusion rates used in these experiments were indeed "maximal" *in vivo* doses, since attempts to increase the dose by a factor of three in several rats proved uniformly lethal.

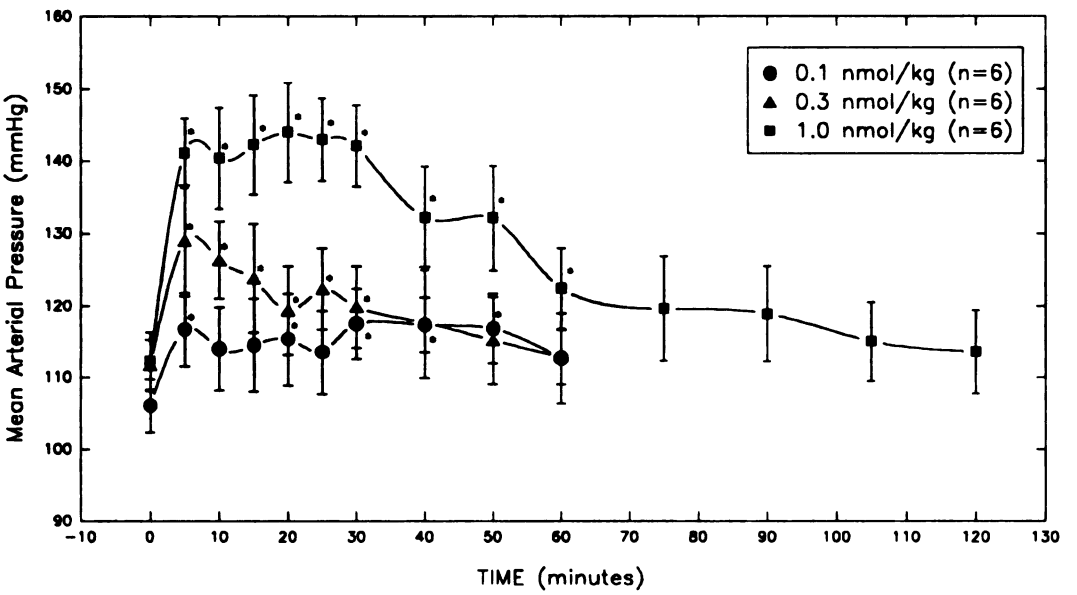

Figure 1. Mean arterial blood pressure responses to bolus injection of ET-1 at doses of 0.1, 0.3, and 1.0 nmol·kg^{-1}, i.v. (n=6 for all groups). Asterisks indicate significant difference ($P<0.05$) from control measurement at 0 minutes. Error bars represent SEM.

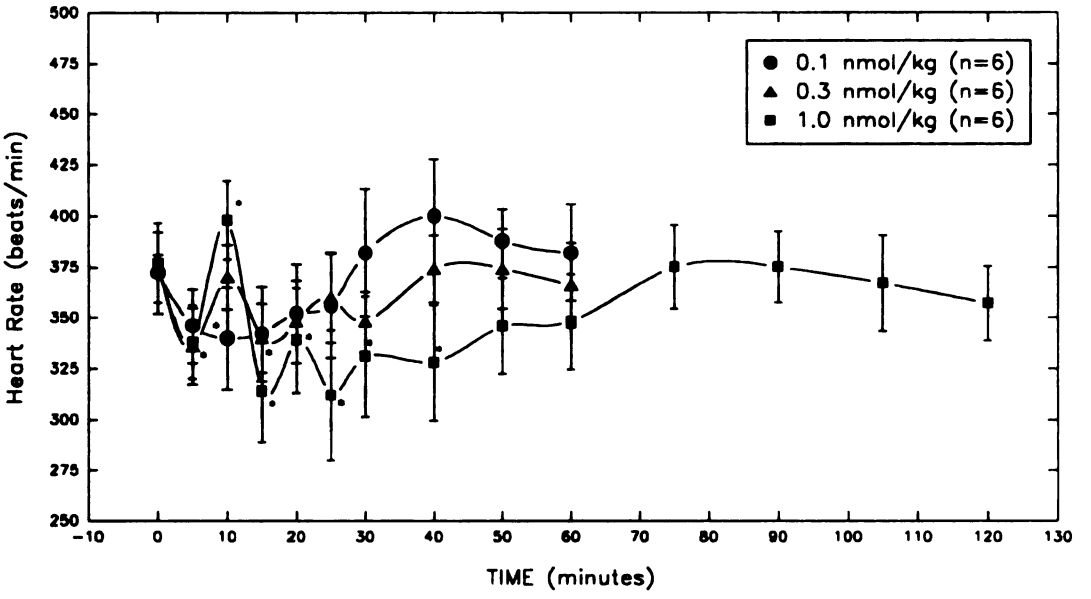

Figure 2. Heart rate responses to bolus injection of ET-1 at doses of 0.1, 0.3, and 1.0 nmol·kg^{-1}, i.v. (n=6 for all groups). Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

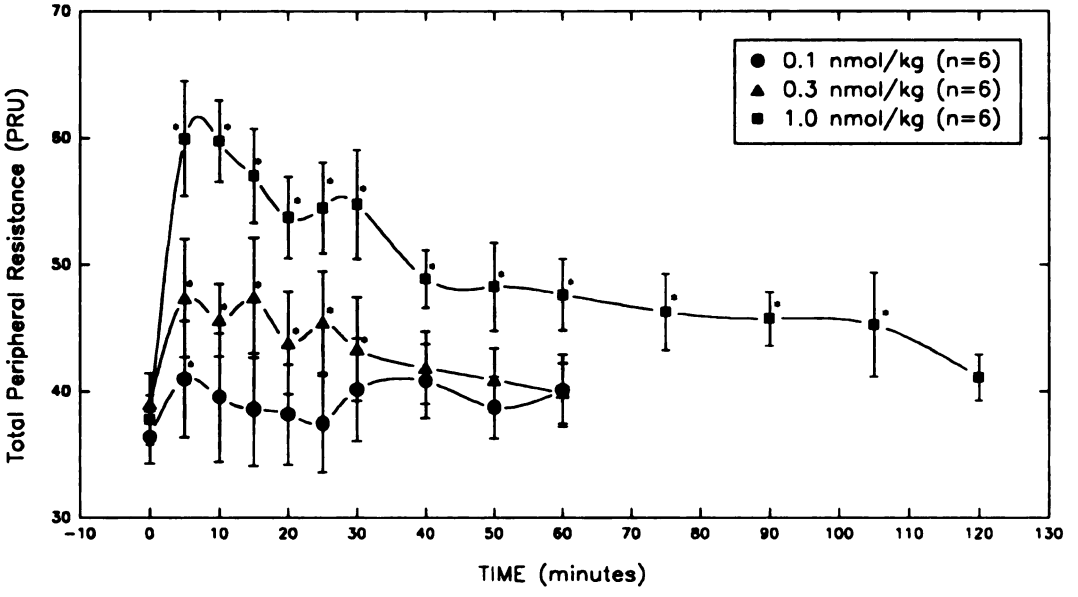

Figure 3. Changes in total peripheral resistance (expressed as peripheral resistance units, PRU [see text]) in response to bolus injection of ET-1 at doses of 0.1, 0.3, and 1.0 nmol·kg^{-1}, i.v. (n=6 for all groups). Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

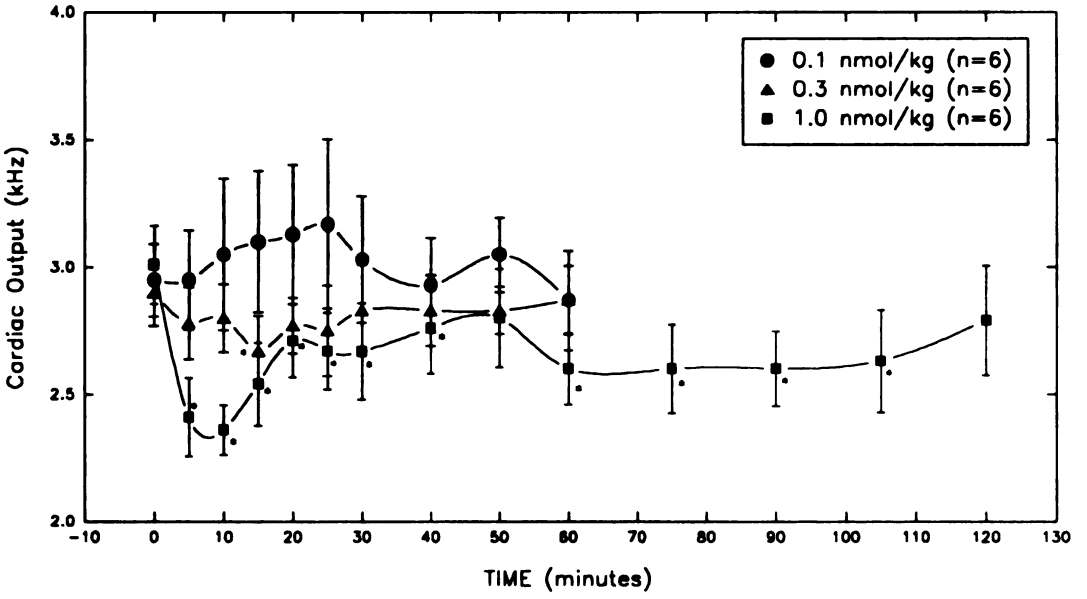

Figure 4. Changes in cardiac output in response to bolus injection of ET-1 at doses of 0.1, 0.3, and 1.0 nmol·kg^{-1}, i.v. (n=6 for all groups). Asterisks indicate significant difference ($P<0.05$) from control measurement at 0 minutes. Error bars represent SEM.

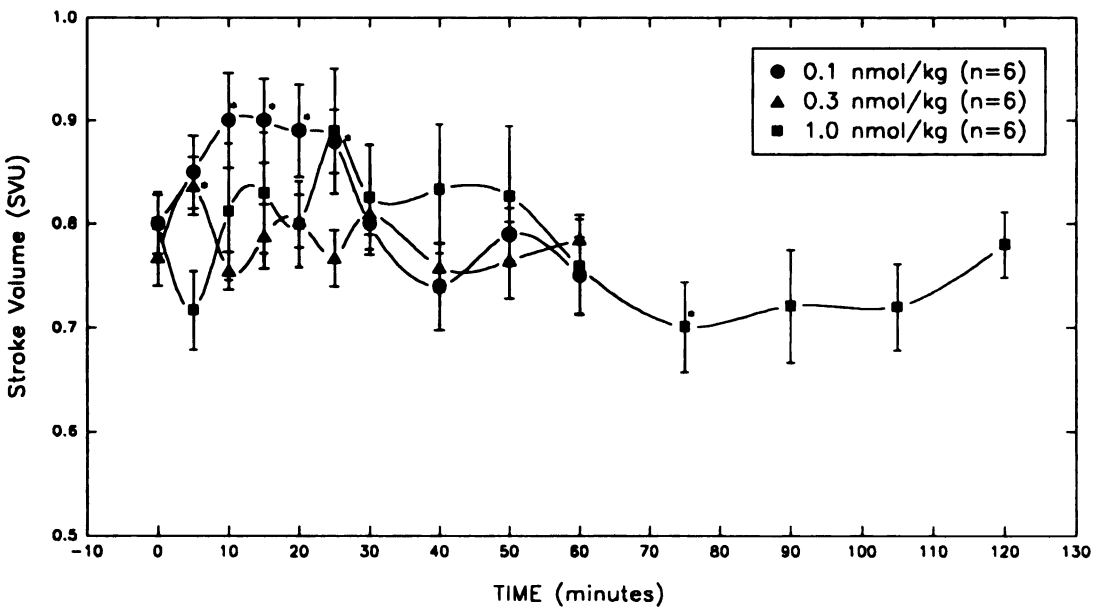

Figure 5. Changes in stroke volume (expressed as stroke volume units, SVU [see text]) in response to bolus injection of ET-1 at doses of 0.1, 0.3, and 1.0 nmol·kg^{-1}, i.v. (n=6 for all groups). Asterisks indicate significant difference ($P<0.05$) from control measurement at 0 minutes. Error bars represent SEM.

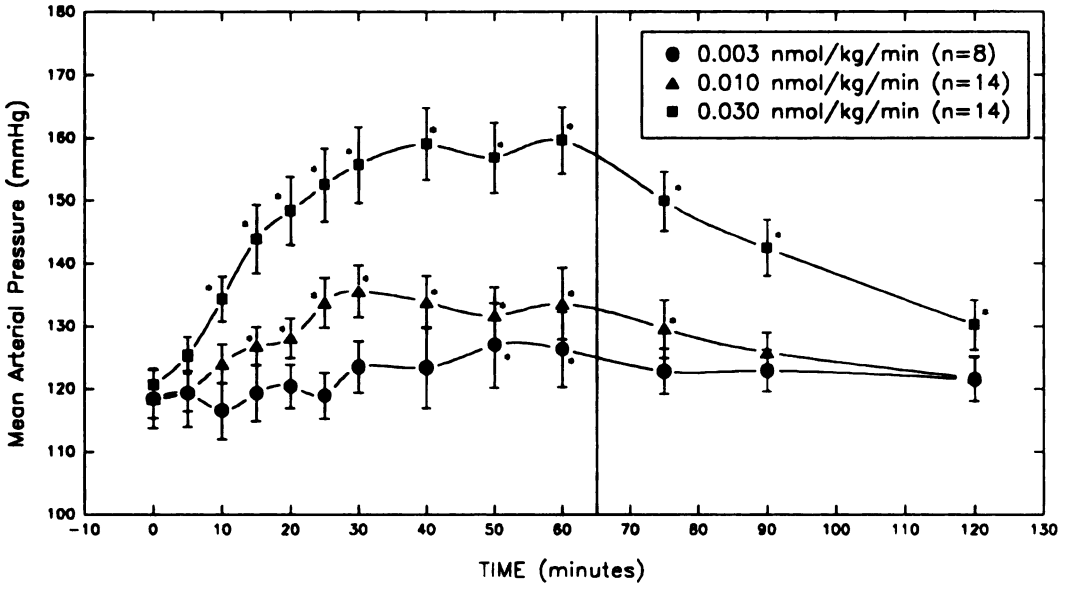

Figure 6. Mean arterial pressure responses to 1-hr acute infusions of ET-1 at dose rates of 0.003 (n=8), 0.010 (n=14), and 0.030 (n=14) nmol·kg^{-1}·min^{-1}, i.v. Infusions were discontinued at the 60-min time point (solid vertical line) and the animals were monitored for an additional hour. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

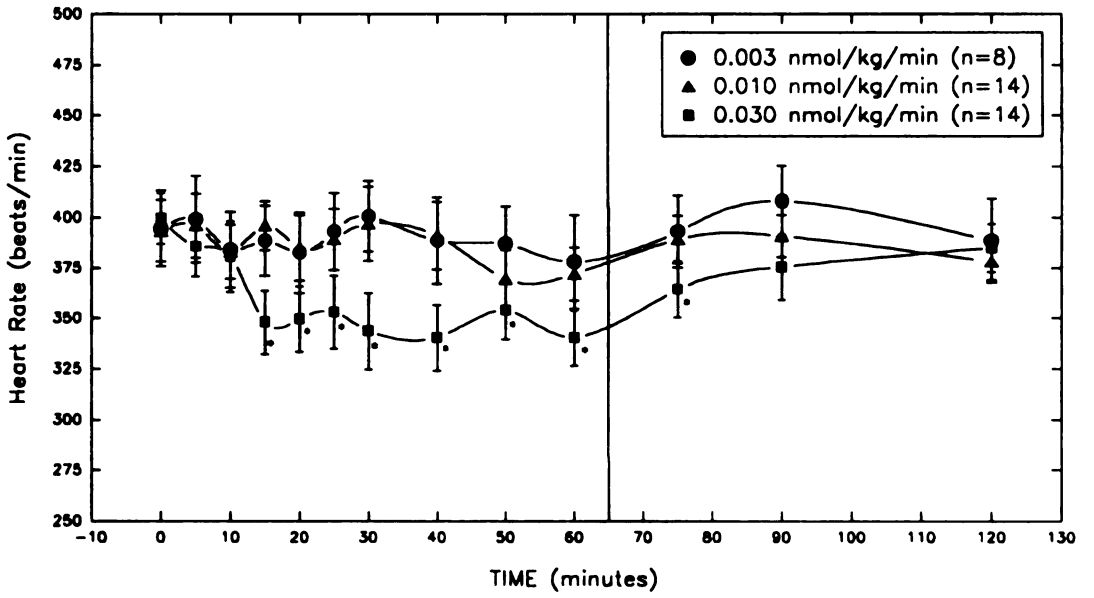

Figure 7. Heart rate responses to 1-hr acute infusions of ET-1 at dose rates of 0.003 (n=8), 0.010 (n=14), and 0.030 (n=14) nmol·kg^{-1}·min^{-1}, i.v. Infusions were discontinued at the 60-min time point (solid vertical line) and the animals were monitored for an additional hour. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

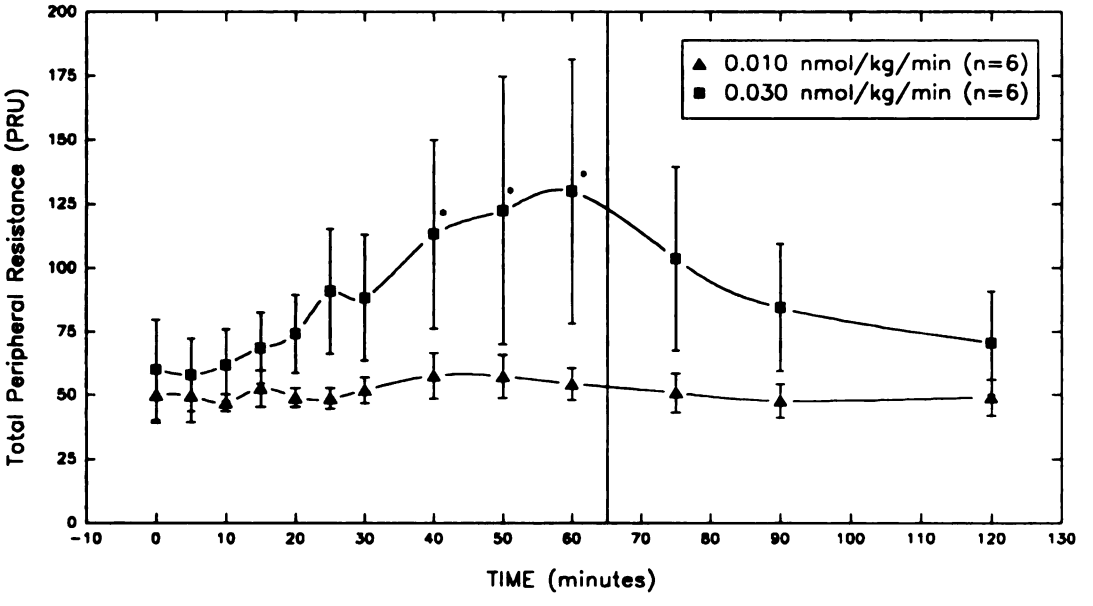

Figure 8. Changes in total peripheral resistance in response to 1-hr acute infusions of ET-1 at dose rates of 0.010 (n=6), and 0.030 (n=6) nmol·kg^{-1}·min^{-1}, i.v. Infusions were discontinued at the 60-min time point (solid vertical line) and the animals were monitored for an additional hour. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

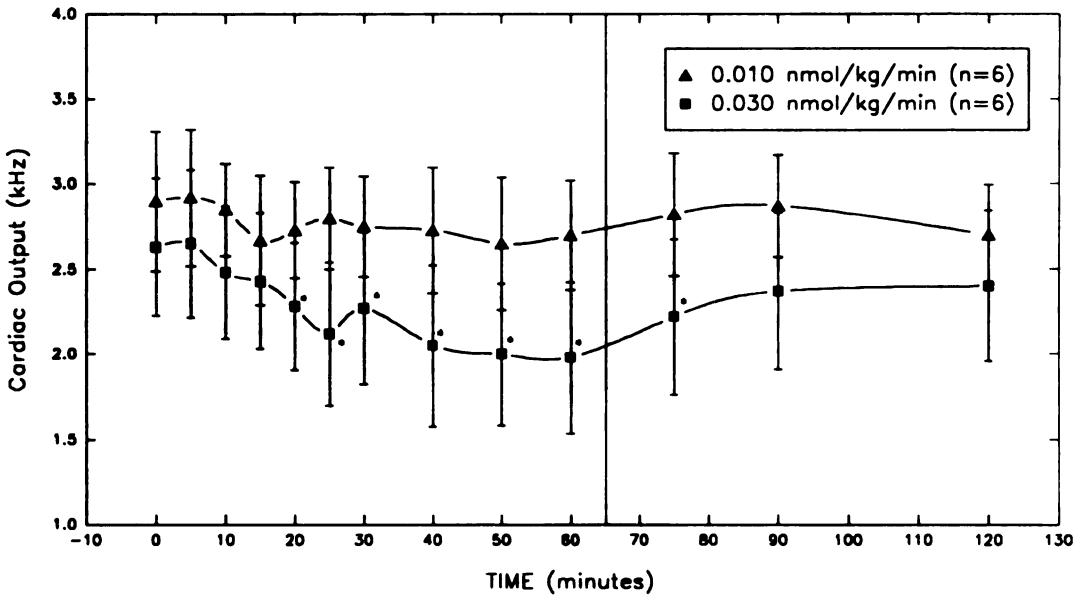

Figure 9. Changes in cardiac output in response to 1-hr acute infusions of ET-1 at dose rates of 0.010 (n=6), and 0.030 (n=6) nmol·kg^{-1}·min^{-1}, i.v. Infusions were discontinued at the 60-min time point (solid vertical line) and the animals were monitored for an additional hour. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

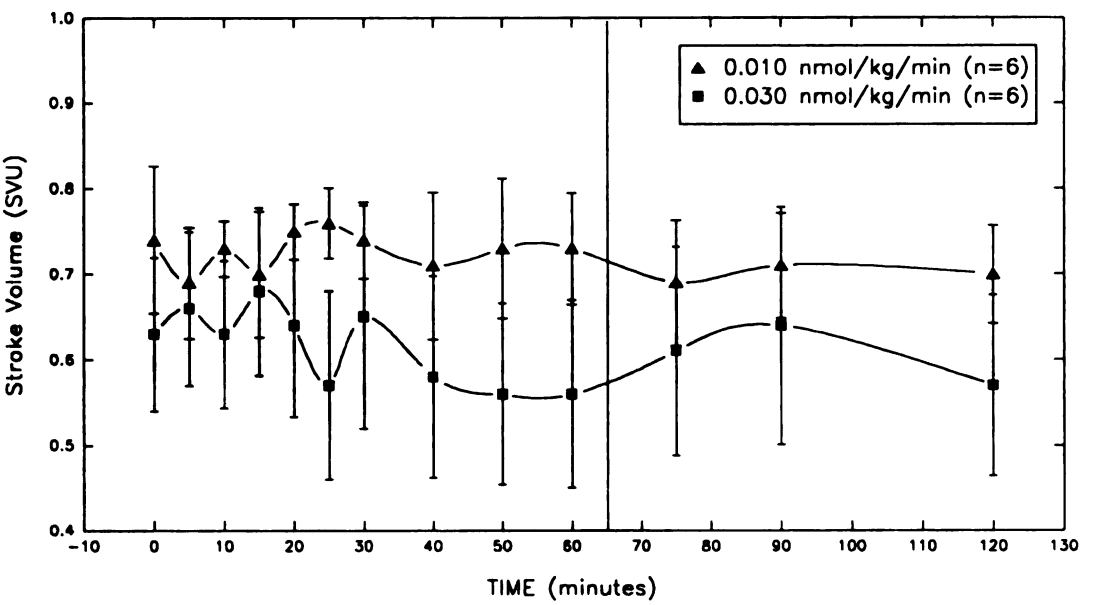

Figure 10. Changes in stroke volume in response to 1-hr acute infusions of ET-1 at dose rates of 0.010 (n=6), and 0.030 (n=6) nmol·kg^{-1}·min^{-1}, i.v. Infusions were discontinued at the 60-min time point (solid vertical line) and the animals were monitored for an additional hour. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

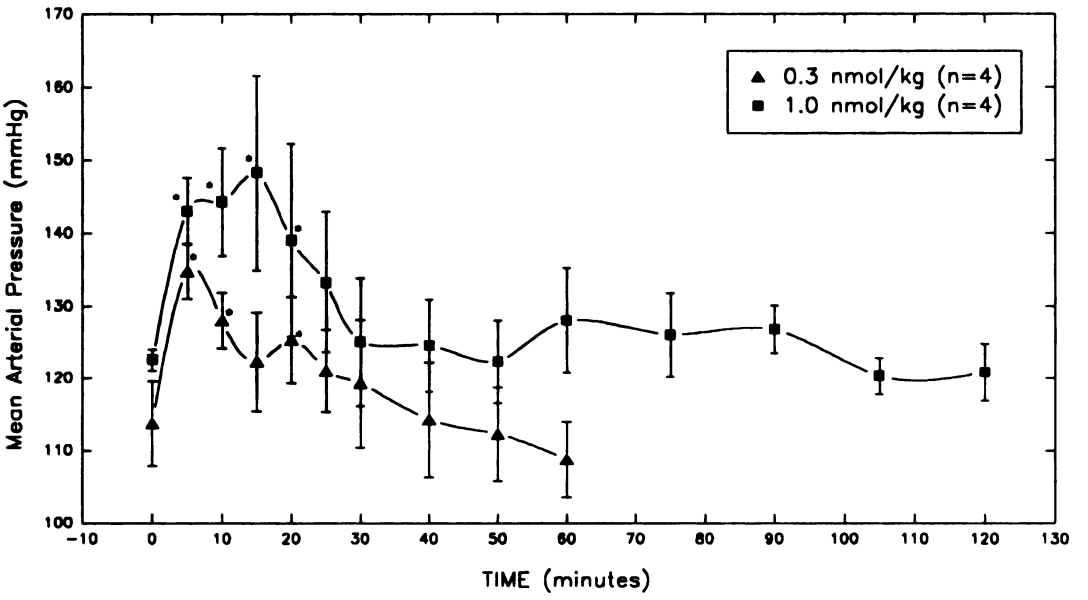

Figure 11. Mean arterial pressure responses to bolus injection of ET-3 at doses of 0.03 (n=4), and 1.0 (n=4) nmol·kg^{-1}, i.v. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

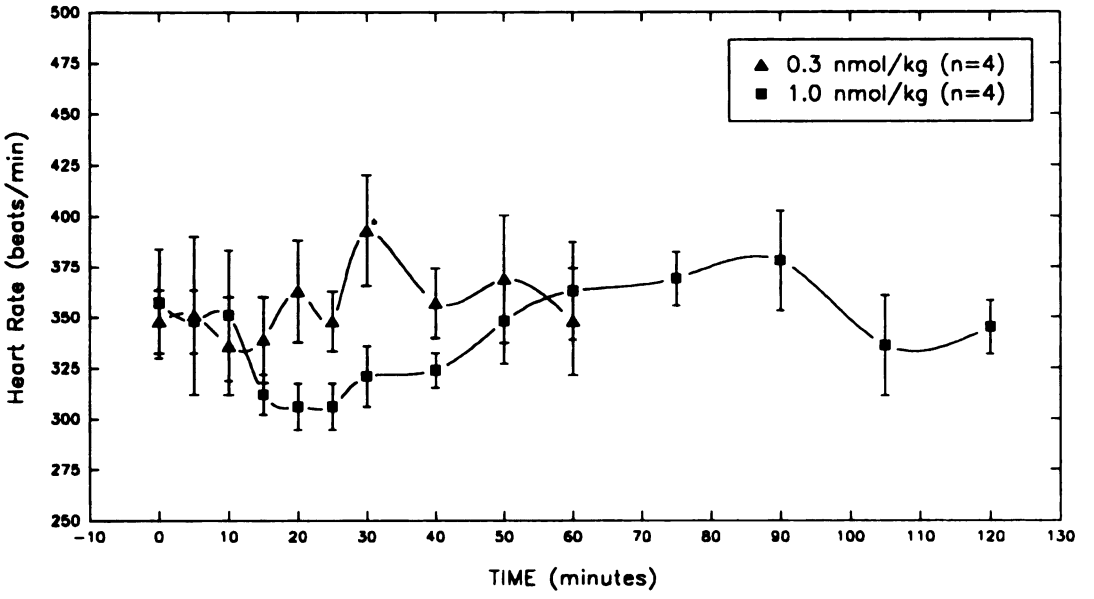

Figure 12. Heart rate responses to bolus injection of ET-3 at doses of 0.3 (n=4) and 1.0 (n=4) nmol·kg^{-1}, i.v. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

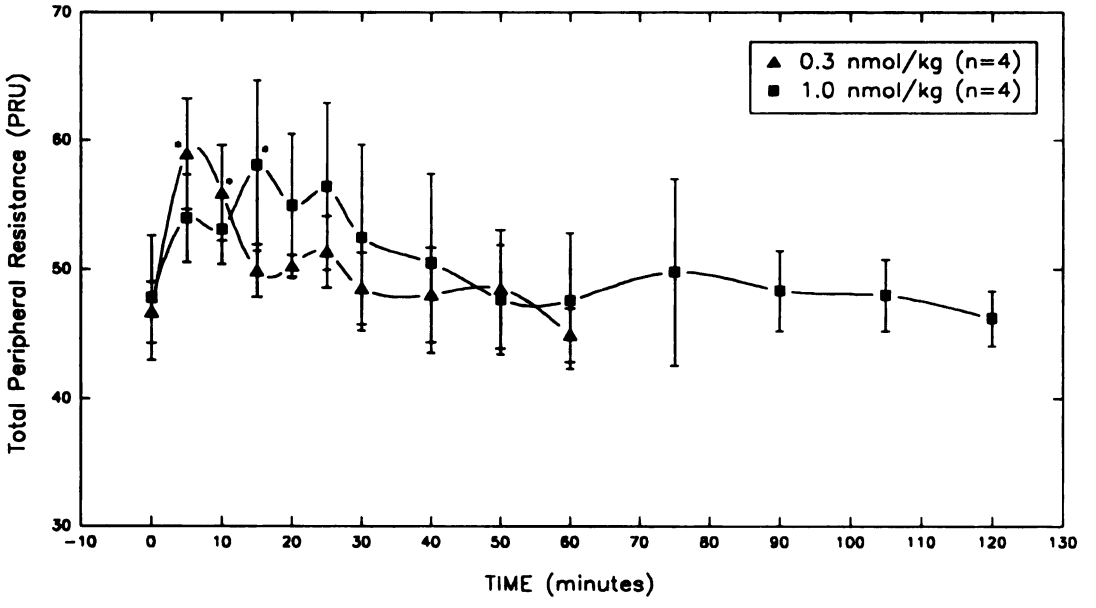

Figure 13. Changes in total peripheral resistance in response to bolus injection of ET-3 at doses of 0.3 (n=4) and 1.0 (n=4) nmol·kg^{-1}, i.v. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

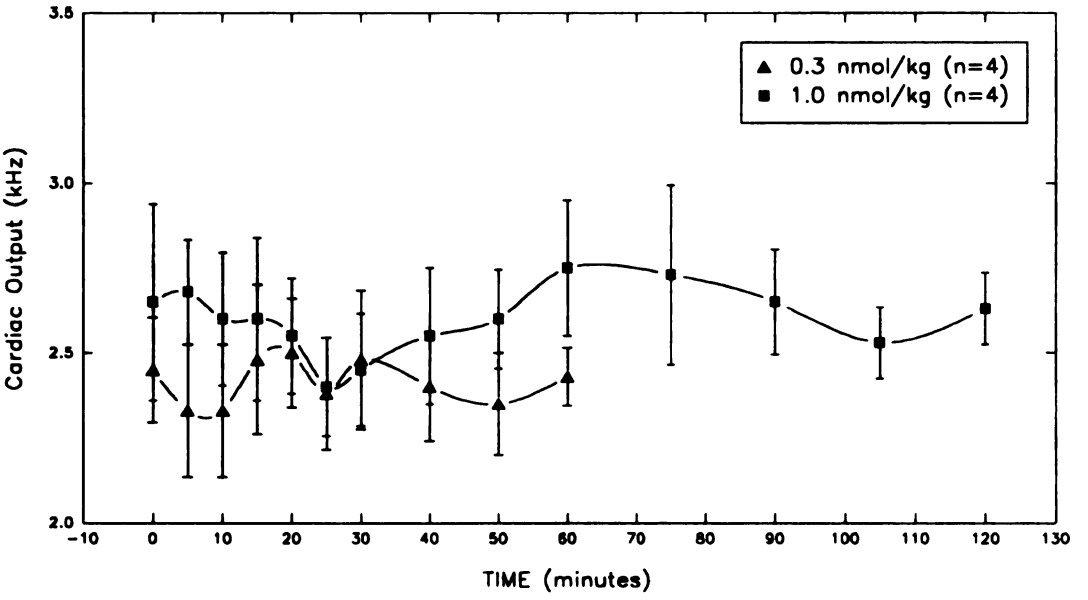

Figure 14. Changes in cardiac output in response to bolus injection of ET-3 at doses of 0.3 (n=4) and 1.0 (n=4) nmol·kg^{-1}, i.v. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

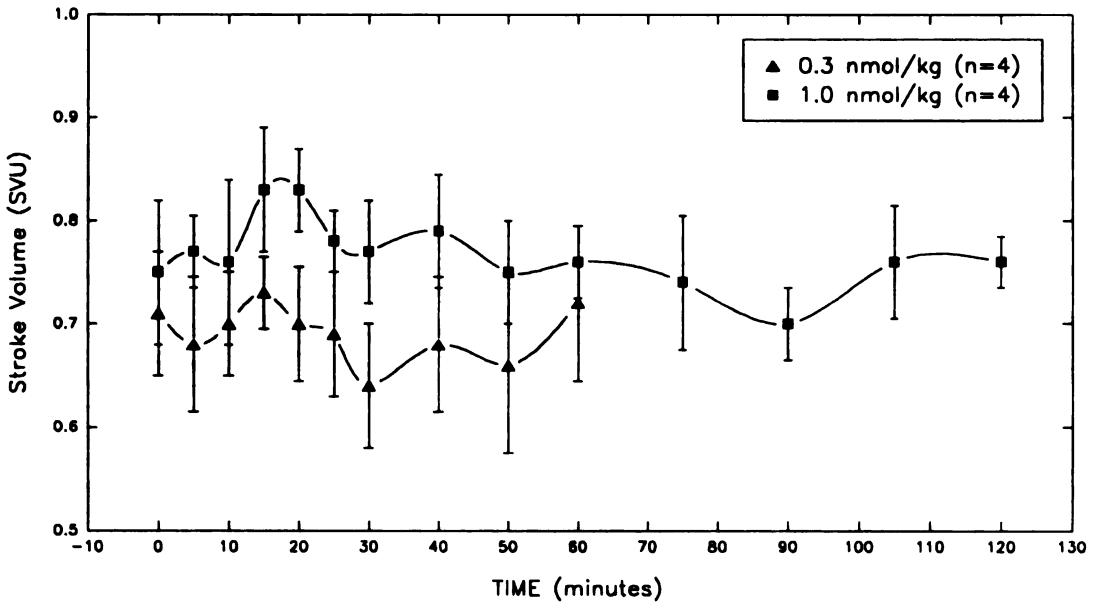

Figure 15. Changes in stroke volume in response to bolus injection of ET-3 at doses of 0.3 (n=4) and 1.0 (n=4) nmol·kg^{-1}, i.v. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

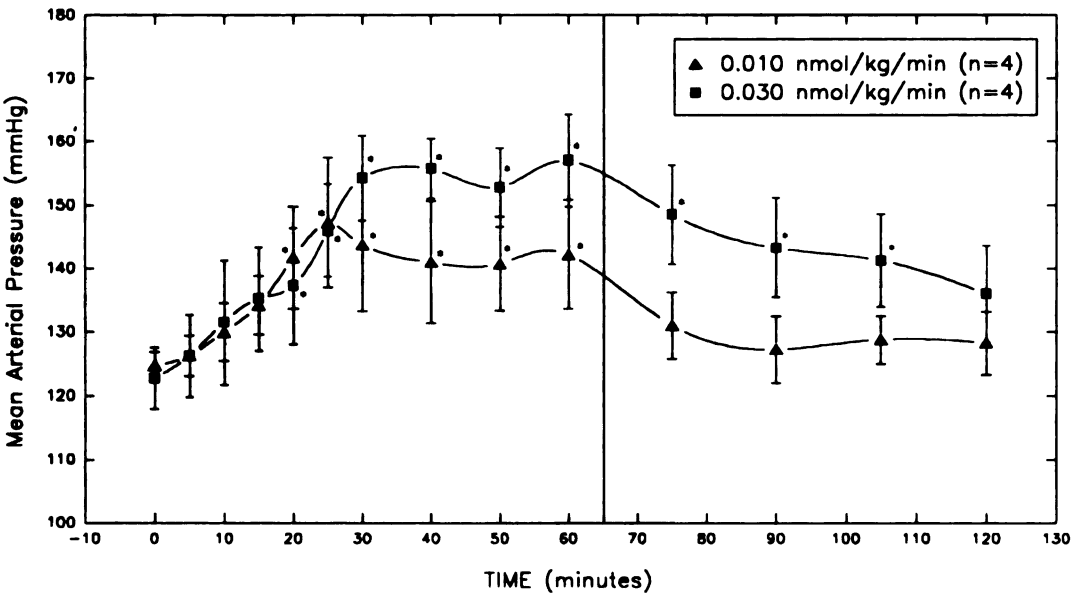

Figure 16. Mean arterial pressure responses to 1-hr acute infusions of ET-3 at dose rates of 0.010 (n=4) and 0.030 (n=4) nmol·kg^{-1}·min^{-1}, i.v. Infusions were discontinued at the 60-min time point (solid vertical line) and the animals were monitored for an additional hour. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

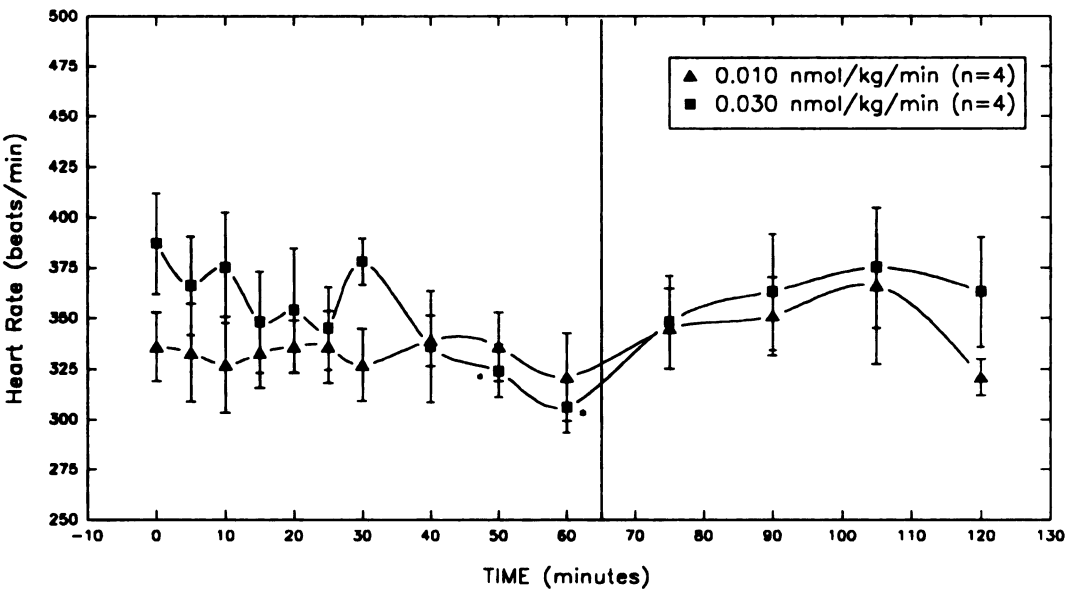

Figure 17. Heart rate responses to 1-hr acute infusions of ET-3 at dose rates of 0.010 (n=4), and 0.030 (n=4) nmol·kg^{-1}·min^{-1}, i.v. Infusions were discontinued at the 60-min time point (solid vertical line) and the animals were monitored for an additional hour. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

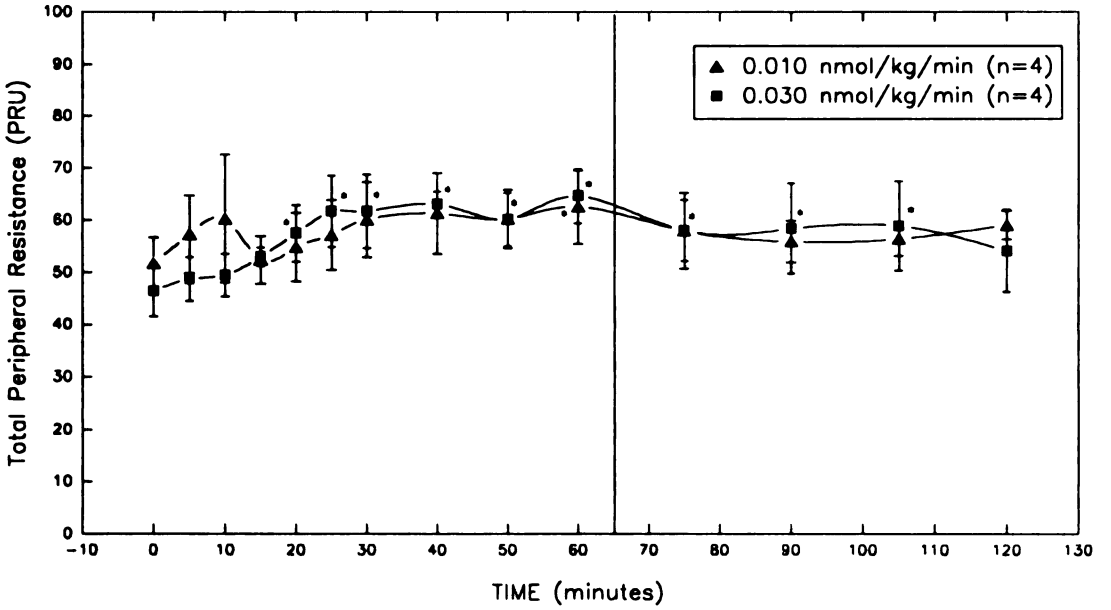

Figure 18. Changes in total peripheral resistance in response to 1-hr acute infusions of ET-3 at dose rates of 0.010 (n=4) and 0.030 (n=4) nmol·kg^{-1}·min^{-1}, i.v. Infusions were discontinued at the 60-min time point (solid vertical line) and the animals were monitored for an additional hour. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

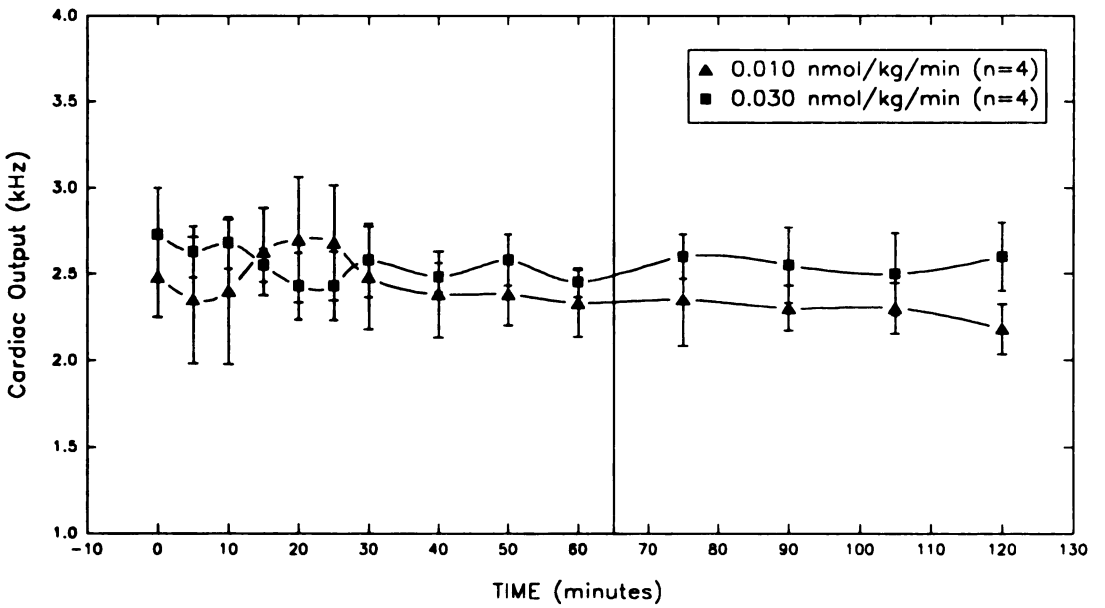

Figure 19. Changes in cardiac output in response to 1-hr acute infusions of ET-3 at dose rates of 0.010 (n=4) and 0.030 (n=4) nmol·kg^{-1}·min^{-1}, i.v. Infusions were discontinued at the 60-min time point (solid vertical line) and the animals were monitored for an additional hour. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

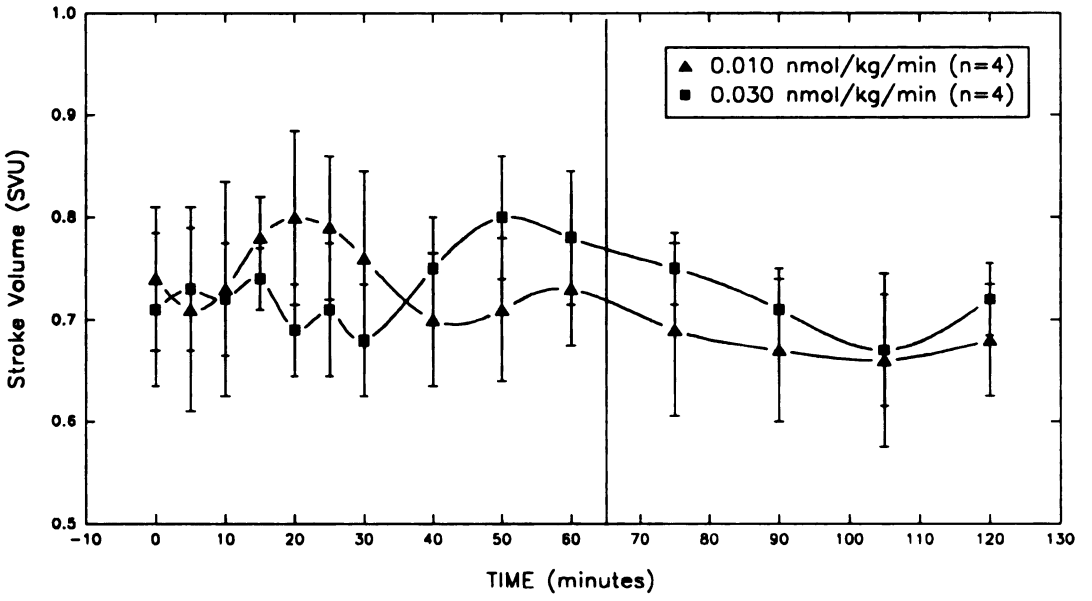

Figure 20. Changes in stroke volume in response to 1-hr acute infusions of ET-3 at dose rates of 0.010 (n=4) and 0.030 (n=4) nmol·kg^{-1}·min^{-1}, i.v. Infusions were discontinued at the 60-min time point (solid vertical line) and the animals were monitored for an additional hour. Asterisks indicate significant difference (P<0.05) from control measurement at 0 minutes. Error bars represent SEM.

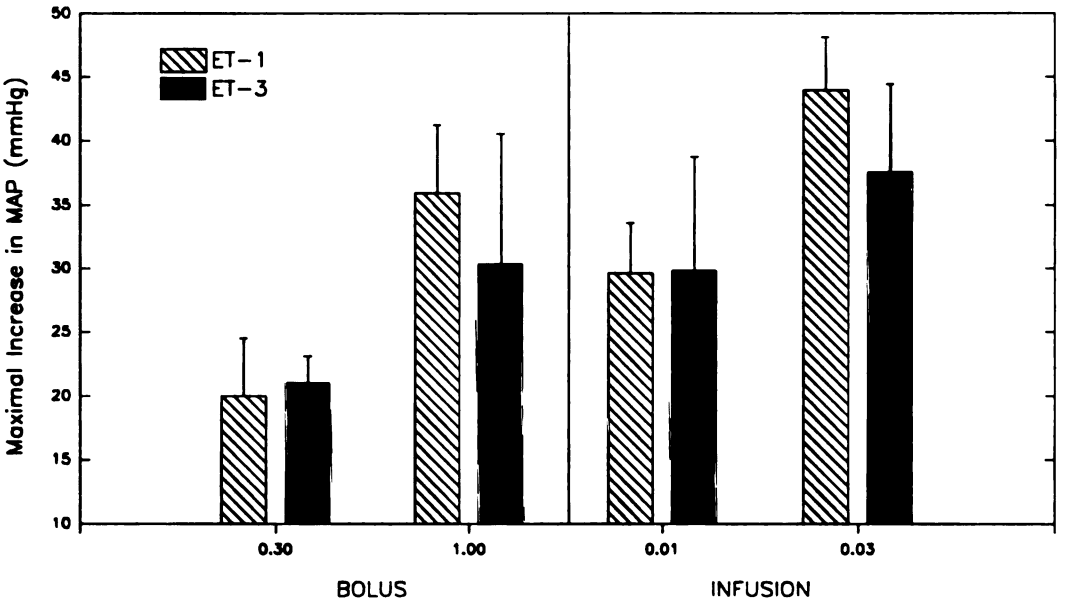

Figure 21. Maximal increases in mean arterial pressure elicited by bolus injection or acute infusion of ET-1 or ET-3. Asterisks indicate significant difference ($P<0.05$) between individual paired groups. When administered as either a bolus injection (0.3 and 1.0 nmol·kg^{-1}) or an infusion (0.01 and 0.03 nmol·kg^{-1}·min^{-1}), ET-1 and ET-3 are not significantly different in terms of the maximal increases in MAP that they elicit, suggesting similar efficacies. Error bars represent SEM.

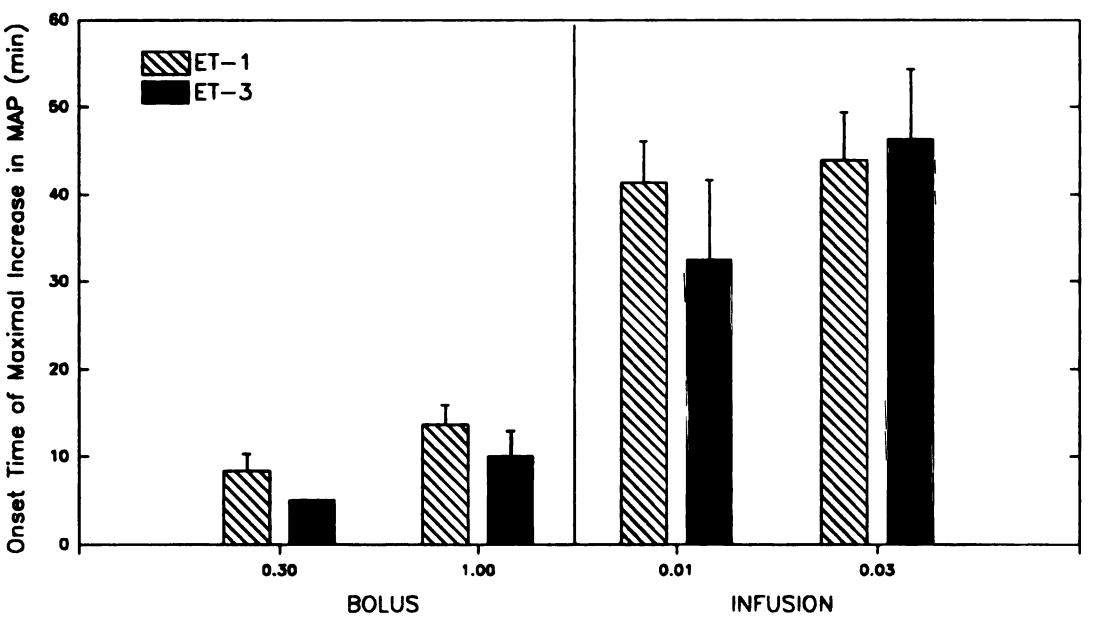

***Figure* 22.** Onset times of maximal increases in mean arterial pressure elicited by bolus injection or acute infusion of ET-1 or ET-3. Asterisks indicate significant difference ($P<0.05$) between individual paired groups. When administered as either a bolus injection (0.3 and 1.0 nmol·kg^{-1}) or an infusion (0.01 and 0.03 nmol·kg^{-1}·min^{-1}), ET-1 and ET-3 are not significantly different in terms of the onset times of maximal increases in MAP that they elicit, again suggesting similar efficacies. Error bars represent SEM.

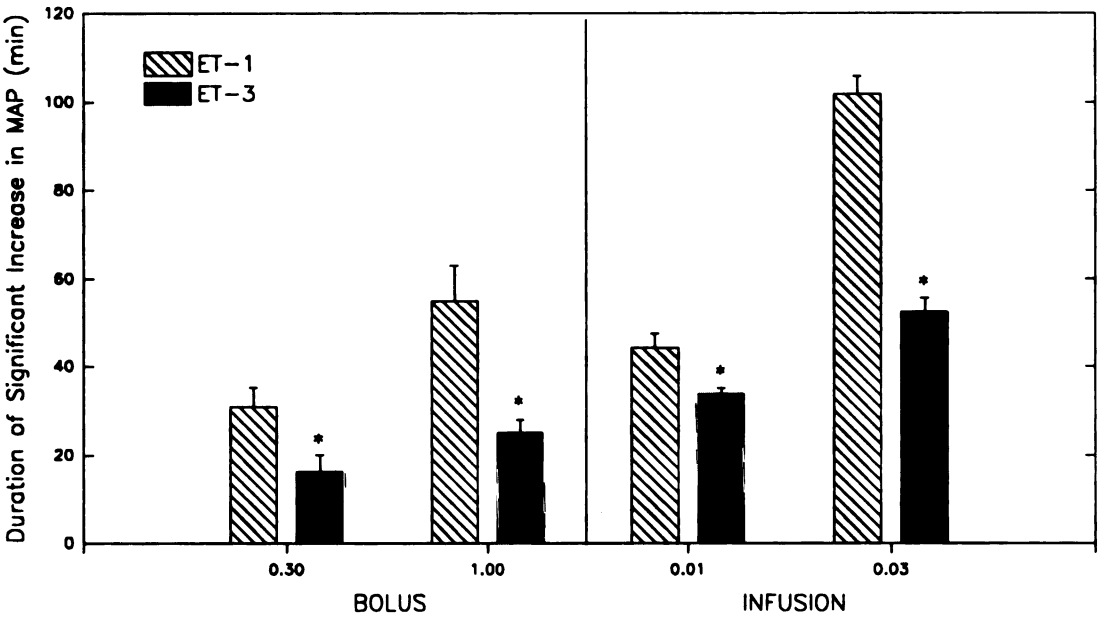

Figure 23. Durations of significant increases in mean arterial pressure elicited by bolus injection or acute infusion of ET-1 or ET-3. Asterisks indicate significant difference ($P<0.05$) between individual paired groups. Comparison of the durations of the blood pressure responses elicited by bolus injection (0.3 and 1.0 $nmol \cdot kg^{-1}$) or infusion (0.01 and 0.03 $nmol \cdot kg^{-1} \cdot min^{-1}$) of either peptide, reveals that ET-3 is significantly ($p<0.05$) less potent than ET-1. Error bars represent SEM.

2. Chronic Intravenous Infusion of ET-1 and ET-3

a. Dose-dependency of Endothelin-Hypertension Initial experiments were performed in rats without aortic flow probes to determine the chronic ET-1/arterial pressure dose-response relationship. Figure 24 illustrates the results of these experiments. Chronic infusion of ET-1 produced dose-dependent increases in MAP which were readily reversible upon termination of the infusion. The infusion of ET-1 at 7.5 $pmol \cdot kg^{-1} \cdot min^{-1}$ caused a 60% mortality within a few days; thus, higher rates were not employed. Subsequent studies with ET-1 were performed using an infusion rate of 5 $pmol \cdot kg^{-1} \cdot min^{-1}$. Figures 25-32 present the hemodynamic and body fluid responses to a fifteen day infusion regimen which included seven days of i.v. infusion of ET-1 at a rate of 5.0 $pmol \cdot kg^{-1} \cdot min^{-1}$ (n=7). Infusion of ET-1 at this rate produced a statistically significant increase in MAP (26%, $P<0.05$, Figure 25). This appeared to be due to statistically insignificant increases in TPR (16%, Figure 27) and CO (11%, (Figure 28), whereas SV (Figure 29), $U_{Na}V$ (Figure 31), U_KV (Figure 32) and WB (Figure 30) remained unchanged throughout the infusion period. HR (Figure 26) was significantly decreased on the first day after starting the ET-1 infusion and a significant tachycardia occurred following termination of the infusion. Increases in MAP reached a plateau by the end of the first day of ET-1 infusion and returned to baseline levels only after the infusion was discontinued. We observed no tachyphylaxis of the MAP response to ET-1 or ET-3 at any time during the infusion period.

Figures 33-40 present the hemodynamic responses from similar experiments using ET-3. The peptide was infused for a 7-day period at a rate of 7.5 $pmol \cdot kg^{-1} \cdot min^{-1}$ (n=5). Compared to values obtained during infusion of ET-1, ET-3 infusion at this higher molar dose produced only a 15.8% increase in MAP ($P<0.05$, Figure 33) which appeared to be primarily due to a statistically insignificant increase in TPR (12.4%, Figure 35). CO (Figure 36) actually decreased 3% (not statistically significant) during the infusion period. ET-3 infusion did not alter HR (Figure 34), SV (Figure 37), $U_{Na}V$ (Figure 39), U_KV (Figure 40) or WB (Figure 38). Increases in MAP reached their maximum levels after the second day of ET-3 infusion. Again, tachyphylaxis to the pressor responses to ET-3 was not noted at any time during the infusion period.

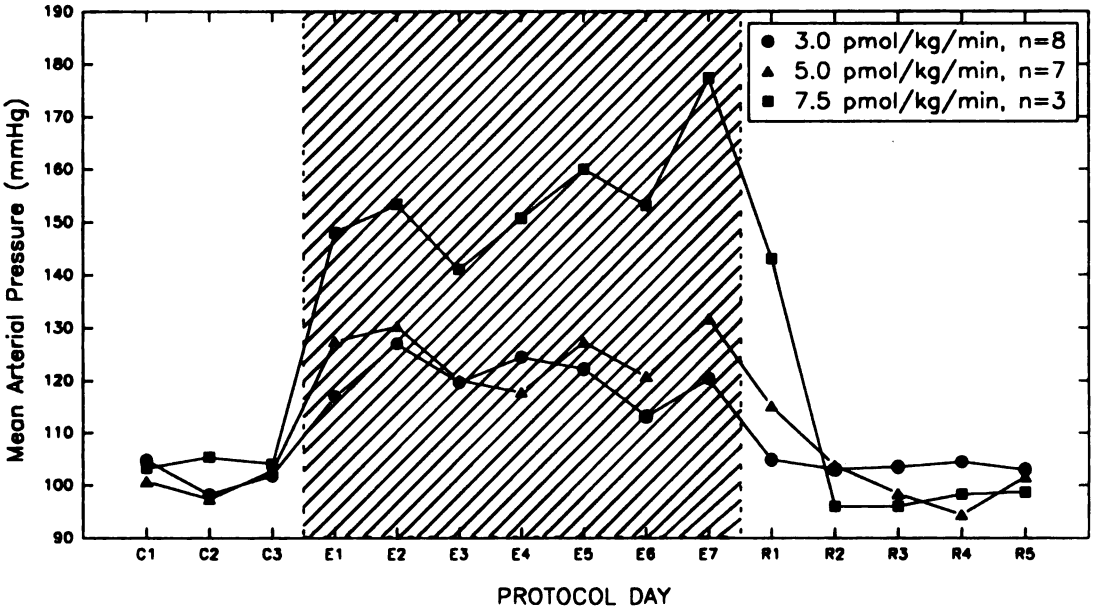

Figure 24. Mean arterial pressure responses to 7-day chronic infusions of ET-1 at dose rates of 3.0 (n=8), 5.0 (n=7) and 7.5 (n=3) pmol·kg^{-1}·min^{-1}, i.v. MAP at all doses of ET-1 was significantly elevated during the ET-1 infusion period as compared to control measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

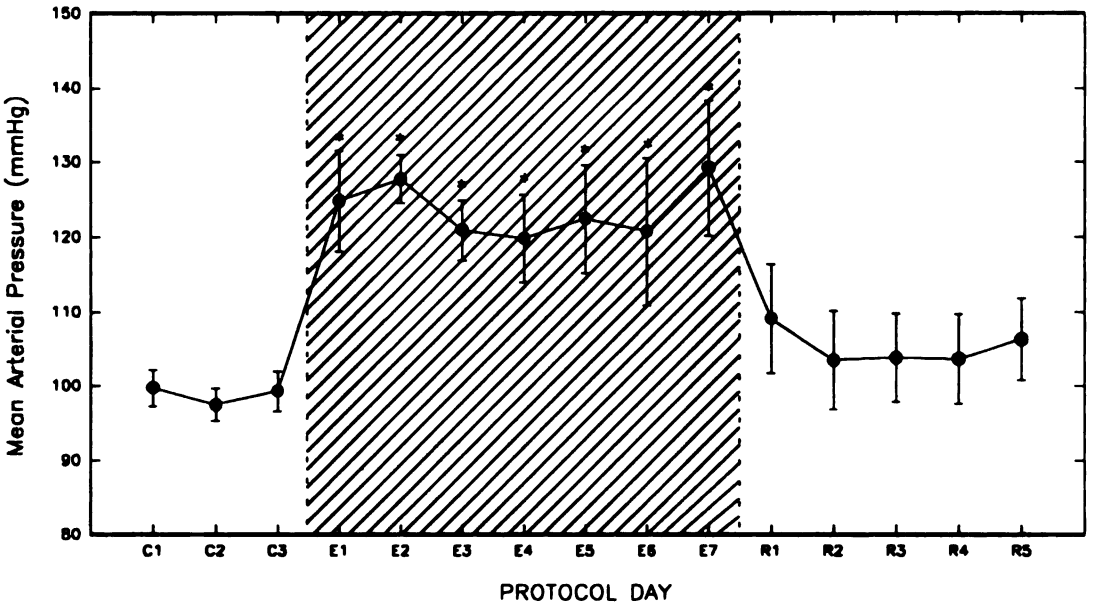

Figure 25. Mean arterial pressure responses to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. (n=7). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

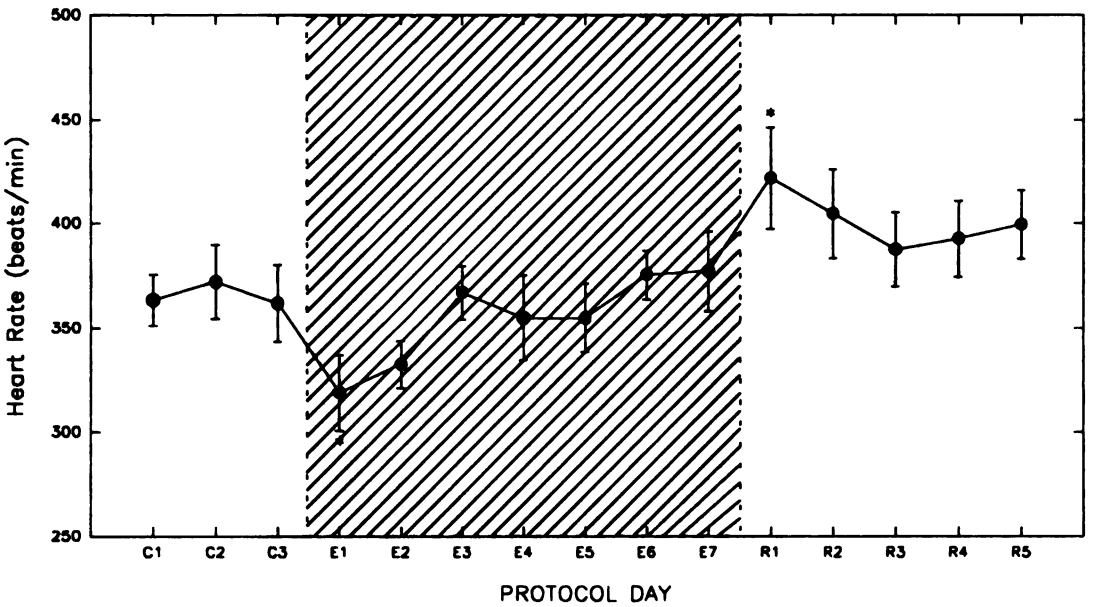

Figure 26. Heart rate responses to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. (n=7). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

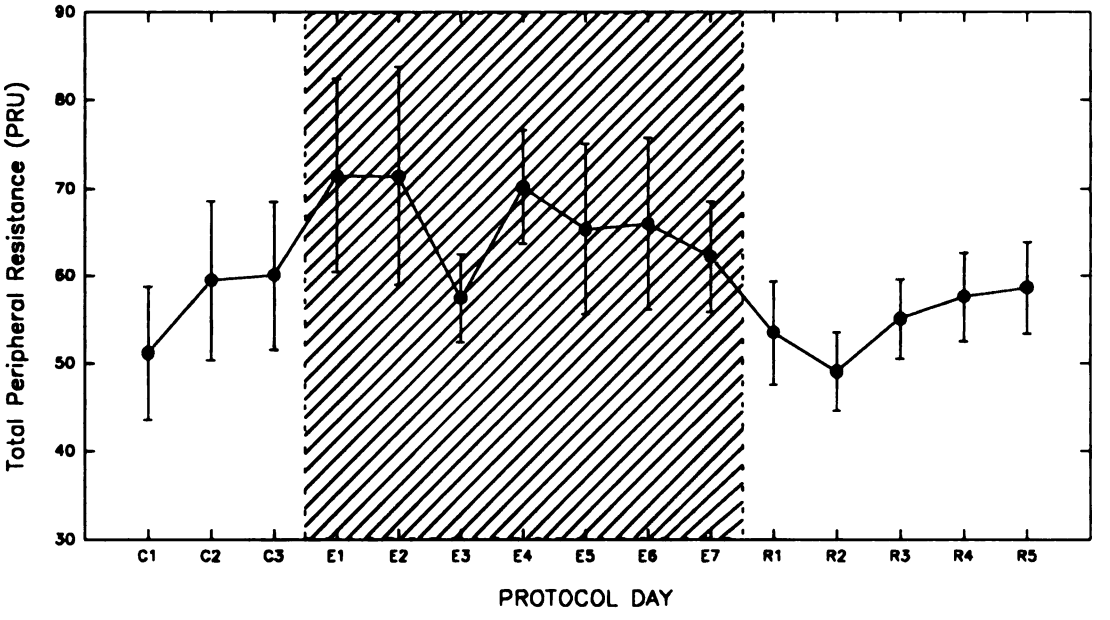

Figure 27. Changes in total peripheral resistance in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. (n=7). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

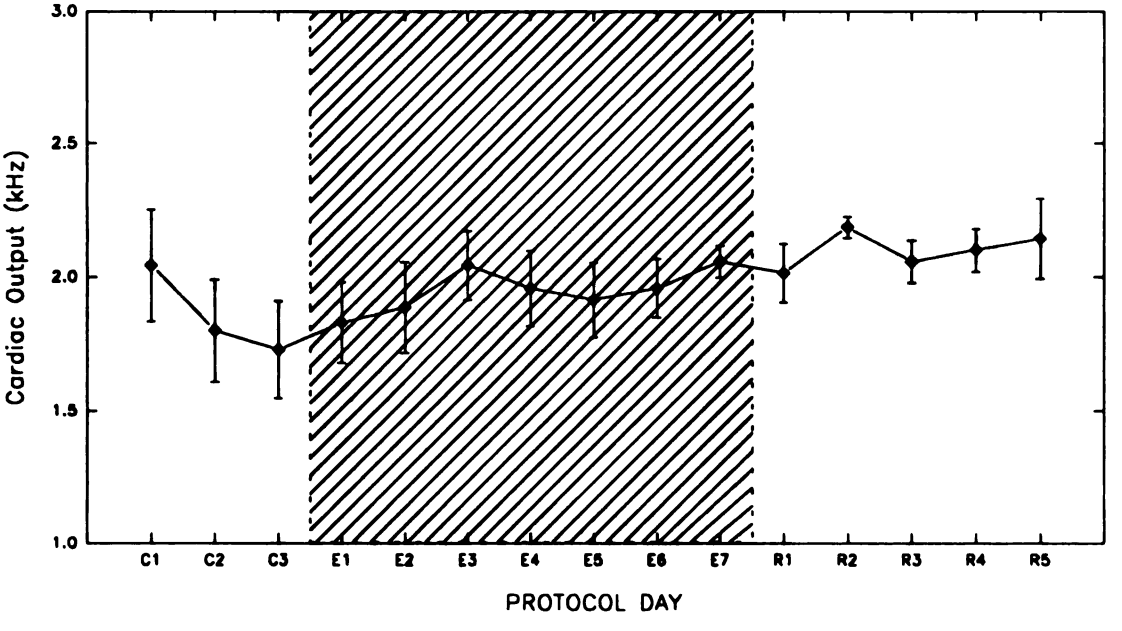

Figure 28. Changes in cardiac output in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. (n=7). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

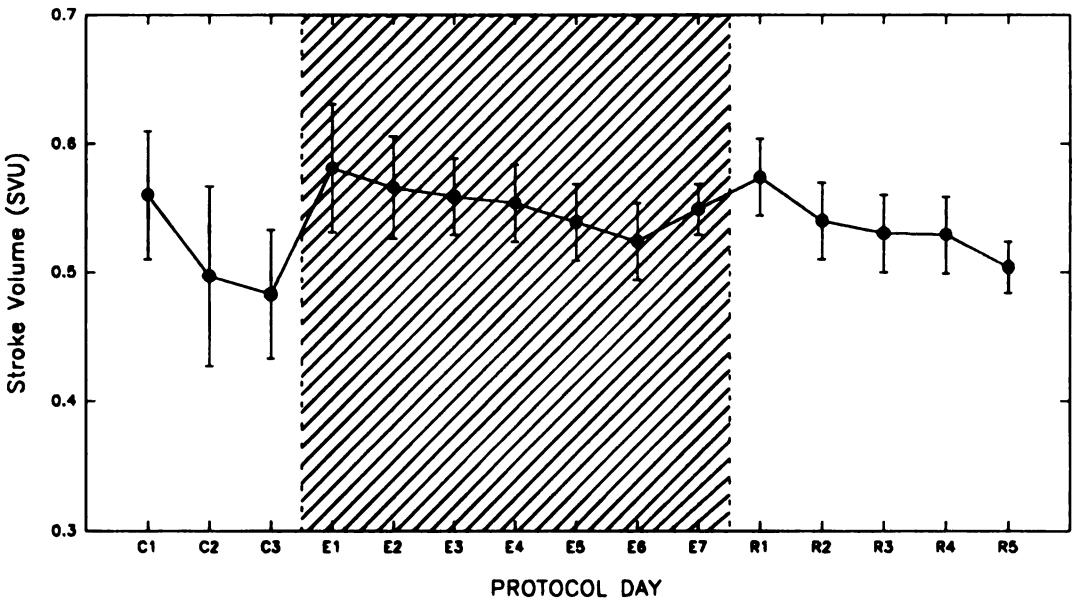

Figure 29. Changes in stroke volume in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. (n=7). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

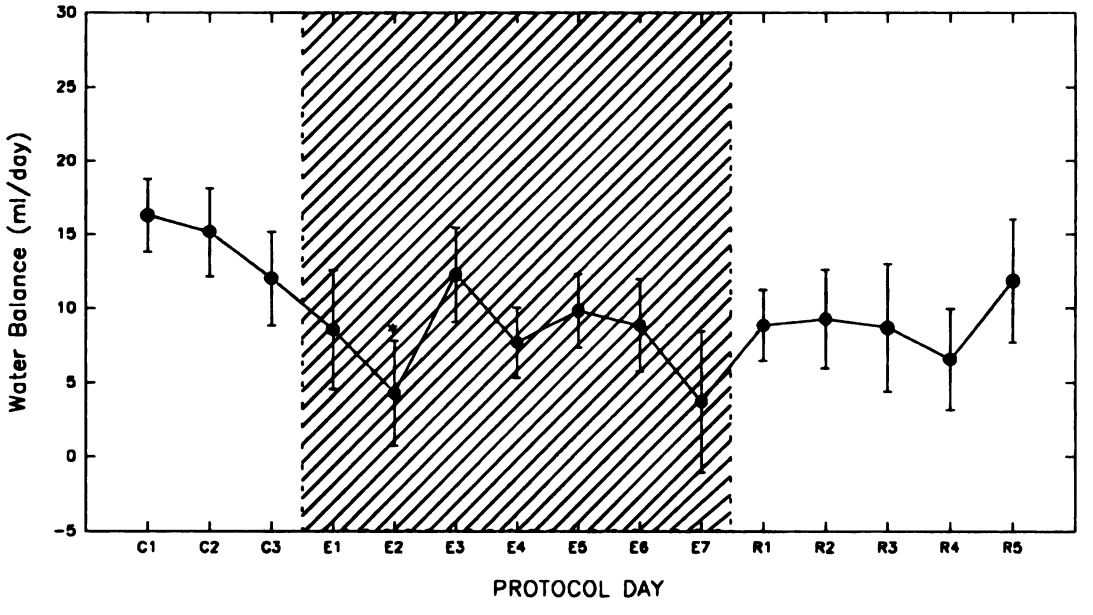

Figure 30. Changes in water balance in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. (n=7). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

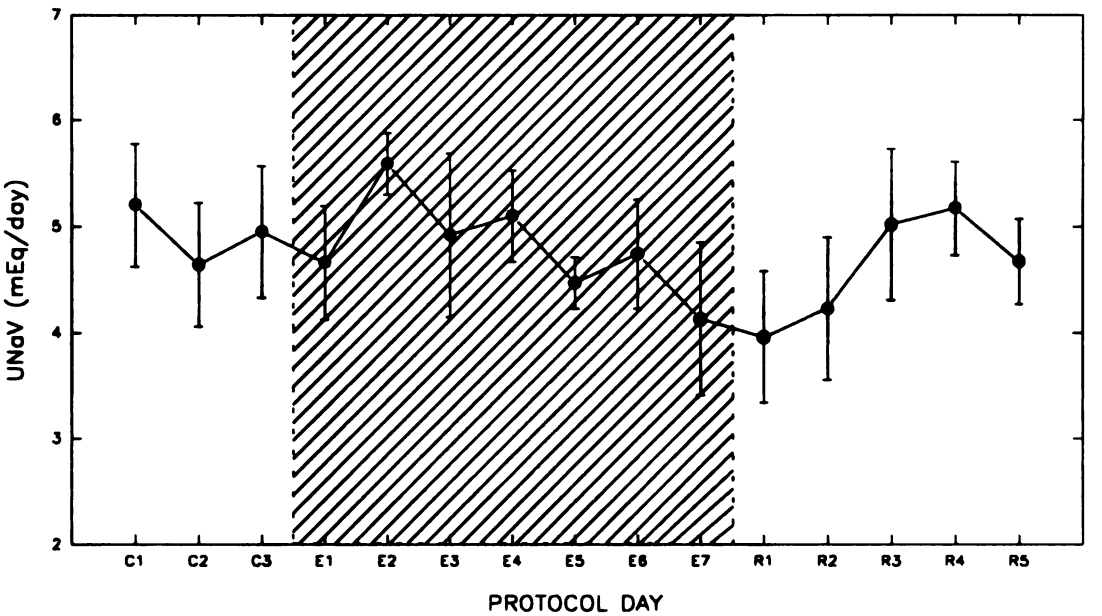

Figure 31. Changes in urinary sodium excretion in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. (n=7). Asterisks indicate significant difference ($P<0.05$) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

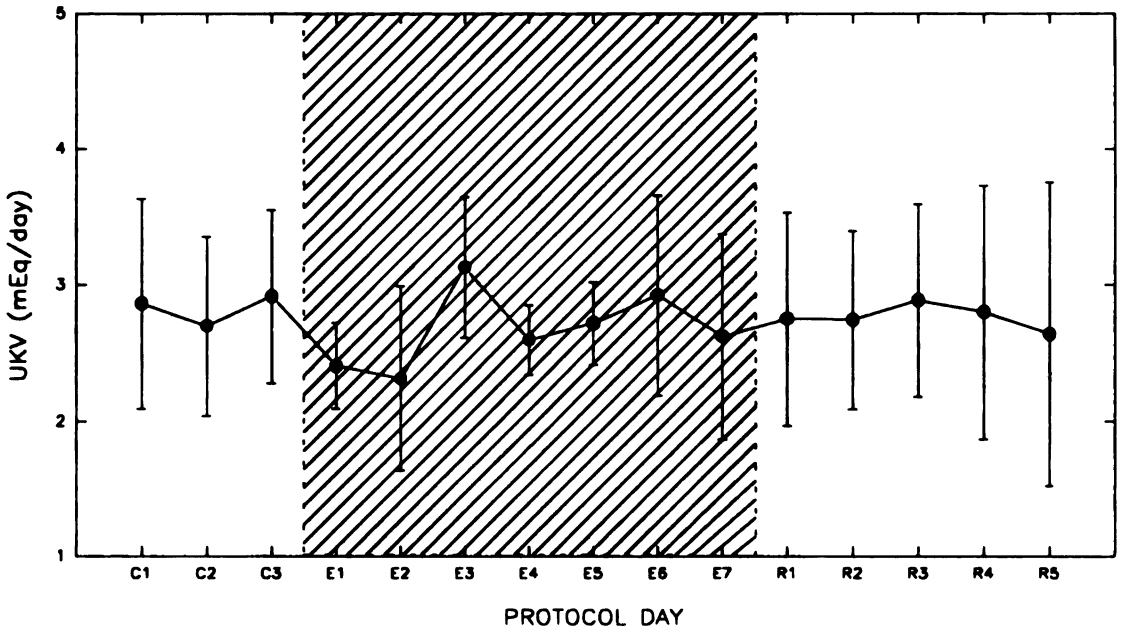

***Figure* 32.** Changes in urinary potassium excretion in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. (n=7). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

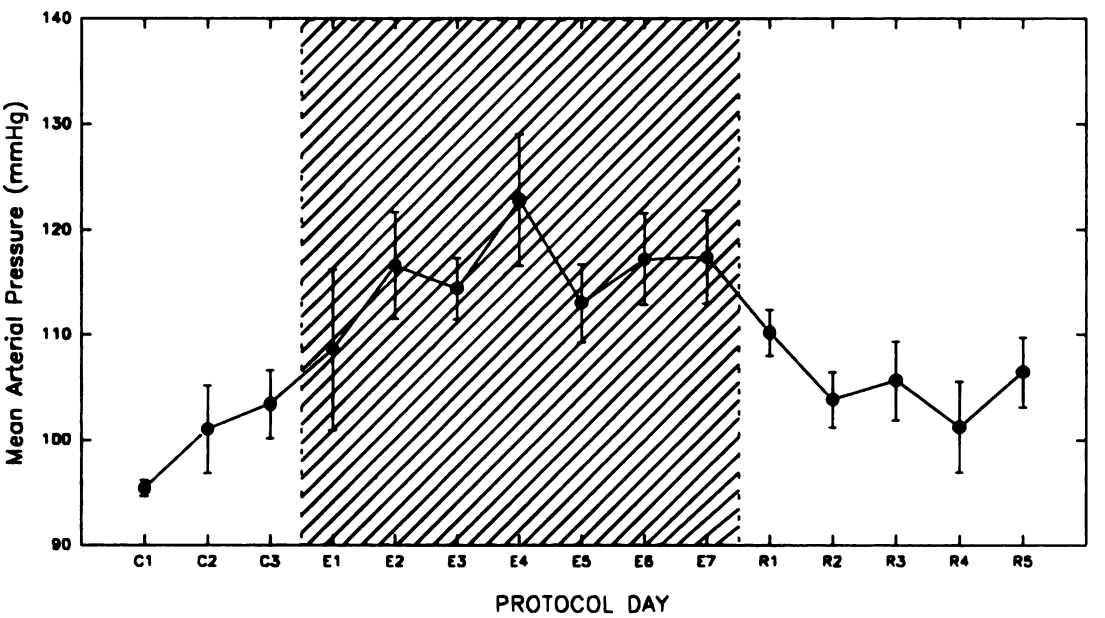

Figure 33. Mean arterial pressure responses to 7-day chronic infusion of ET-3 at a dose rate of 7.5 pmol·kg^{-1}·min^{-1}, i.v. (n=5). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

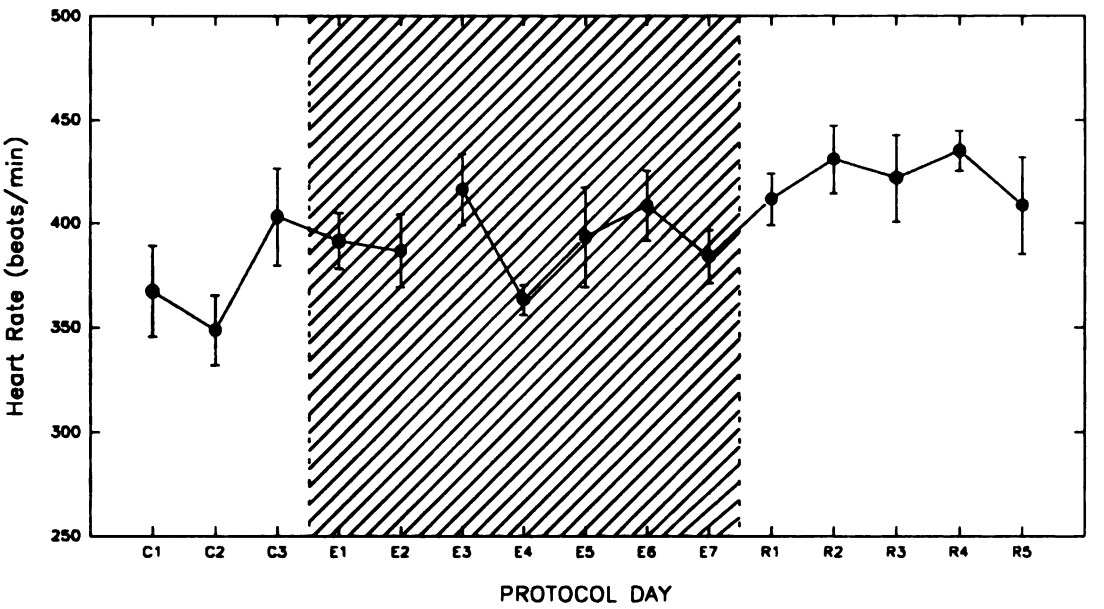

Figure 34. Heart rate responses to 7-day chronic infusion of ET-3 at a dose rate of 7.5 pmol·kg^{-1}·min^{-1}, i.v. (n=5). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

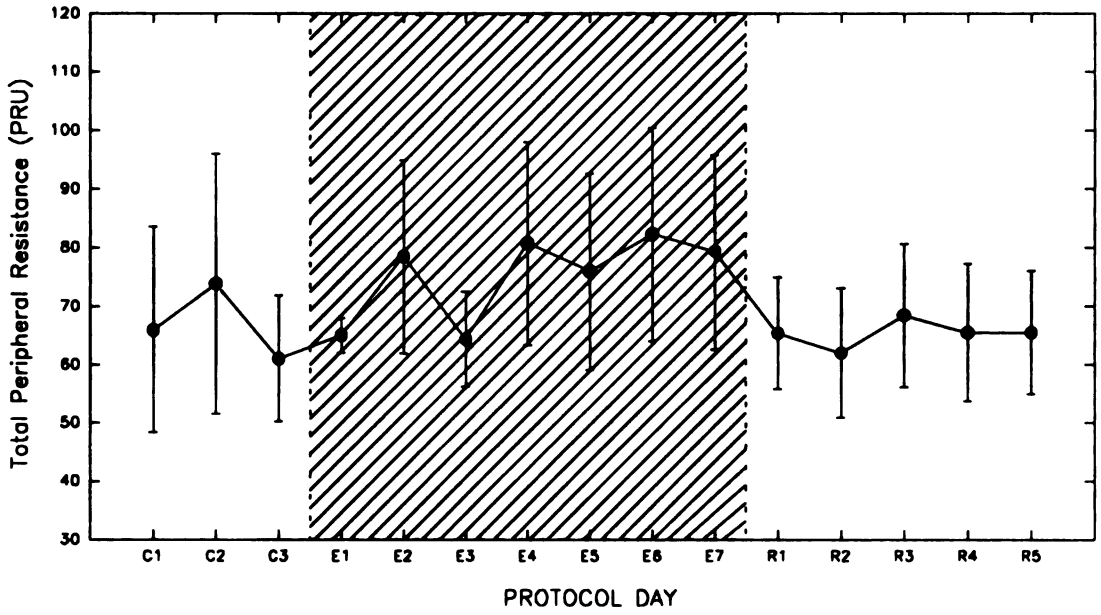

Figure 35. Changes in total peripheral resistance in response to 7-day chronic infusion of ET-3 at a dose rate of 7.5 pmol·kg^{-1}·min^{-1}, i.v. (n=5). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

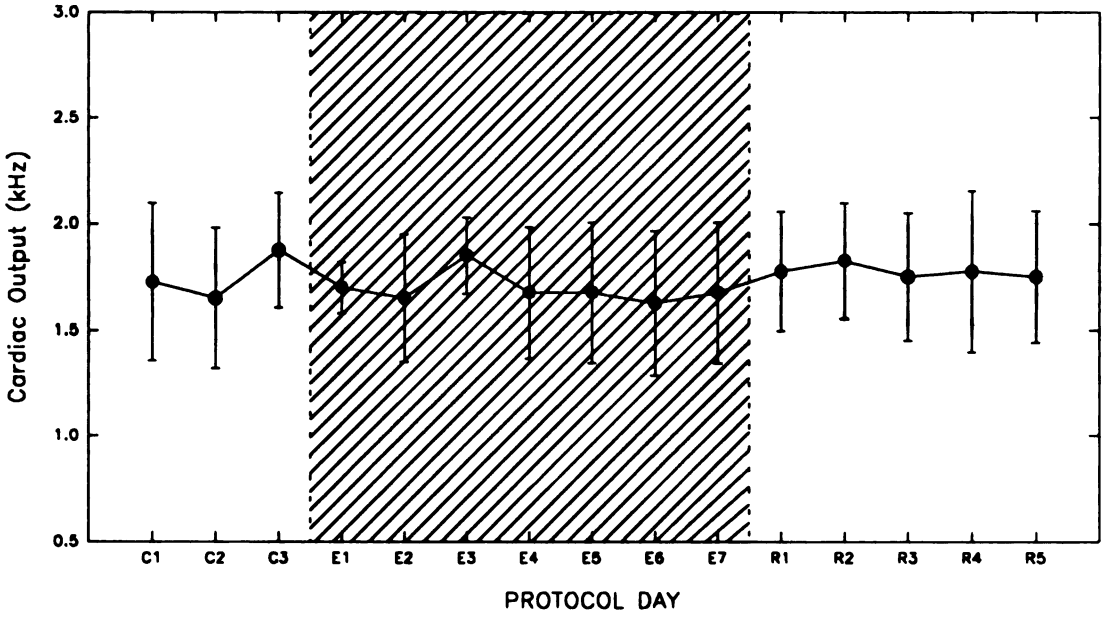

Figure 36. Changes in cardiac output in response to 7-day chronic infusion of ET-3 at a dose rate of 7.5 pmol·kg^{-1}·min^{-1}, i.v. (n=5). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

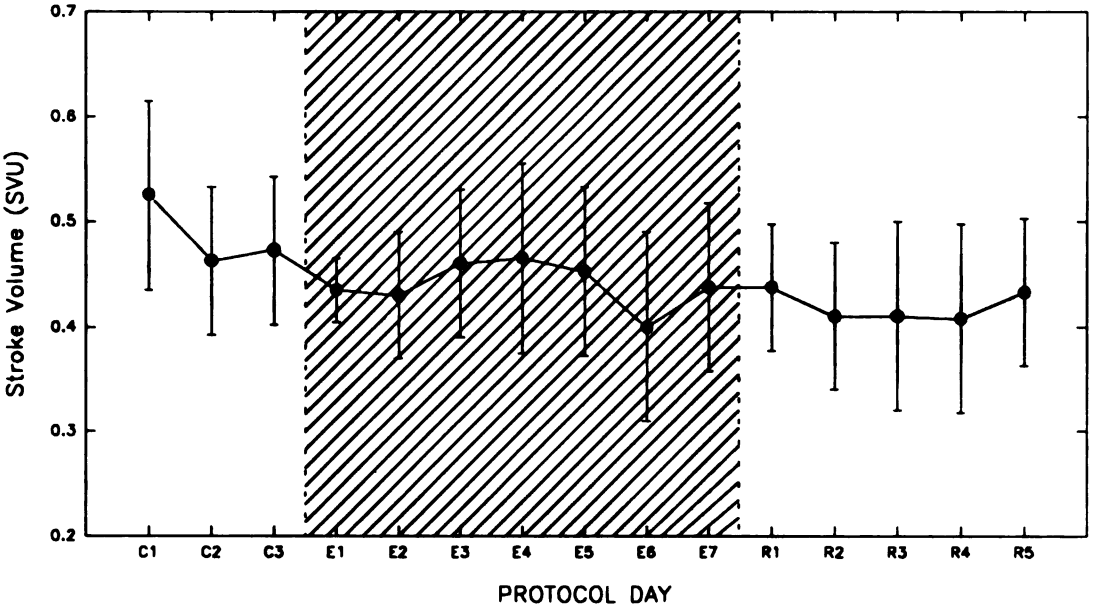

Figure 37. Changes in stroke volume in response to 7-day chronic infusion of ET-3 at a dose rate of 7.5 pmol·kg^{-1}·min^{-1}, i.v. (n=5). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

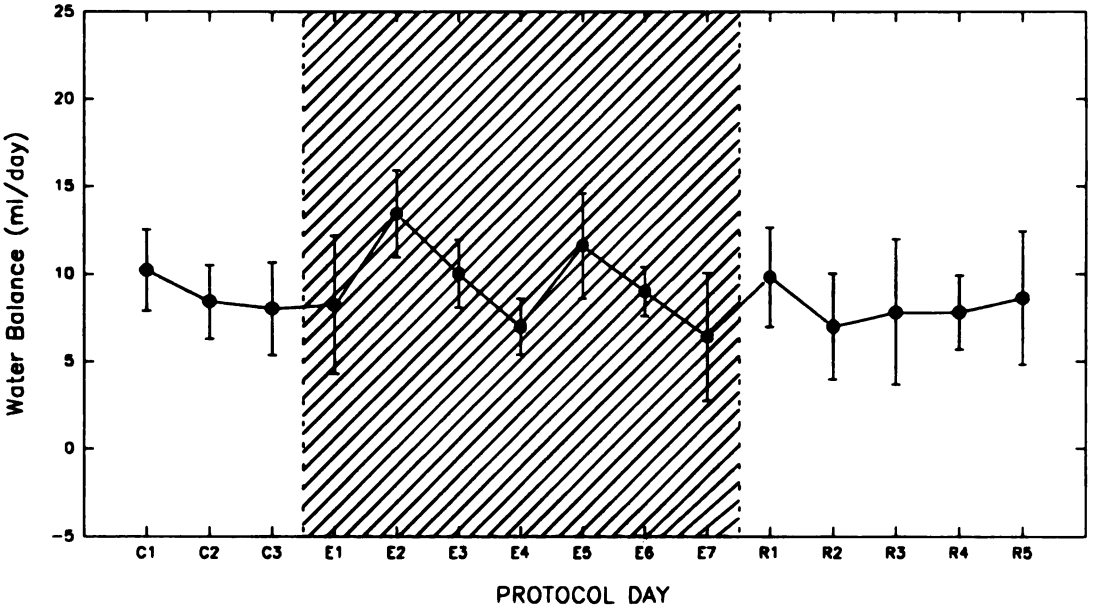

Figure 38. Changes in water balance in response to 7-day chronic infusion of ET-3 at a dose rate of 7.5 pmol·kg^{-1}·min^{-1}, i.v. (n=5). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

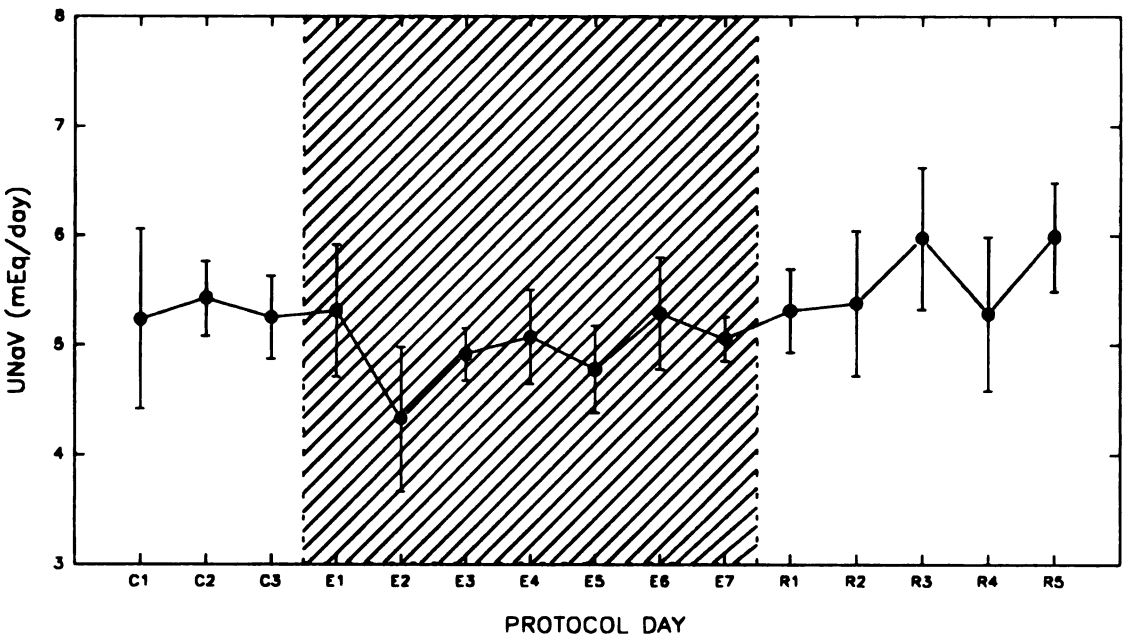

Figure 39. Changes in urinary sodium excretion in response to 7-day chronic infusion of ET-3 at a dose rate of 7.5 pmol·kg^{-1}·min^{-1}, i.v. (n=5). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

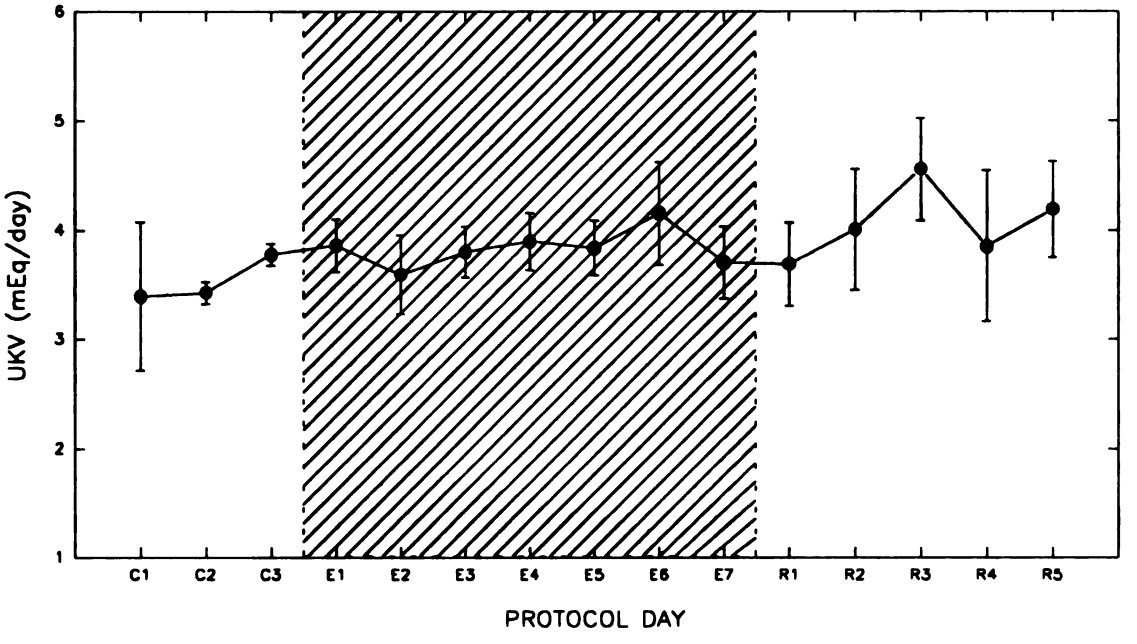

Figure 40. Changes in urinary potassium excretion in response to 7-day chronic infusion of ET-3 at a dose rate of 7.5 pmol·kg^{-1}·min^{-1}, i.v. (n=5). Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

b. Salt-dependency of Endothelin-Hypertension Cardiovascular data from these experiments are summarized in Figures 41-48. Continuous, seven-day, i.v. infusion of ET-1 at a rate of 5.0 $pmol \cdot kg^{-1} \cdot min^{-1}$ produced significant, sustained and reversible increases (~25.1%) in MAP (Figure 41) in high salt infused (6.0 $mEq \cdot day^{-1}$, HS) rats (n=12). Maximal increases in MAP were attained within 24 hrs after initiation of ET-1 infusion; tachyphylaxis was not observed throughout the ET-1 infusion period. In agreement with previous findings, the increase in MAP was primarily the result of an increased TPR (~17.8%, Figure 43) during ET-1 infusion, since SV (Figure 45) and CO (Figure 44) were not significantly affected. The increased MAP in HS rats receiving ET-1 was also associated with transient bradycardia; likewise, the fall in MAP on termination of the ET-1 infusion produced transient tachycardia (Figure 42). Infusion of ET-1 in HS rats produced no significant changes in WB (Figure 46), $U_{Na}V$ (Figure 47) or U_KV (Figure 48). Plasma electrolytes (control values: P_{Na}, 143.6±4.7; P_K, 4.12±0.14) also were not affected by ET-1 infusion (data not shown). In contrast, infusion of ET-1 at 5.0 $pmol \cdot kg^{-1} \cdot min^{-1}$ into normal salt infused (2.0 $mEq \cdot day^{-1}$, NS) rats (n=7) produced no significant changes in any cardiovascular or urinary variable (Figures 41-48). Both NS (n=5) and HS (n=7) rats not receiving ET-1 (controls) exhibited stable values for all measured variables throughout the fifteen day protocol. No significant differences between the control NS and HS groups were found for any variable except urinary sodium excretion (a reflection of sodium intake).

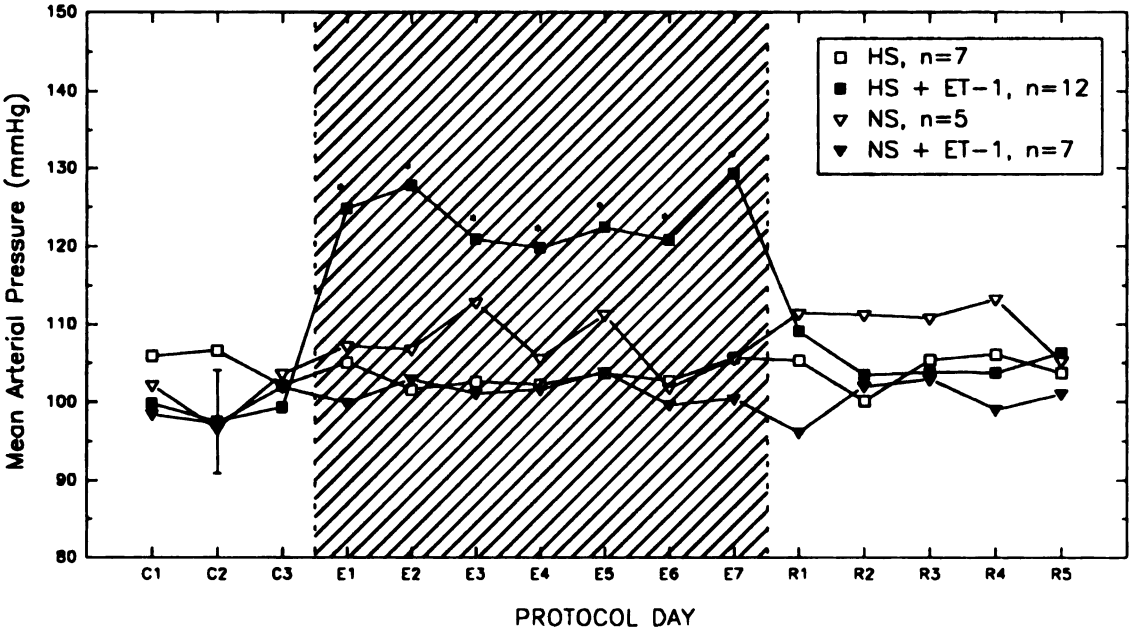

Figure 41. Mean arterial pressure responses to 7-day chronic infusion of ET-1 at a dose rate of 0.0 or 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats on either a high sodium (HS) or normal sodium (NS) intake. Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

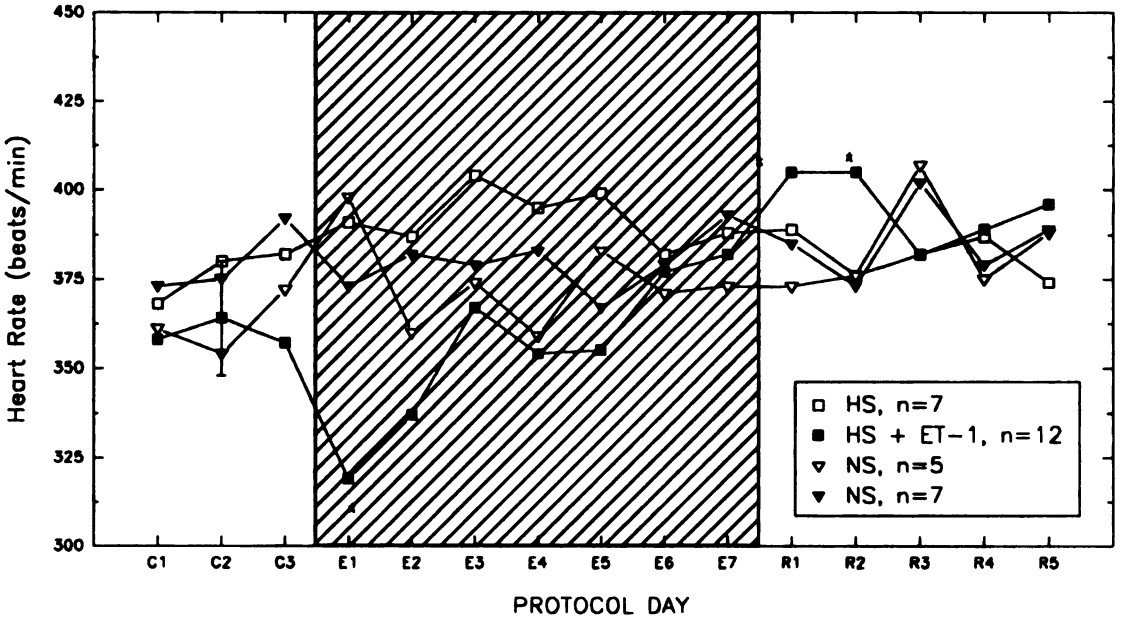

Figure 42. Heart rate responses to 7-day chronic infusion of ET-1 at a dose rate of 0.0 or 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats on either a high sodium (HS) or normal sodium (NS) intake. Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

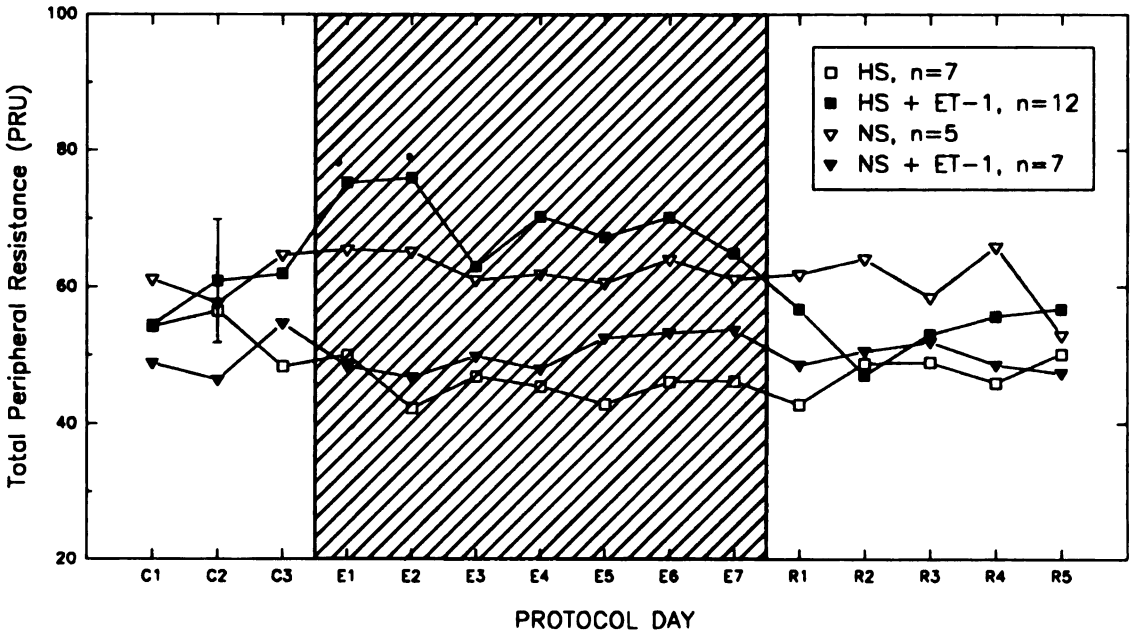

Figure 43. Changes in total peripheral resistance in response to 7-day chronic infusion of ET-1 at a dose rate of 0.0 or 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats on either a high sodium (HS) or normal sodium (NS) intake. Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

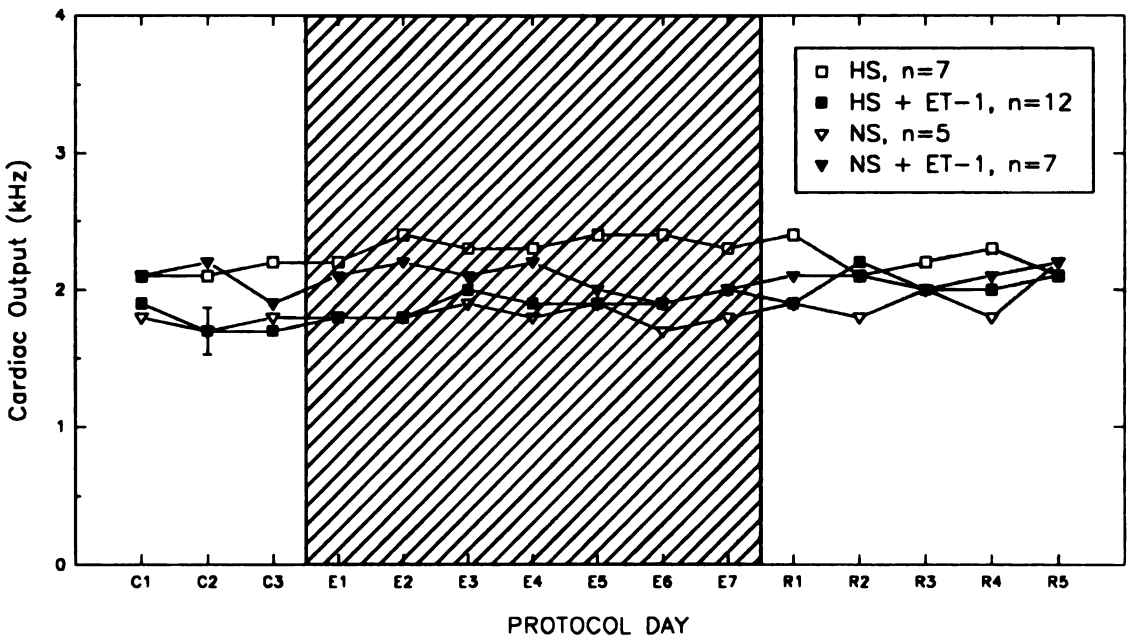

Figure 44. Changes in cardiac output in response to 7-day chronic infusion of ET-1 at a dose rate of 0.0 or 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats on either a high sodium (HS) or normal sodium (NS) intake. Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

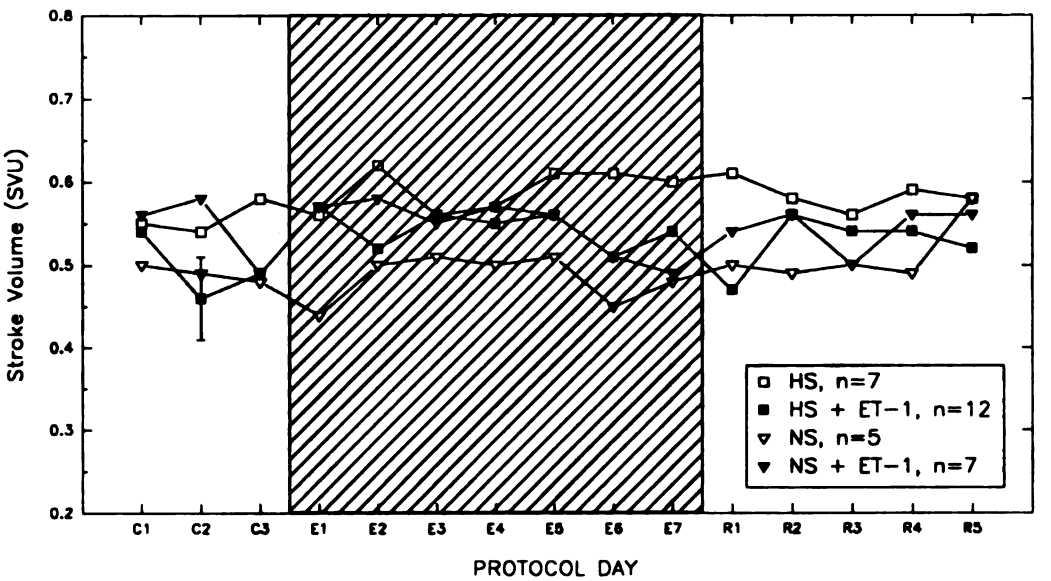

Figure 45. Changes in stroke volume in response to 7-day chronic infusion of ET-1 at a dose rate of 0.0 or 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats on either a high sodium (HS) or normal sodium (NS) intake. Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

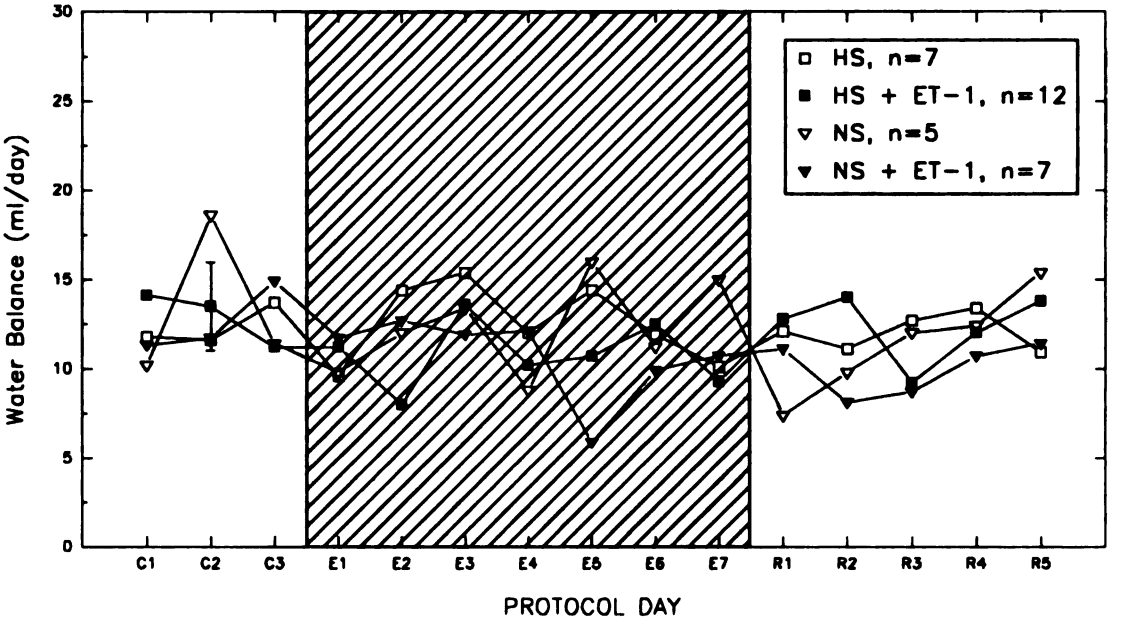

Figure 46. Changes in water balance in response to 7-day chronic infusion of ET-1 at a dose rate of 0.0 or 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats on either a high sodium (HS) or normal sodium (NS) intake. Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

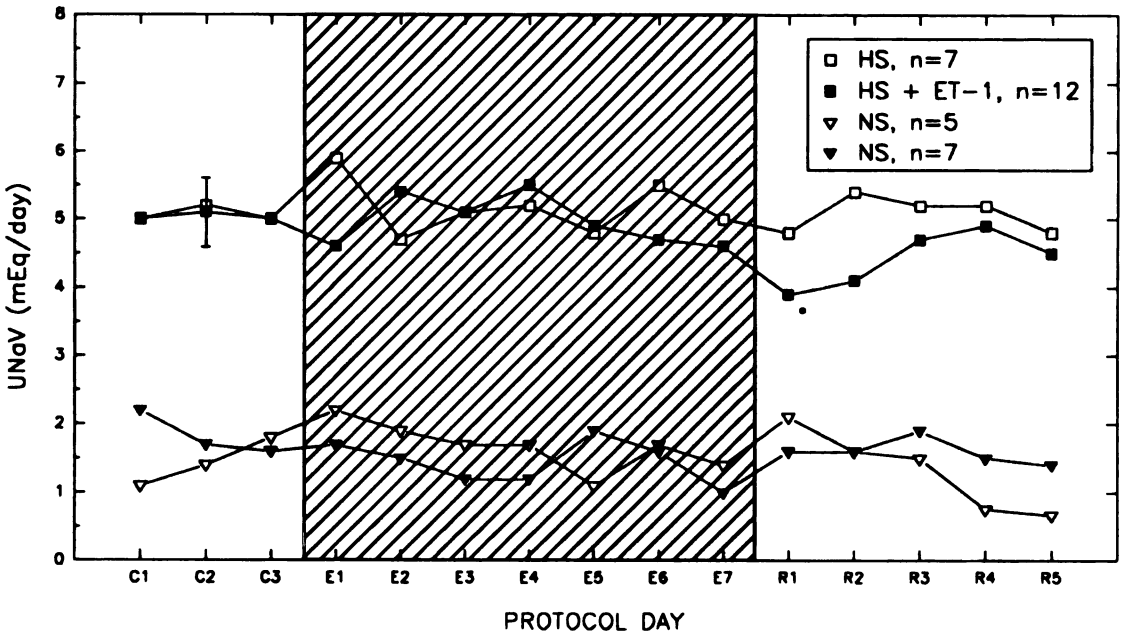

Figure 47. Changes in urinary sodium excretion in response to 7-day chronic infusion of ET-1 at a dose rate of 0.0 or 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats on either a high sodium (HS) or normal sodium (NS) intake. Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

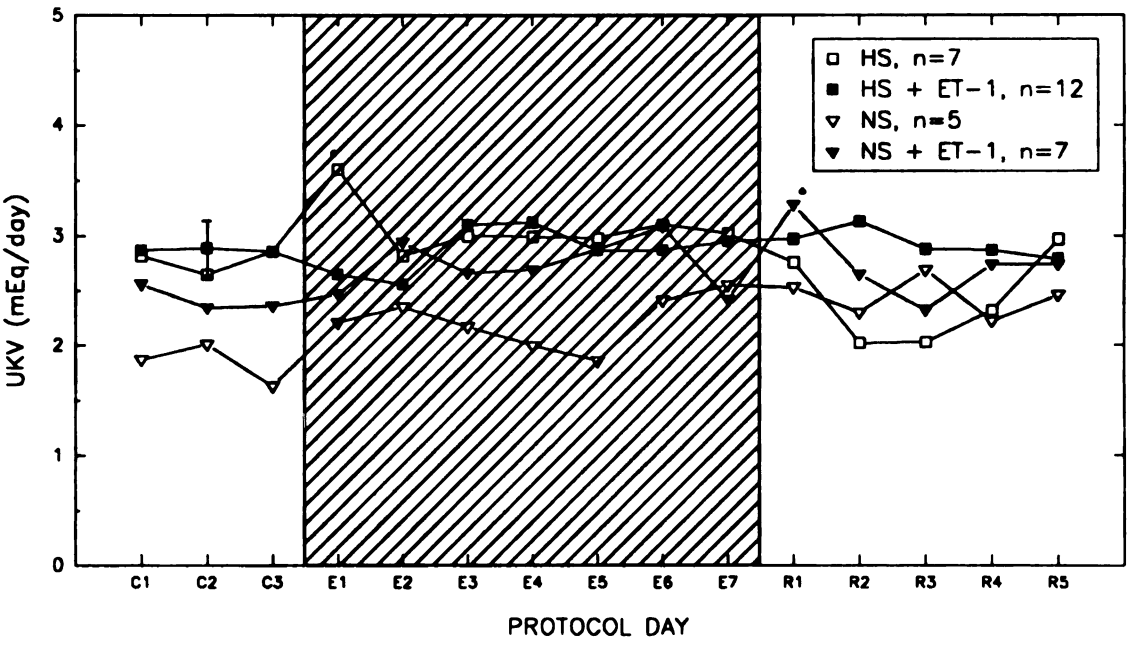

Figure 48. Changes in urinary potassium excretion in response to 7-day chronic infusion of ET-1 at a dose rate of 0.0 or 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats on either a high sodium (HS) or normal sodium (NS) intake. Asterisks indicate significant difference (P<0.05) from control day measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

3. Endothelin-Hypertension: Contributions of the Sympathetic Nervous System

a. Baroreceptor reflex afferents: Infusion of ET-1 into baroreceptor-intact rats, at a rate of 5.0 $pmol \cdot kg^{-1} \cdot min^{-1}$, produced a sustained increase in MAP (Figure 49), averaging +17 mmHg, without affecting blood pressure lability (Figure 50). In SAD rats, ET-1 produced a much larger ($P<0.05$) sustained increase in MAP (Figure 49), averaging +57 mmHg, without further increasing lability (Figure 50). Daily HR (Figure 51), WB (Figure 52), $U_{Na}V$ (Figure 53) and U_KV (Figure 54) measurements were not consistently different between the two groups at any time during the protocol. Plasma ET-1 concentrations, measured by radioimmunoassay (RIA), were significantly different between the intact and SAD rats only during the initial control period (C3, Figure 55). Compared to their own controls, measurements obtained on day C3, infusion of ET-1 at 5.0 $pmol \cdot kg^{-1} \cdot min^{-1}$ produced significant ($P<0.05$) increases in circulating plasma levels of ET-1 only in SAD rats (days E3 and E7); these values were not significantly different from control values obtained for intact rats.

The commercially available radioimmunoassay was consistently unreliable in terms of inter- and intra-assay variability and standard curve reproducibility. Additionally, the methodology of extraction was unsuited for the measurement of ET-1 in plasma due to consistent loss of measurable protein. These problems may help to explain the differences in $[ET\text{-}1_P]$ between the rat groups during the control period.

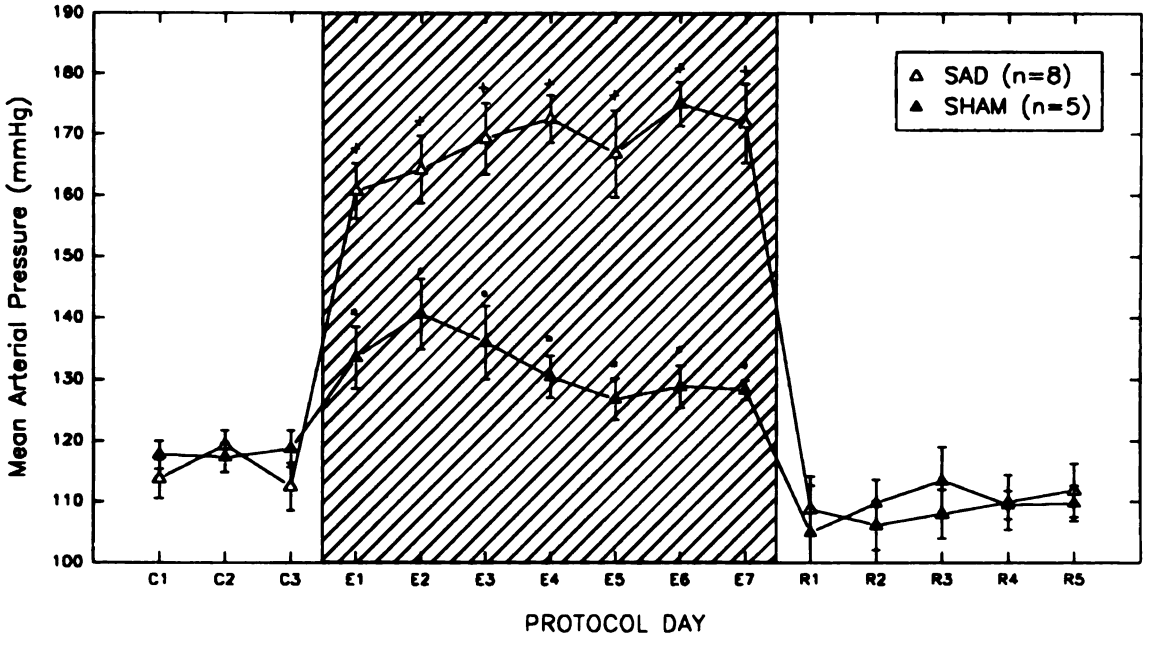

Figure 49. Mean arterial pressure responses to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in normal (SHAM, n=5) or sino-aortic denervated (SAD, n=8) rats. Asterisks indicate significant difference (P<0.05) from control day measurements. Crosses indicate significant difference from daily paired group measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

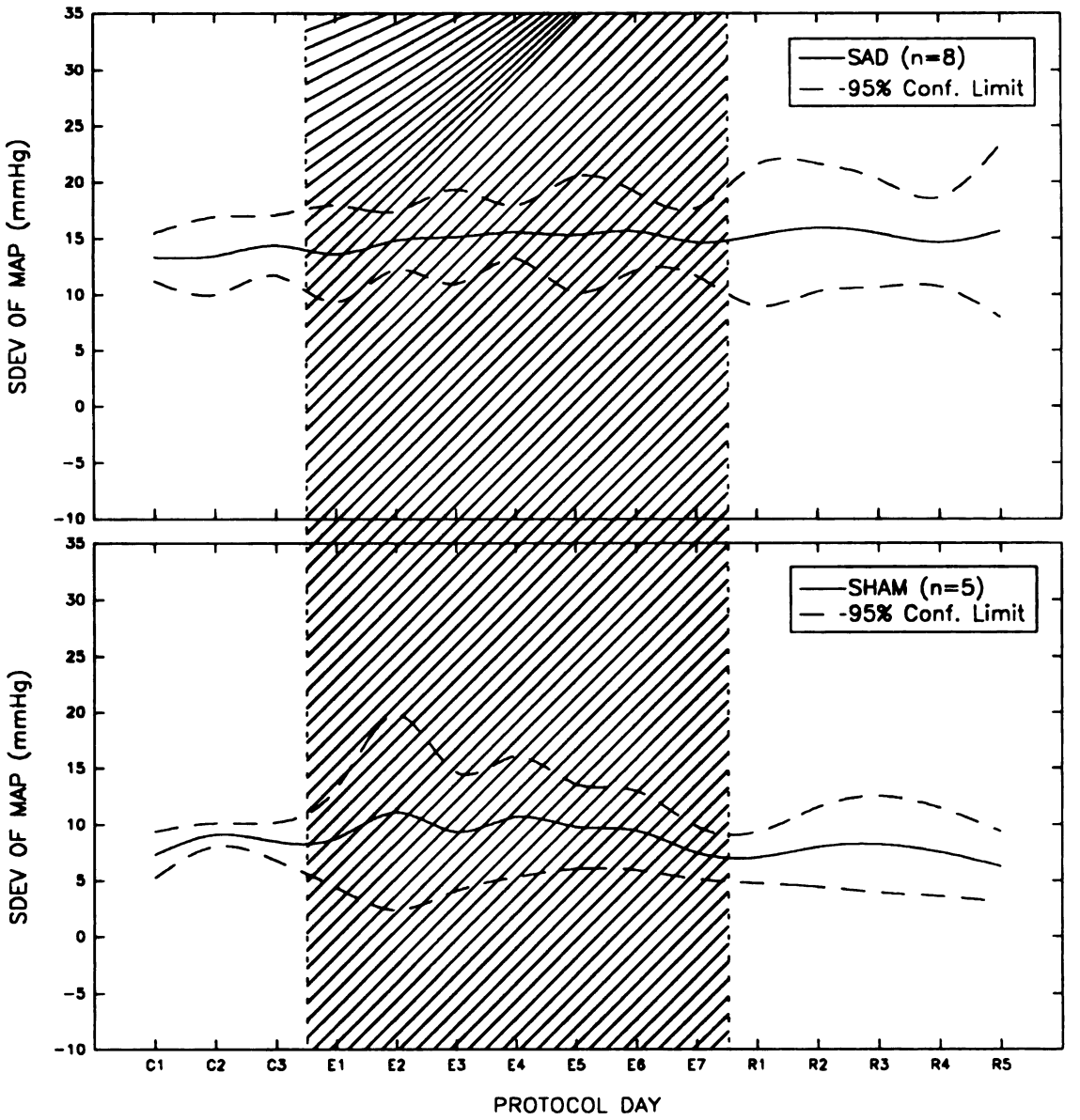

Figure 50. Blood pressure lability was determined daily by computer-aided sampling and averaging to obtain a standard deviation of mean arterial pressure (SDEV). SAD rats (SDEV≈14mmHg, top panel) demonstrated a consistently higher SDEV of MAP compared to SHAM rats (SDEV≈7mmHg, bottom panel) throughout the fifteen day protocol. Standard deviations are displayed with upper and lower 95% confidence limits. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

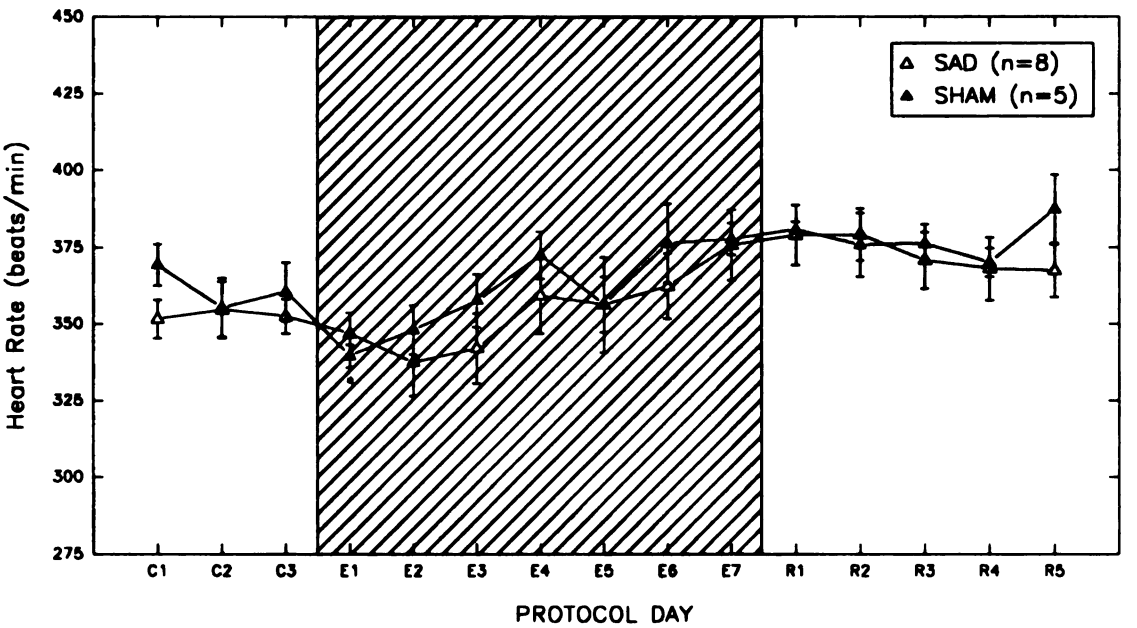

Figure 51. Heart rate responses to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in normal (SHAM, n=5) or sino-aortic denervated (SAD, n=8) rats. Asterisks indicate significant difference ($P<0.05$) from control day measurements. Crosses indicate significant difference from daily paired group measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

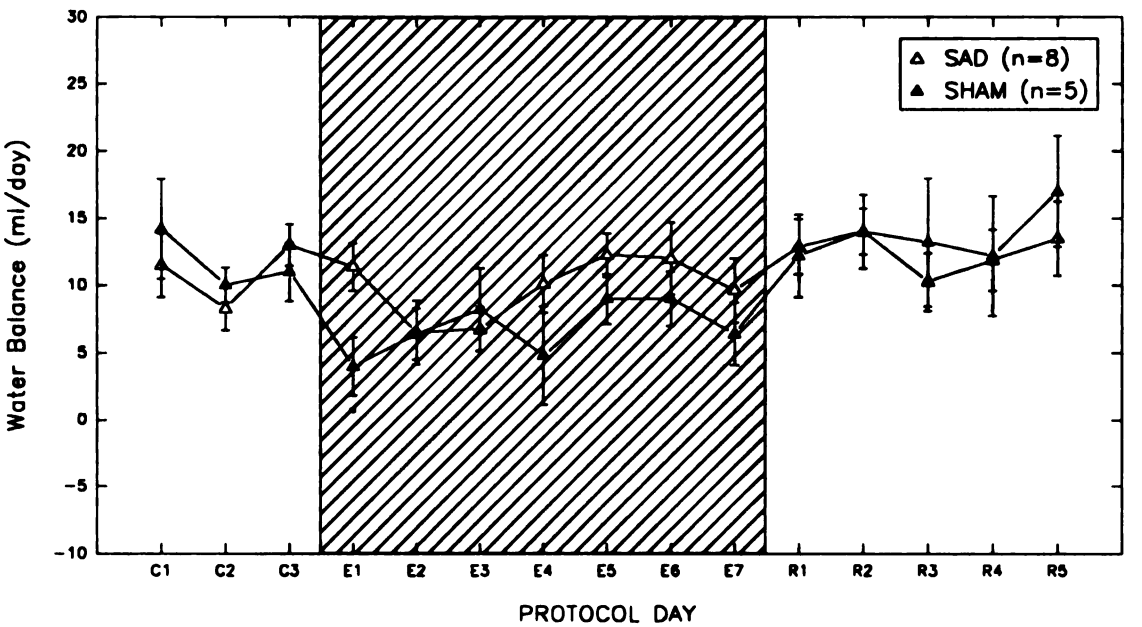

Figure 52. Changes in water balance in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in normal (SHAM, n=5) or sino-aortic denervated (SAD, n=8) rats. Asterisks indicate significant difference ($P<0.05$) from control day measurements. Crosses indicate significant difference from daily paired group measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

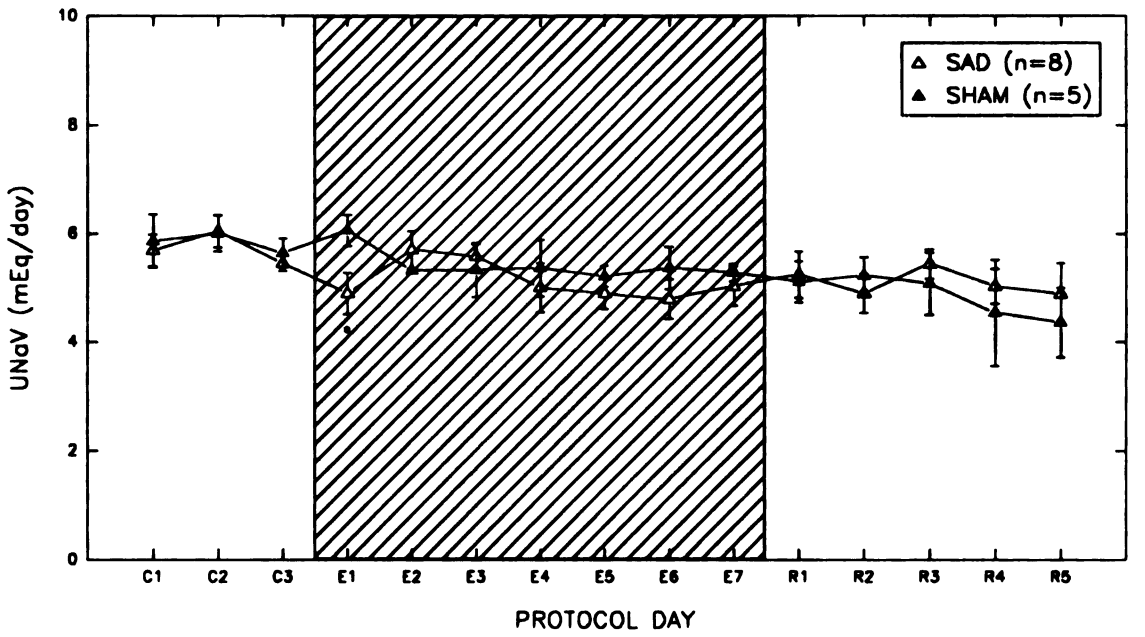

Figure 53. Changes in urinary sodium excretion in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in normal (SHAM, n=5) or sino-aortic denervated (SAD, n=8) rats. Asterisks indicate significant difference ($P<0.05$) from control day measurements. Crosses indicate significant difference from daily paired group measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

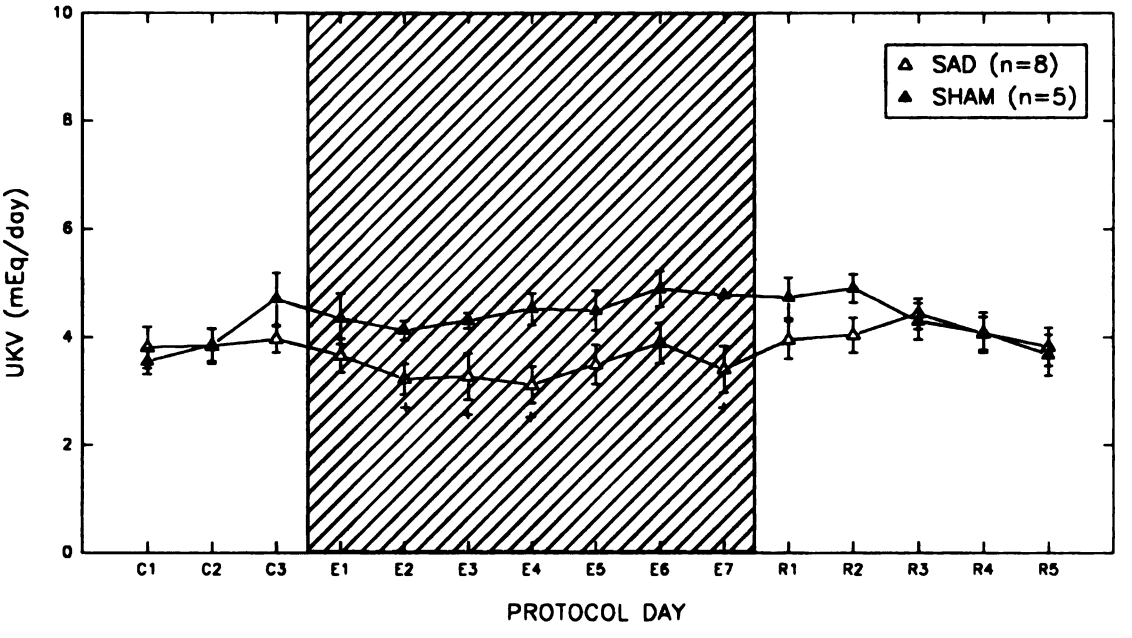

Figure 54. Changes in urinary potassium excretion in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in normal (SHAM, n=5) or sino-aortic denervated (SAD, n=8) rats. Asterisks indicate significant difference (P<0.05) from control day measurements. Crosses indicate significant difference from daily paired group measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

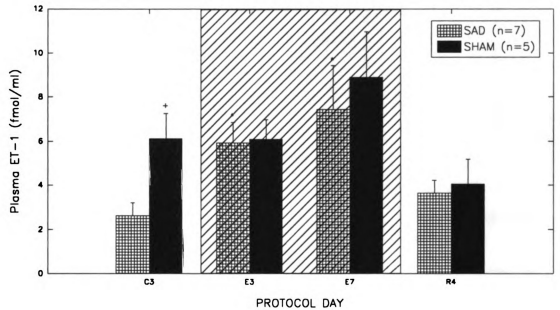

Figure 55. Changes in plasma ET-1 concentration, $[ET\text{-}1]_P$, in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 $pmol \cdot kg^{-1} \cdot min^{-1}$, i.v. in normal (SHAM, n=5) or sino-aortic denervated (SAD, n=7) rats. $[ET\text{-}1]_P$ was measured once during the control period (C3), twice during the ET-1 infusion period (E3 and E7) and once during the recovery period (R4). $[ET\text{-}1]_P$ was significantly higher in SHAM rats during the control period (~3x) and was not significantly effected by ET-1 infusion. $[ET\text{-}1]_P$ in SAD rats, however, was significantly elevated (~3x) in response to ET-1 infusion and was reversible upon cessation of the infusion during the recovery period. (+) and (*) indicate significant difference from SAD control value.

b. Area Postrema ablation: Endothelin-hypertension was not altered by APX. As shown in Figure 56, during the ET-1 infusion period, mean arterial pressures in APX rats were not significantly different from those of SHAM rats. Additionally, Figures 57, 58, and 59 demonstrate that this absence of an altered MAP was observed despite significant but inconsistent changes in HR, water balance and potassium excretion, respectively. Sodium excretion (Figure 59) remained unchanged throughout the protocol in both groups and was not altered by ET-1 infusion or area or APX.

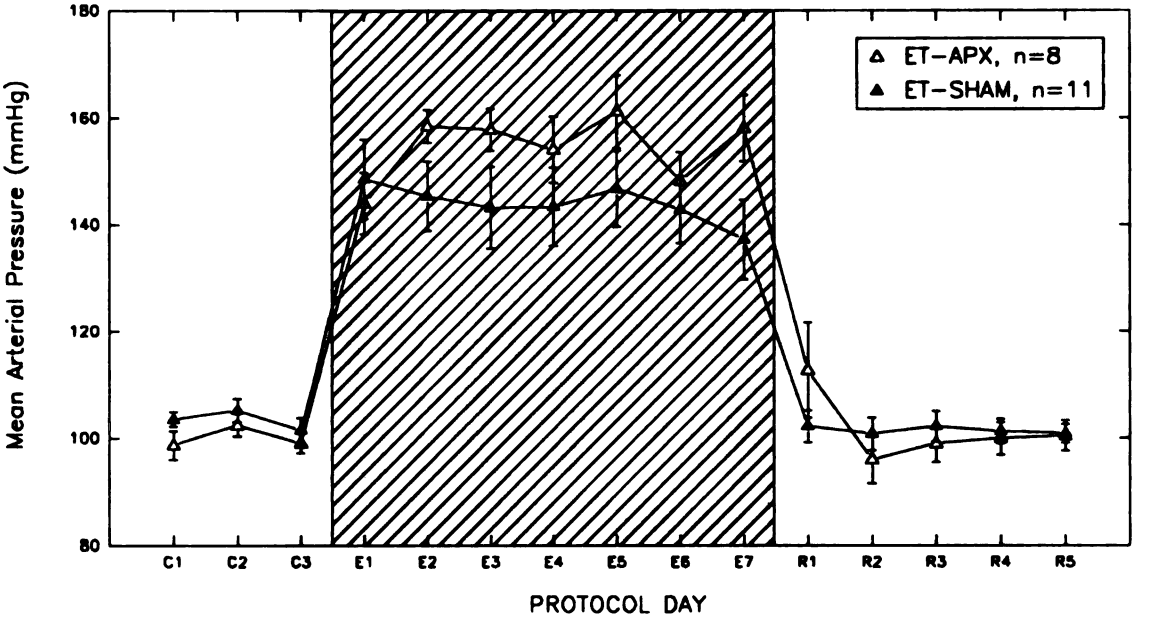

Figure 56. Mean arterial pressure responses to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in normal (ET-SHAM, n=11) or area postrema ablated (ET-APX, n=8) rats. Mean arterial pressure was significantly elevated, compared to control day measurements, in both groups of animals throughout the ET-1 infusion period. Asterisks indicate significant difference (P<0.05) from daily paired group measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

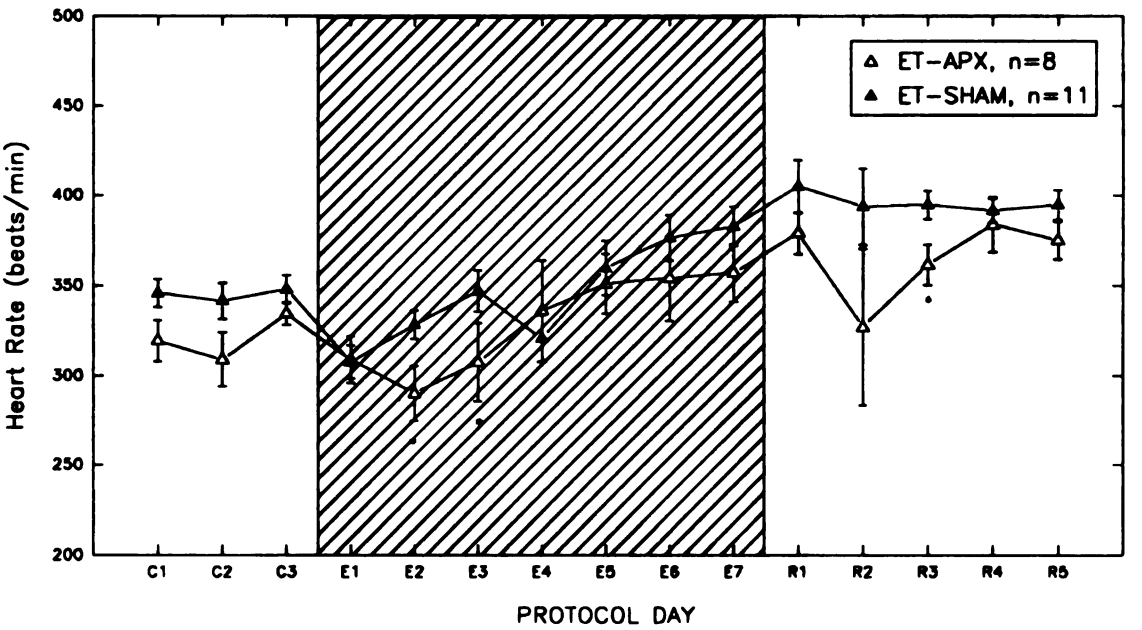

Figure 57. Heart rate responses to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in normal (ET-SHAM, n=11) or area postrema ablated (ET-APX, n=8) rats. Asterisks indicate significant difference (P<0.05) from daily paired group measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

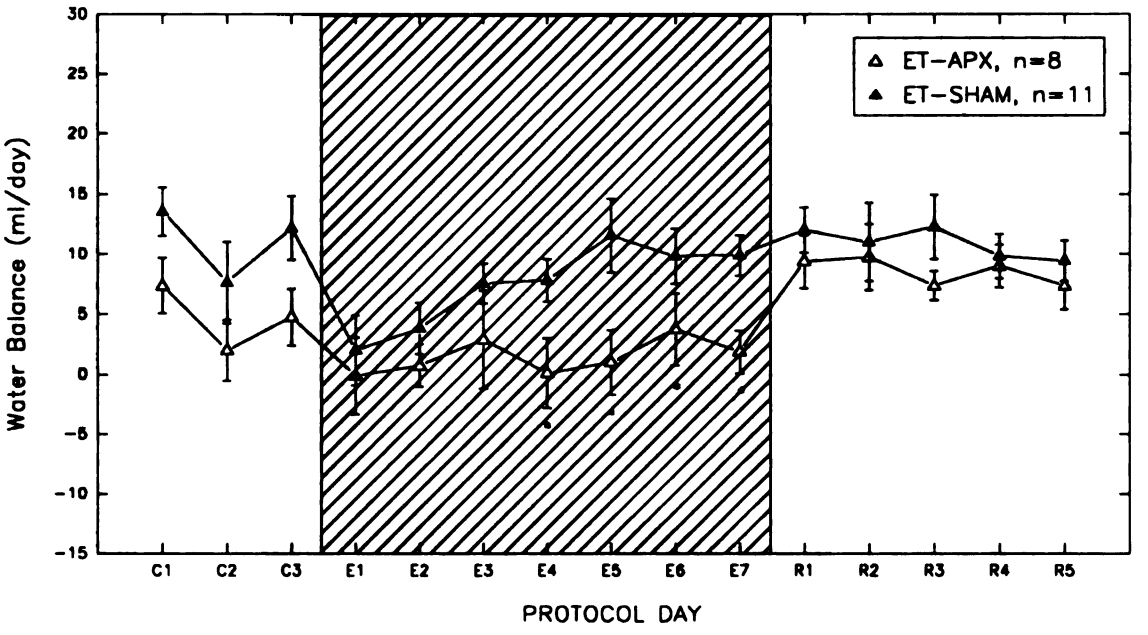

Figure 58. Changes in water balance in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in normal (ET-SHAM, n=11) or area postrema ablated (ET-APX, n=8) rats. Asterisks indicate significant difference (P<0.05) from daily paired group measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

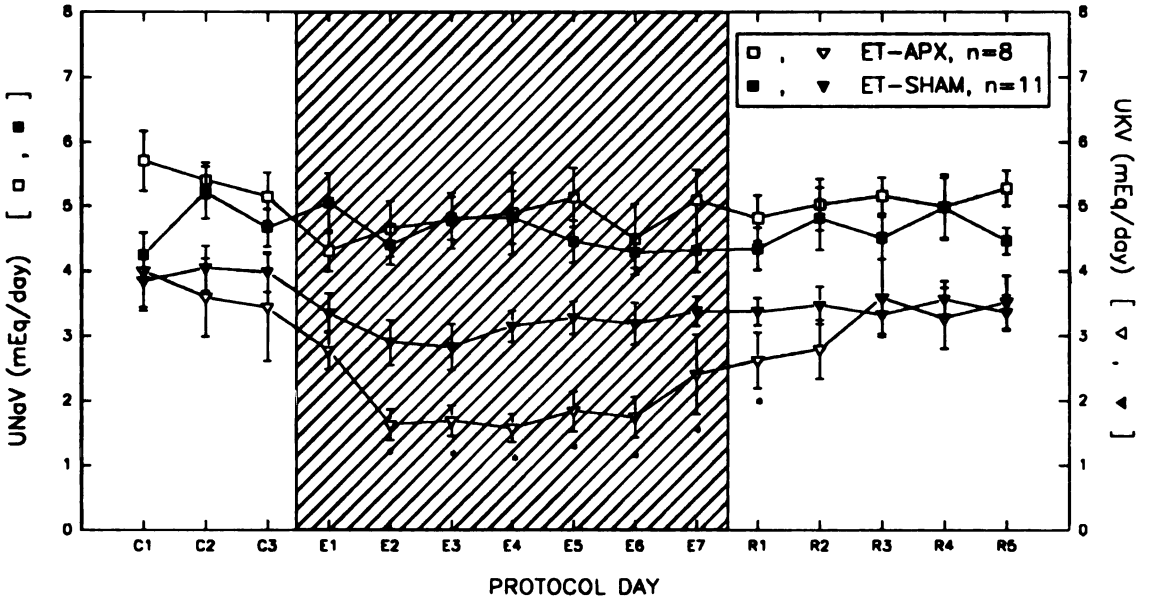

Figure 59. Changes in urinary sodium and potassium excretion in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in normal (ET-SHAM, n=11) or area postrema ablated (ET-APX, n=8) rats. Asterisks indicate significant difference (P<0.05) from daily paired group measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

4. Endothelin-Hypertension: Contributions of the Renin-Angiotensin System

a. Chronic infusion of angiotensin converting enzyme inhibitors: Figures 60-63 demonstrate that i.v. infusion of ET-1, alone (n=7), at 5.0 $pmol \cdot kg^{-1} \cdot min^{-1}$ produced a significant increase in MAP that was sustained throughout the ET-1 infusion period (days E_1-E_7) and was reversible upon cessation of ET-1 infusion (day R_1) (Figure 60). As shown earlier, the onset (day E_1) and regression (day R_1) of ET-1 hypertension were accompanied by brief and statistically insignificant reflex bradycardia and tachycardia (Figure 61), respectively. Sodium, potassium and water retention were not observed in this hypertensive model since water balance (Figure 62) and electrolyte excretions (Figure 63) were maintained at steady state throughout the protocol.

Addition of captopril to the chronic infusion regimen inhibited the development of endothelin-hypertension (Figure 60). This group of animals (n=8), however, did not exhibit the transient HR changes observed in the animals administered ET-1 alone (Figure 61). The captopril-treated animals also demonstrated no significant changes in fluid (Figure 62) or electrolyte balance (Figure 63) throughout the protocol. Additionally, use of the non-sulfhydryl, ACE inhibitor enalapril yielded a similar preventive action against the development and maintenance of endothelin-hypertension (Figure 60, n=6).

In another group of animals, infusion of ET-1 at 5.0 $pmol \cdot kg^{-1} \cdot min^{-1}$ similarly produced a significant and sustained rise in MAP but produced no statistically significant alterations in plasma AngII levels, $[AngII]_P$ (Figure 64).

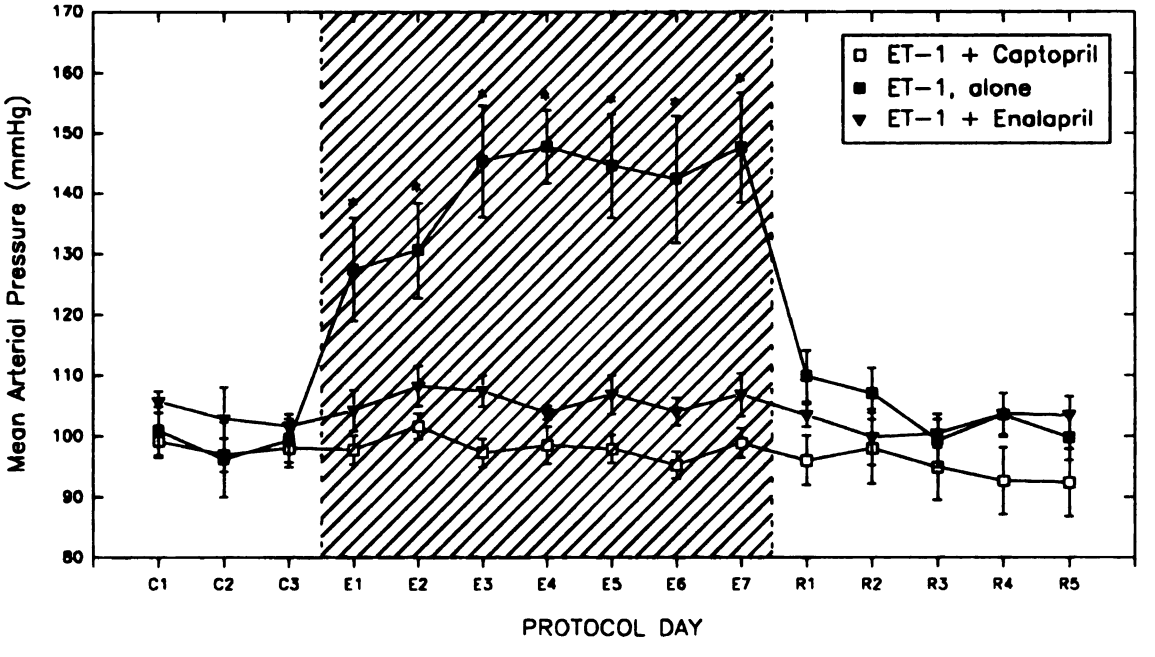

Figure 60. Mean arterial pressure responses to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats receiving ET-1 alone (n=7), ET-1 and captopril (n=8), or ET-1 and enalapril (n=6). Asterisks indicate significant difference (P<0.05) from control period measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

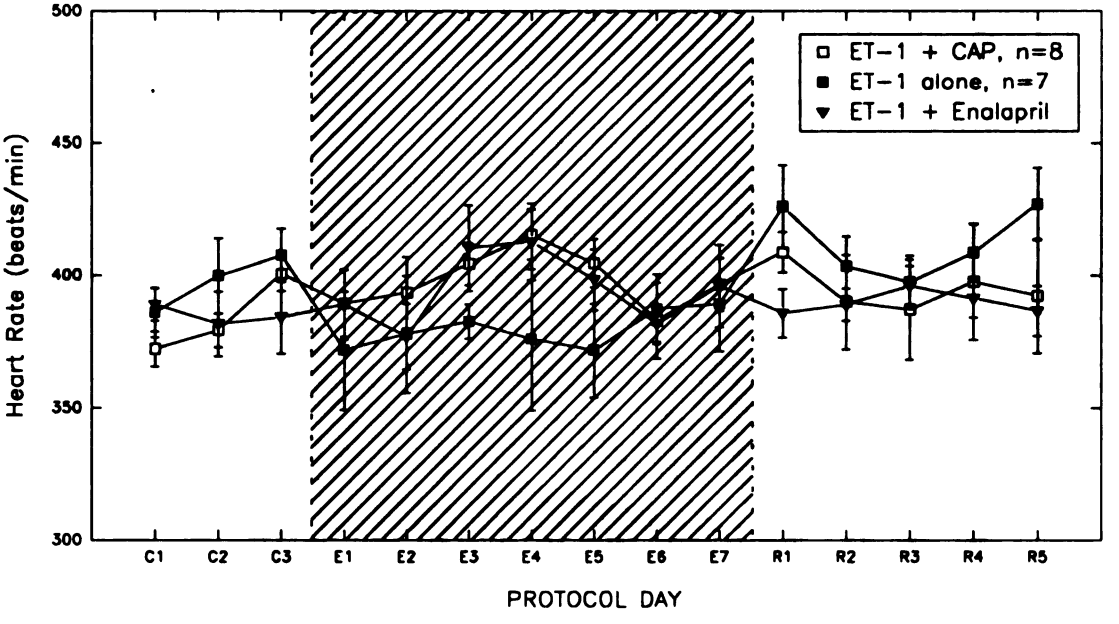

Figure 61. Heart rate responses to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats receiving ET-1 alone (n=7), ET-1 and captopril (n=8), or ET-1 and enalapril (n=6). Asterisks indicate significant difference (P<0.05) from control period measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

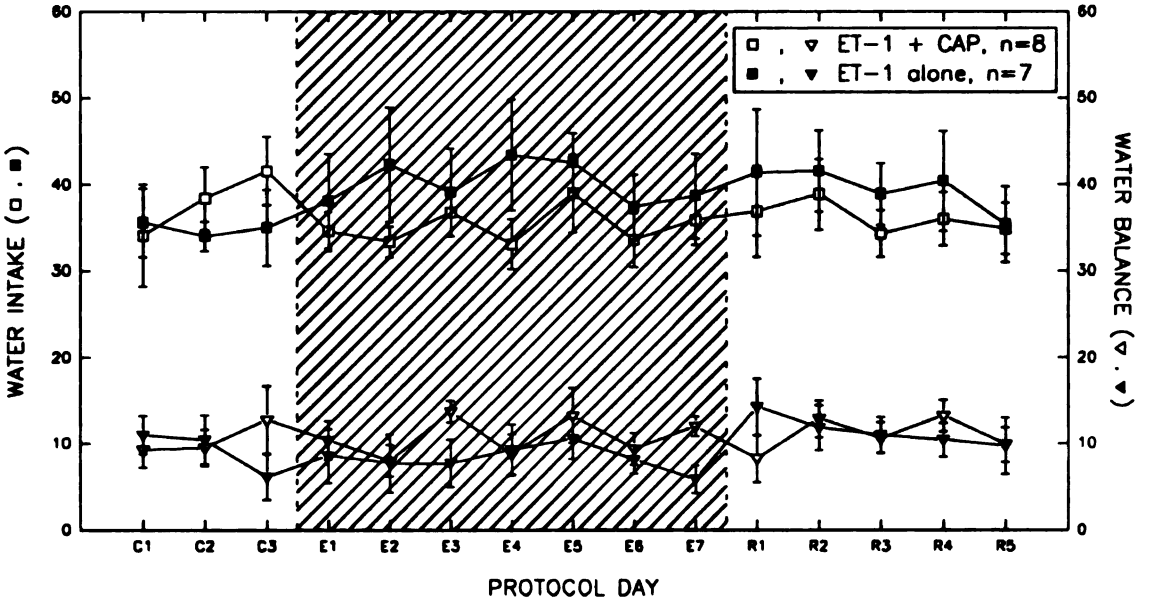

Figure 62. Changes in water balance in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats receiving ET-1 alone (n=7), ET-1 and captopril (n=8), or ET-1 and enalapril (n=6). Asterisks indicate significant difference (P<0.05) from control period measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

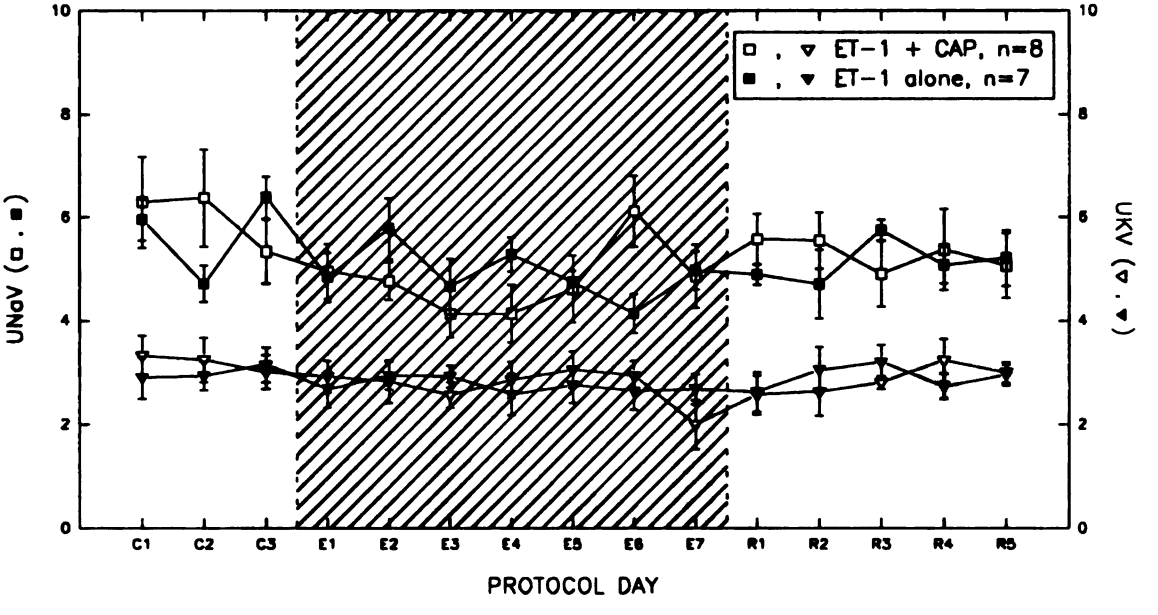

Figure 63. Changes in urinary sodium and potassium excretion in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats receiving ET-1 alone (n=7), ET-1 and captopril (n=8), or ET-1 and enalapril (n=6). Asterisks indicate significant difference (P<0.05) from control period measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

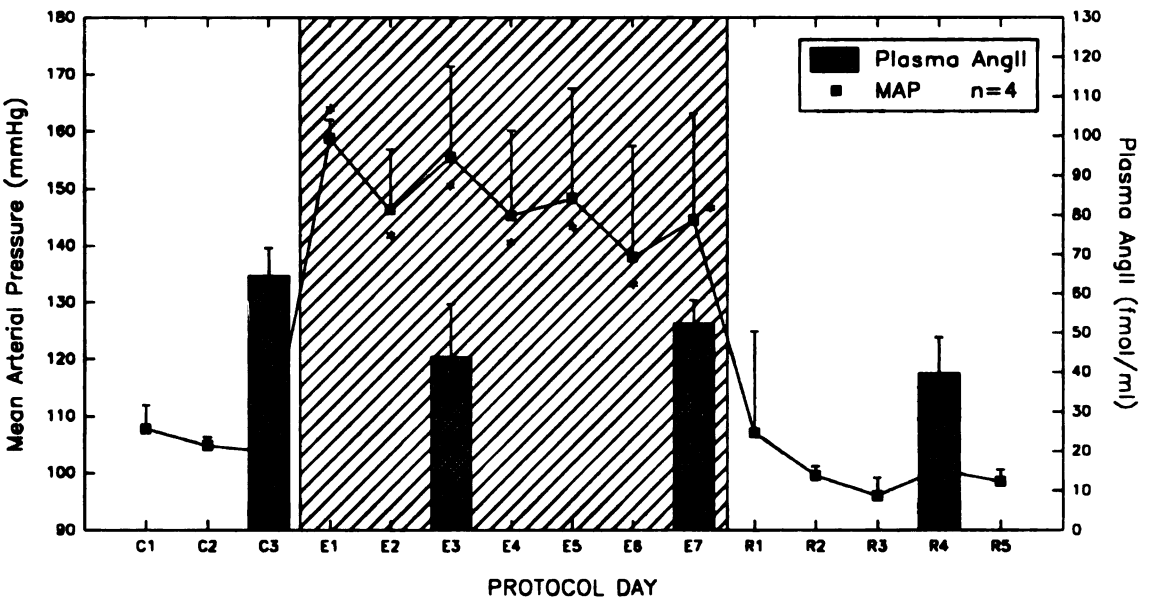

Figure 64. Changes in mean arterial pressure and plasma AngII concentration (vertical bars) in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats receiving ET-1 alone (n=4). Asterisks indicate significant difference ($P<0.05$) from control period measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

b. Bolus and chronic infusion administration of an AT_1 receptor antagonist

Bolus losartan administration: In the first set of experiments, losartan, at a dose of 3.0 $mg \cdot kg^{-1}$, i.v. was administered to each rat, once during the control period, on two separate days during the ET-1 infusion period (DAYS 2-3 and DAYS 4-5 of the ET-1 infusion period), and once during the recovery period. MAP and HR were measured at 0-, 15-, and 30- min, and 1-, 2-, 6- and 24-hr time-points after losartan was administered. Figure 65 and Figure 66 demonstrate the results of losartan administration during these four days. The seven bars for each day represent the time course of measurement from control to 24-hr after losartan treatment. ET I and ET II represent the two days of losartan administration during which animals were also receiving endothelin. As shown in Figure 65, MAP was significantly elevated during the ET-1 infusion period, as shown by the control bars. Though losartan administration had little effect on normal resting pressure during the control and recovery periods, losartan was able to produce significant reductions in MAP for periods up to 24- hr after administration during endothelin-hypertension. On each of these two days, a significant fall in MAP was observed as soon as 30-min after dosing and a maximal decrease was achieved approximately 6-hr after losartan was given. There were no significant effects of bolus losartan treatment on HR throughout the protocol (Figure 66).

Chronic losartan administration: Unpublished results from this laboratory demonstrated that losartan's antagonist action at AT_1 receptors are sustained

for periods up to two to three days. We, therefore, administered losartan once a day throughout the same 15-day protocol, described previously, as a bolus dose of 3.0 $mg \cdot kg^{-1} \cdot day^{-1}$. In addition, bolus administration of pressor doses of AngII at various days throughout the protocol failed to produce a pressor response in animals administered losartan at the dose above; again assuring complete AT_1 receptor blockade.

Figure 67 demonstrates the blood pressure response to seven days of ET-1 infusion at a rate of 5.0 $pmol \cdot kg^{-1} \cdot min^{-1}$ (solid squares, n=6). Again, ET-1 produces a sustained and reversible increase in MAP upon continuous infusion. When continuous losartan is added to the protocol regimen, solid triangles (n=6), there is no longer an anti-hypertensive action of losartan on endothelin-hypertension, as was observed in the previous experiment during shorter time interval measurements. HR (Figure 68), WB (Figure 69), UNaV (Figure 70) and UKV (Figure 70) were also examined and also were not consistently altered by this treatment protocol.

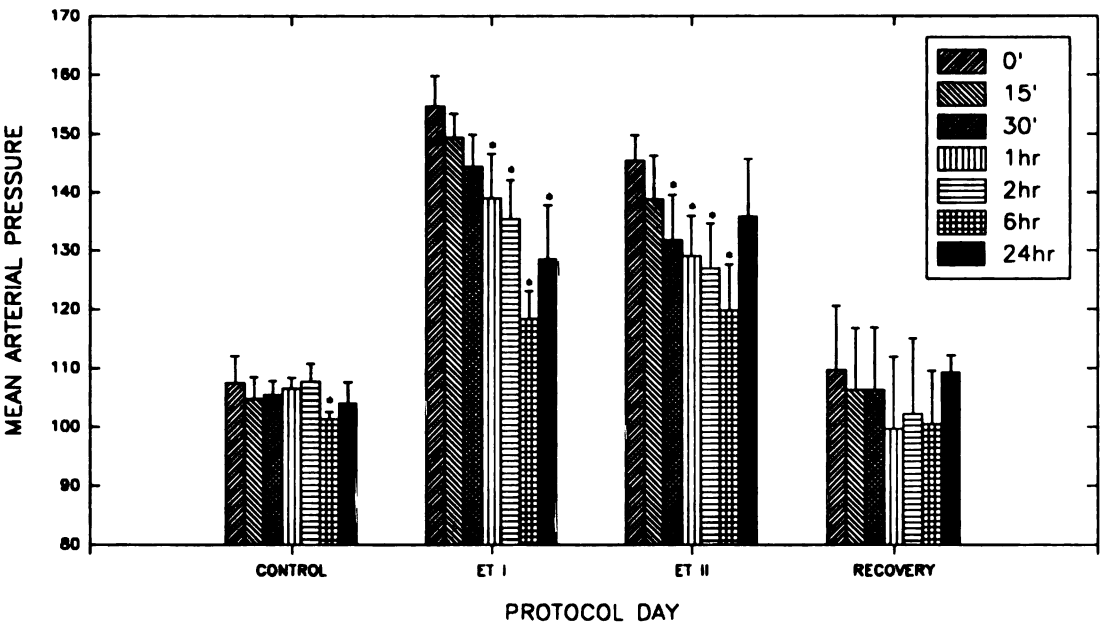

Figure 65. Mean arterial pressure responses to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats receiving losartan at a bolus dose of 3.0 mg·kg^{-1}, i.v. Asterisks indicate significant difference (P<0.05) from individual daily control period measurements. Losartan was administered to each rat, once during the control period, on two days during the ET-1 infusion period (DAYS 2-3 [E I] and DAYS 4-5 [E II] of the ET-1 infusion period), and once during the recovery period. After losartan administration, MAP was continuously monitored for the time periods indicated.

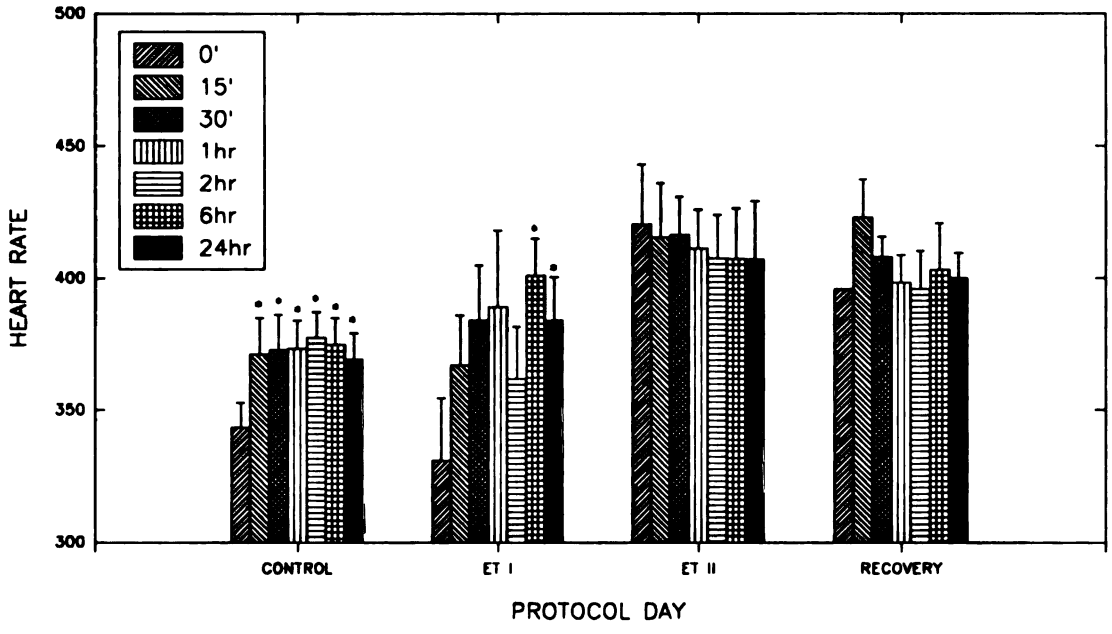

Figure 66. Heart rate responses to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats receiving losartan at a bolus dose of 3.0 mg·kg^{-1}, i.v. Asterisks indicate significant difference ($P<0.05$) from individual daily control period measurements. Losartan was administered to each rat, once during the control period, on two days during the ET-1 infusion period (DAYS 2-3 [E I] and DAYS 4-5 [E II] of the ET-1 infusion period), and once during the recovery period. After losartan administration, HR was continuously monitored for the time periods indicated.

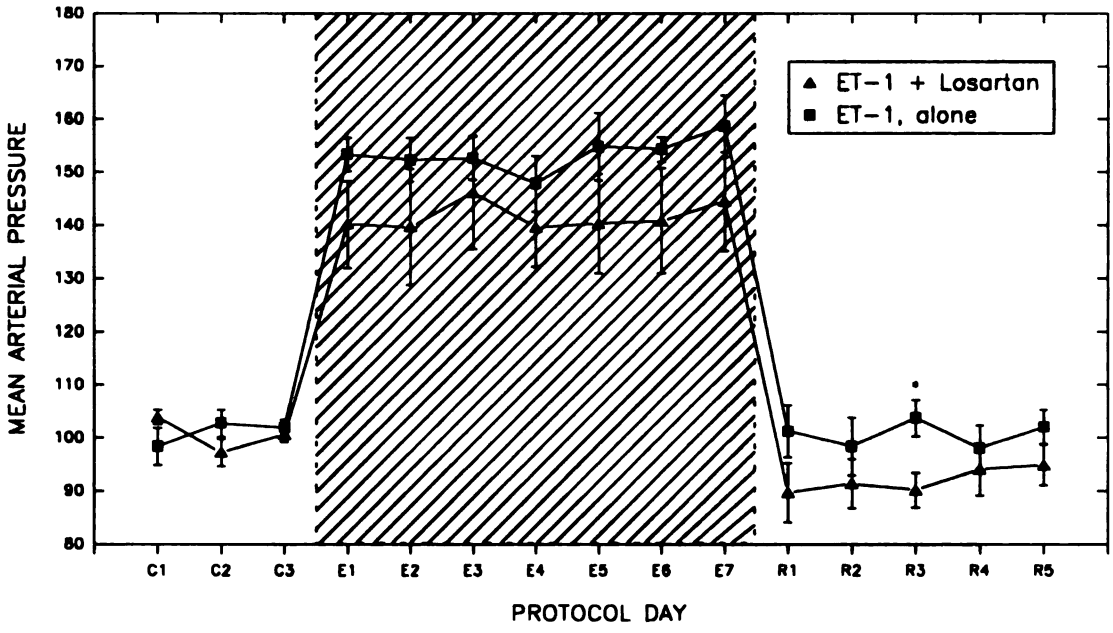

Figure 67. Mean arterial pressure responses to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats receiving ET-1 alone (n=6) or ET-1 and losartan (n=6). Asterisks indicate significant difference (P<0.05) from daily paired group measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

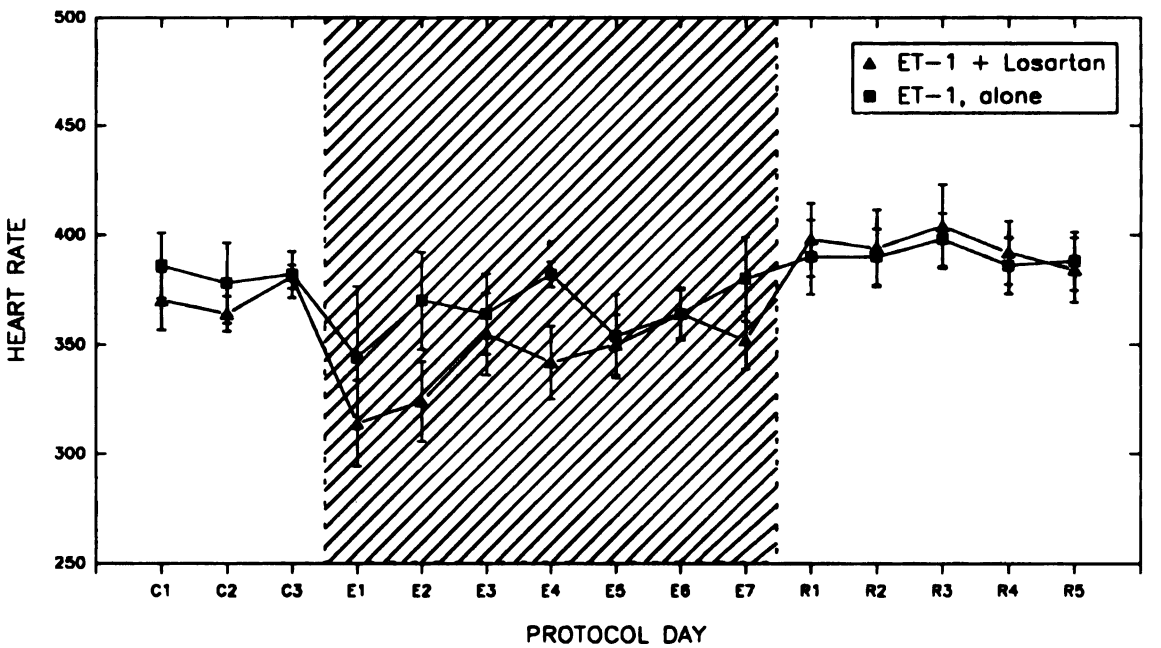

Figure 68. Heart rate responses to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats receiving ET-1 alone (n=6) or ET-1 and losartan (n=6). Asterisks indicate significant difference (P<0.05) from daily paired group measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

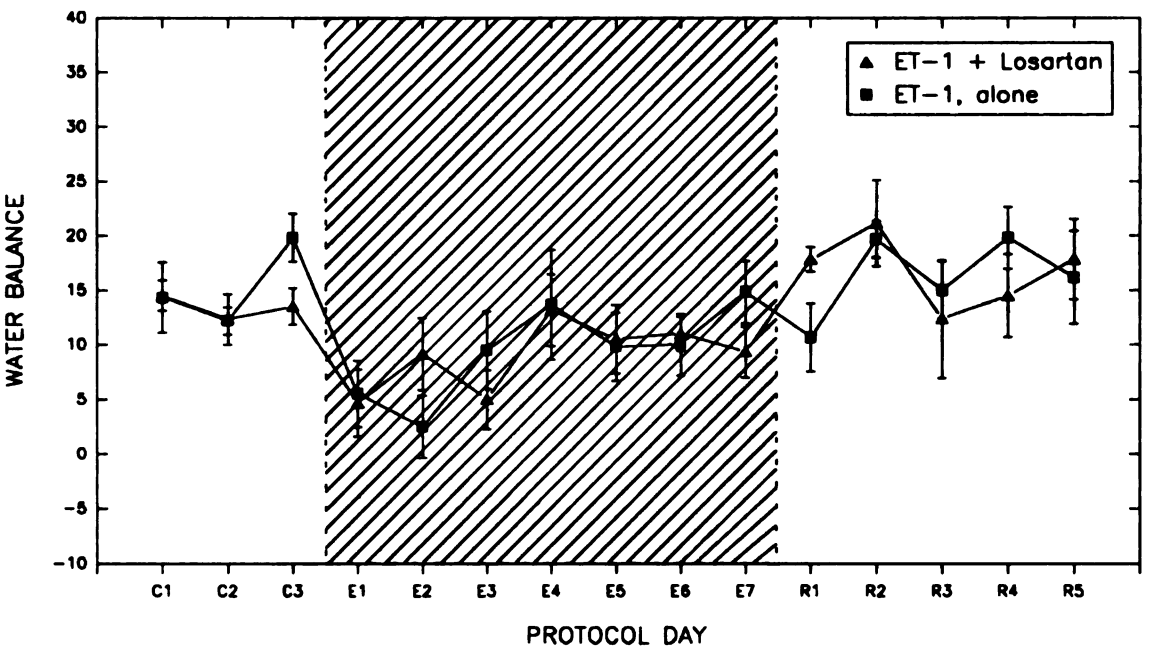

Figure 69. Changes in water balance in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats receiving ET-1 alone (n=6) or ET-1 and losartan (n=6). Asterisks indicate significant difference (P<0.05) from daily paired group measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

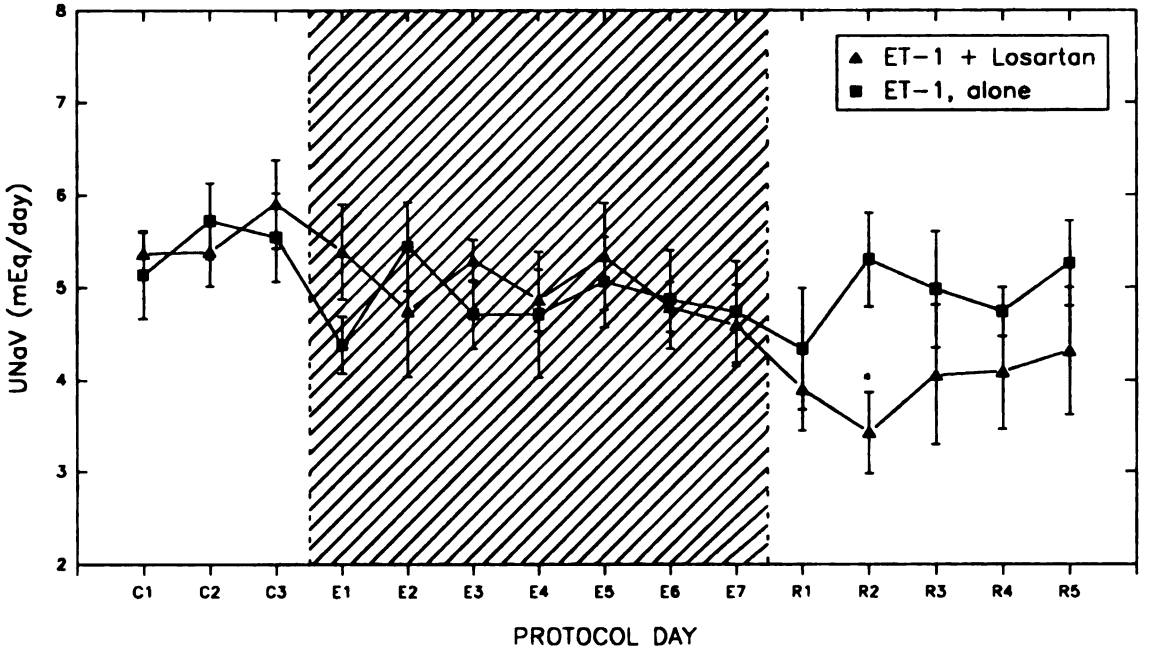

Figure 70. Changes in urinary sodium excretion in response to 7-day chronic infusion of ET-1 at a dose rate of 5.0 pmol·kg^{-1}·min^{-1}, i.v. in rats receiving ET-1 alone (n=6) or ET-1 and losartan (n=6). Asterisks indicate significant difference (P<0.05) from daily paired group measurements. C1-C3, control days; E1-E7, ET-1 infusion days; R1-R5, recovery days [see text].

DISCUSSION

A. Cardiovascular Responses to *Bolus and Acute Infusions* of the Endothelins

Rat sequence endothelin (ET-3) has been shown to be similar to human sequence endothelin (ET-1) except for six NH_2-terminal amino acid substitutions, three of which are substitutions by chemically similar amino acid residues. Yanagisawa *et al.*, (1988) demonstrated that ET-3 constricted rat aortic strips with less potency than does ET-1 and that, more importantly, the decay of the coronary pressor effect of ET-3 was much more rapid than that of ET-1. Their biochemical reasoning as to why ET-3 is less potent than ET-1 was based on the fact that ET-3 is chemically more polar than ET-1 because of the presence of a charged lysine, which replaces a hydrophobic methionine found in ET-1. This substitution may render ET-3 more susceptible to being "washed off" of relevant receptors due to its more polar chemical nature. The *in vivo* data presented in this study suggest that, as a pressor agent, ET-3 is as efficacious as ET-1; its duration of action, however, is significantly shorter. The reasons for this are presently unclear, though one can speculate that ET-3 is more easily removed from the endothelin receptor *per* the chemical reasoning above or that ET-3 is simply more susceptible to degradative enzymes in the rat circulation than is ET-1.

The results are consistent with the suggestion that both ET-1 and ET-3 induce their pressor effect in rats through a mechanism involving an elevation in TPR since, regardless of which peptide was used, CO was either decreased or remained unchanged in temporal association with increases in MAP.

Several investigators have reported that ET-1 has potent coronary vasoconstrictor (Yanagisawa *et al.*, 1988) and atrial inotropic effects (Hu *et al.*, 1988, Ishidawa *et al.*, 1988). It cannot be determined from the current results whether these actions were expressed *in vivo* in rats receiving endothelin. The fall in CO observed with the highest doses of ET-1, but not ET-3, could reflect an important difference in the biological actions of the two peptides in conscious rats. For example, ET-1 may impair cardiac contractility and thereby reduce CO more than ET-3 simply due to a more potent or long-lasting coronary constrictor action (Yanagisawa *et al.*, 1988). Alternatively, ET-1 and ET-3 may possibly have differing effects on venous capacitance or cardiac contractile force by acting on different putative receptor subtypes. In the current study it appears that bradycardia, most closely associated temporally with a rise in MAP, is the primary cause for the observed fall in CO produced by ET-1. Since ET-3 administration produced less consistent bradycardia than ET-1, it is also possible that the two peptides have different direct chronotropic effects or disparate influences on baroreflex mechanisms. Additional studies will be required to establish the validity of any of these possibilities.

As reported by several other laboratories (Lippton *et al.*, 1988, Wright *et al.*, 1988), ET-1 does appear to produce a bi-phasic blood pressure response; that is,

a short-lived hypotension followed by a sustained pressor response. It must be noted that this bi-phasic response was only noticeable when the animals were given *bolus injections* of ET-1 or ET-3. Such a response was not seen upon *acute infusion* of either ET-1 or ET-3 at any infusion rate. The reasons for this initial hypotensive effect of the endothelins upon bolus injection are unclear; however, it has been speculated that the peptide, when given in high concentrations, causes the release of autocoidal vasodilators such as EDRF and prostacyclin which may produce the initial short-lived depressor response (Ohlstein *et al.*, 1990). Likewise, endothelins, acting on different receptor subtypes or acting in a species-dependent manner, may produce a vessel-dependent vasodilation as seen by several investigators in *in vivo* studies measuring feline renal arterial perfusion pressure (Lippton *et al.*, 1988), rat carotid arterial blood flow, and rat abdominal aortic blood flow (Wright *et al.*, 1988).

In summary, this set of experiments demonstrates that both ET-1 and ET-3 are potent and long-lasting vasoconstrictive agents when administered intravenously as boluses or acute infusions to intact, conscious rats. Although a transient decrease in blood pressure can be observed after bolus administration of the peptides, the predominant and consistent cardiovascular response to the either peptide is a sustained elevation in MAP accompanied by an increase in TPR. The differing hemodynamic patterns of the responses to ET-1 and ET-3 could reflect differential actions of the peptides on putative receptor subtypes (Hoyer *et al.*, 1989).

B. Cardiovascular Responses to *Chronic Infusion* of the Endothelins

As with bolus or acute infusion studies with the endothelins, the results of this portion of the study indicate that ET-1 and ET-3 produce a dose-dependent increase in arterial pressure when infused intravenously into normal rats for a period of seven days. An important finding is that acute pressor effects of the endothelins demonstrated previously by ourselves (above) and others (Brain, 1989, Han *et al.*, 1989, Goetz *et al.*, 1988, Hinojosa-Laborde *et al.*, 1989, Minkes *et al.*, 1989) are well maintained during long-term peptid e infusion. It appears that the normal cardiovascular homeostatic processes which prevent certain acute pressor agents such as norepinephrine and epinephrine from inducing chronic increases in arterial pressure are not sufficiently engaged to effectively oppose the long-term vasoconstrictor effects of the endothelins.

There is disagreement in the literature as to whether the vascular actions of the endothelins exhibit tachyphylaxis (Öhlen *et al.*, 1989, Hirata *et al.*, 1988) but no indication of such an effect was observed in the current experiments. Likewise, there is evidence that the endothelins alter baroreflex function (Chapleau *et al.*, 1989) but only transient bradycardia was observed during ET-1 infusion in this study suggesting that at least the cardiac component of the baroreflex is appropriately reset during chronic endothelin infusion. Detailed studies by Knuepfer *et al* (1989), also found no effect of acute endothelin infusion on the cardiac component of the baroreflex in conscious rats. Increases in arterial pressure can also be effectively countered by elevated renal fluid excretion: the pressure diuresis/natriuresis mechanism (Guyton *et al.*, 1972). Abundant data

suggest, however, that infused endothelins bring about marked reductions in renal blood flow, glomerular filtration rate and sodium excretion (Goetz *et al.*, 1988, López-Farré *et al.*, 1989, Miller *et al.*, 1989). The failure of endothelin-induced hypertension to cause the expected sodium and water loss in this portion of the study may reflect the importance of these renal actions of endothelin.

As described above and by others, the hemodynamic mechanism of the acute pressor action of the endothelins is a rise in vascular resistance (Yanagisawa *et al.*, 1988, Brain, 1989, Han *et al.*, 1989, Minkes *et al.*, 1989). This portion of the study demonstrates that long-term infusion of the endothelins also raises arterial pressure primarily through increases in TPR although the variability of this mathematically-derived measure precluded demonstration of a statistically significant increase. A significantly elevated CO during chronic endothelin infusion might have been expected as a result of the inotropic effects of the peptide (Watanabe *et al.*, 1989, Shah *et al.*, 1989), or from volume expansion secondary to renal sodium and water retention (Goetz *et al.*, 1988, López-Farré *et al.*, 1989). However, neither of these factors are likely to have contributed to the modest increases in CO observed during ET-1 infusion since renal fluid retention did not occur and SV did not increase.

Three mature (21-residue) endothelin isoforms (ET-1, ET-2 and ET-3) are encoded in the human, rat and pig genomes (Inoue *et al.*, 1989). Endothelial cells produce only ET-1 and it is this isoform that is detectable in the circulating blood of humans (Ando *et al.*, 1989, Saito *et al.*, 1989). Both ET-2 and ET-3 may be expressed in a tissue specific manner; for example, ET-3 has been suggested to be

the "neuronal" endothelin isoform (Inoue *et al.*, 1989). Multiple endothelin receptor subtypes also have been inferred based on a number of pharmacological differences between ET-1 and ET-3 (Inoue *et al.*, 1989). Possible disparities in the cardiovascular responses to chronic ET-1 and ET-3 infusion therefore were explored in the current investigation. The effects of ET-3 on hemodynamic and body fluid variables were found to closely resemble those caused by infusion of ET-1; the only differences of note were that the pressor action of ET-3 developed more slowly and required a higher infusion rate. Thus, these results do not strongly support the suggestion that distinct receptor subtypes mediate the chronic cardiovascular effects of the endothelins.

The data from these studies also indicate that endothelin-hypertension is salt-sensitive. When infused intravenously for seven days into conscious rats at 5.0 $pmol \cdot kg^{-1} \cdot min^{-1}$, ET-1 produced a significant, sustained and reversible elevation in MAP when rats were maintained on a fixed salt intake of 6.0 $mEq \cdot day^{-1}$ (HS), but not when they were maintained on a salt intake of 2.0 $mEq \cdot day^{-1}$ (NS). This hypertensive response was attributable to an increase in TPR; CO, SV and HR were not consistently or significantly influenced by the ET-1/HS infusion protocol.

There are several potential mechanisms by which a high sodium/ET-1 combination could produce this observed increase in arterial pressure. An obvious possibility is the direct vasoconstrictor action of ET-1 since endothelins are known potent vasoconstrictors both *in vivo* (Yokokawa *et al.*, 1991) and *in vitro* (Franco-Cereceda, 1989). This constriction has been shown to occur secondary to

an increase in intracellular calcium concentrations derived from an increase in transmembrane calcium flux and/or a release of intracellular stored calcium (Van Renterghem *et al.*, 1988). Two considerations, however, suggest that a direct vasoconstrictor mechanism may not be the only means by which ET-1 infusion produces an elevated MAP and TPR in this study: first, the infusion rate of ET-1 used in this study has been previously (above) shown to produce only a very small increase in MAP during acute 1-hr infusions; second, if direct vascular constriction was the main action of ET-1, it is not clear why an increase in MAP and TPR occurred only in ET-1 infused rats on the higher salt intake.

Evidence from recent studies, however, suggests that a high sodium intake may augment the vascular response of the whole animal to the acute pressor effects of ET-1 (Folta *et al.*, 1991, Thompson *et al.*, 1991). Thus, in the current study, it is possible that rats on the high sodium intake simply had greater direct vasoconstrictor responses to the infused ET-1. There remains the question, though, of why an increased salt intake would enhance the vascular responsiveness to ET-1. In the intact animal, chronic ET-1 infusion may, for example, produce higher plasma concentrations of ET-1 during high sodium versus normal sodium intake conditions. Currently, however, we have no information to either confirm or refute this possibility. Alternatively, a high sodium intake could alter physiological properties of vascular smooth muscle cells to enhance the contractile responsiveness to ET-1. An increased sensitivity to contractile agonists is one proposed action of putative ouabain-like, natriuretic factors released in response to a high sodium intake (Haddy, 1990), and the

sensitivity of aortic strips to ET-1 *in vitro* has been shown to be increased by ouabain (Tomobe *et al.*, 1991). Thus, a simple increase in vascular responsiveness to ET-1 cannot be ruled out as an explanation for the increased MAP and TPR observed here during chronic infusion of ET-1 in rats on a high sodium intake.

A sustained increase in circulating concentrations of mineralocorticoids (Komanicky *et al.*, 1982) or AngII (Krieger *et al.*, 1989) has also been shown to produce a salt-sensitive hypertension in rats. Infusion of endothelins into intact animals for short periods has been reported to increase plasma renin activity (PRA, Ysuchiya *et al.*, 1990) and raise plasma aldosterone concentration (Cozza *et al.*, 1989). It is conceivable, therefore, that long-term infusion of ET-1 in this investigation produced a persistent elevation in plasma levels of AngII and/or aldosterone, thereby leading to a salt-sensitive hypertension. This possibility is discussed later.

There is now some evidence to implicate neural blood pressure control mechanisms in ET-1-induced hypertension. Binding sites (receptors?) for the endothelins have been found in brain nuclei known to participate in neural cardiovascular regulation (Ferguson *et al.*, 1991, Hoyer *et al.*, 1989), and blood-borne ET-1 has been shown to activate some of these neuronal populations (Ferguson *et al.*, 1991). Endothelins administered selectively into the brain at low doses elicit an increase in arterial pressure dependent on activation of the sympathetic nervous system. Endothelins also may facilitate the release of norepinephrine from sympathetic nerve terminals (Tabuchi *et al.*, 1990). ET-1 has also been reported to alter baroreflex function in some studies (Chapleau *et al.*,

1989) but not in others (Kneupfer *et al.*, 1989). It is not known, however, whether the central or peripheral neural actions of ET-1 are differentially affected by dietary salt intake. Thus, it is premature to speculate on a possible neurogenic basis for the salt-sensitivity to the chronic hypertensive action of ET-1.

It is also well established that a variety of different experimental manipulations of the kidney, including reductions of renal mass or renal perfusion pressure and the administration of sodium retaining hormones, produce a hypertension which is usually more severe in animals maintained on a high sodium intake (de Wardener *et al.*, (I and II) 1990). Although the precise mechanism of these forms of hypertension is not well established, there is general agreement that the initiating event is an excessive sodium chloride intake relative to renal excretory capacity. This has been postulated to result in either: 1) an initial increase in CO followed by autoregulatory increments in TPR; or 2) release into the circulation of natriuretic factors which also have vasoconstrictor activity. It is notable, then, that the endothelins have been found to exert very potent effects on renal function, including reductions in renal blood flow, glomerular filtration rate and urinary sodium excretion (Yamada *et al.*, 1991). In support, autoradiographic studies have identified high-density binding sites for ^{125}I-endothelin in glomeruli, vasa recta and inner medullary structures of rat kidney (Hoyer *et al.*, 1989, Koseki *et al.*, 1989). Circulating ET-1 is, therefore, likely to exert direct effects on the kidney leading to reduced sodium chloride excretory ability. The results of the current experiments reported here, however, indicate that renal actions of ET-1 are not a primary cause of an increased MAP during

chronic low-dose infusion in rats, since daily collections of urine did not reveal a significant reduction in sodium or water excretion during ET-1 infusion in this or the previous studies, above. An increase in CO also was not observed at any time during ET-1 infusion, as would have been predicted from at least one of the common theories concerning salt-sensitive hypertension (Guyton *et al.*, 1969). Although it might be reasonably argued that the rise in arterial pressure countered anti-natriuretic effects of ET-1 in the rats on high sodium intake, this would not account for the failure to observe sodium retention in the rats on normal sodium intake in whom ET-1 did not increase arterial pressure. Thus, though hypertension produced by exogenous infusion of ET-1 into conscious rats appears to be salt-dependent, the contributions of the renal effects of chronic ET-1 infusion in rats remain to be elucidated.

C. The Sympathetic Nervous System and Endothelin-Hypertension

As previously described, ET-1 may elicit many of its cardiovascular actions by stimulation of physiological processes within the central nervous system. Specifically, tissue autoradiographic studies described the presence of specific binding sites for ET-1 in various regions in rat and human brain stem (Jones *et al.*, 1989), basal ganglia, and cerebellum (Koseki *et al.*, 1989). Furthermore, immunoreactive ET-1 and ET-3 as well as their mRNAs were shown to be present in the central nervous system of rat (MacCumber *et al.*, 1989, Matsumoto *et al.*, 1989), pig (Shinmi *et al.*, 1989, Yoshizawa *et al.*, 1989, Yoshizawa *et al.*, 1990), and humans (Giaid *et al.*, 1989). Likewise, the presence of immunoreactive ET-1 in

normal human cerebrospinal fluid was reported to be seven-fold higher than in the plasma (Hirata *et al.*, 1990, Hoffman *et al.*, 1989). Central injection of ET-1 produces marked changes in various physiological parameters: intracerebroventricular injection of ET-1 induces changes in body posture, pupil diameter, and convulsions (Moser *et al.*, 1989); ET-1 influences central dopaminergic (Kataoka *et al.*, 1989) and vasopressinergic (Schichiri *et al.*, 1989) systems. Of particular interest, though, are the many reports demonstrating that intracerebroventricular injection of ET-1 induces potent changes in peripheral blood pressure and heart rate in conscious or anesthetized rats (Ferguson *et al.*, 1990, Ouchi *et al.*, 1989, Seto *et al.*, 1989). All of these data suggest that ET-1 is a functional central neurohormone that may play a role in many pathophysiological states (Hirata *et al.*, 1990, Hoffman *et al.*, 1989).

Few other agents have been shown to have the sustained pressor action of ET-1 when chronically administered (Fink *et al.*, 1987, Kanagy *et al.*, 1990) since physiological compensatory (homeostatic) mechanisms, such as the baroreceptor reflex, appear to effectively counter most adverse elevations in blood pressure through neurogenic regulation of TPR. Those agents that appear to circumvent these homeostatic mechanisms, have been shown to have direct neurogenic activity as well as direct peripheral mechanisms of action. For example, Cowley *et al* (1976) and Pawloski (1990) have shown that, initially, the arterial baroreflex effectively attenuates the rise in arterial pressure due to continuous AngII infusion (since SAD animals demonstrate a rapid rise (1-day) in MAP whereas intact animals become hypertensive over a period of days). After a period of a few

days, however, the blood pressures of intact animals achieves the levels seen in SAD animals suggesting that AngII has induced a "resetting of the baroreflex"; has decreased baroreceptor sensitivity; and/or has directly stimulated central sympathetic activity (Luft *et al.*, 1989)(thereby circumventing the baroreceptor reflex). This ability of the arterial baroreflexes to "reset" rapidly in response to changes in MAP has made their role in pressure regulation in hypertension controversial. For example, baroreceptor denervation in most experimental hypertensive models increases only short- term pressure lability (minute-to-minute) but not average long-term pressure levels. Pawloski (1990) further provided evidence that the full expression of the neurogenic actions of AngII are observable only in SAD animals, or during periods of AngII infusion sufficiently sustained to allow substantial resetting of the baroreflex.

Despite conflicting evidence concerning the influence of ET-1 on baroreflex function (Kneupfer *et al.*, 1989, Kuwaki *et al.*, 1990, Itch *et al.*, 1991), inhibition of the baroreflex clearly enhances the acute (25-min) pressor action of i.v. ET-1 in rats (Hinojosa-Laborde *et al.*, 1989). However, as described above, the chronic effects of baroreceptor denervation on hormonal regulation of blood pressure cannot always be predicted from these acute studies. In the present study, during chronic infusion of ET-1, it appears that the cardiac component of the baroreflex is reset within 48 hrs in the presence of sustained hypertension. Additional evidence that the baroreflex operates normally during ET-1 hypertension is the finding that the minute-to-minute variation in MAP is not altered in SHAM rats throughout the experimental protocol. Alternatively, since baroreflex denervation

produces a sustained enhancement of the pressor response to ET-1, it appears that the vascular component of the baroreflex is not similarly reset. Thus, in the current study, the intact baroreflex appears to effectively attenuate the long-term blood pressure effects of circulating ET-1 at the dose administered. Additionally, the differences in the magnitude of the blood pressure response to ET-1 infusion between SHAM and SAD rats cannot be explained by alterations in $[ET\text{-}1]_P$ since these were similar in both groups during ET-1 infusion.

The demonstration of high-density, ET-1 binding sites in many brain regions, including the dorsal vagal complex (Jones *et al.*, 1989), subfornical organ (Koseki *et al.*, 1989a), median eminence (Koseki *et al.*, 1989b) and area postrema (Ferguson *et al*, 1991), further indicate a central site of action for the peptide. Additionally, studies demonstrating cardiovascular actions of ET-1 following microinjection of the peptide into the area postrema support the concept that ET-1 may act as a chemical messenger within this particular circumventricular region (Ferguson *et al.*, 1990). A combination of the central morphology and recent investigational evidence suggests that ET-1 may have specific actions on neurons within the area postrema.

As a part of the dorsal vagal complex, the rat area postrema is a midline circumventricular structure located on the dorsal surface of the medulla. Characteristically, it is highly vascularized and lacks a normal blood brain barrier. Likewise, compared to other mesencephalic loci, it has been demonstrated to contain the highest densities of binding sites for various circulating peptides including AngII (Mendelsohn *et al.*, 1984), atrial natriuretic factor (ANF, Bianchi

et al., 1986) and ET-1 (Koseki *et al.*, 1989a). The suggested role of the area postrema as a site of action for the central effects of circulating compounds is further supported by anatomical tracing studies which have demonstrated efferent neural connections from the area postrema to the adjacent nucleus tractus solitarius (NTS), the parabrachial nucleus in the pons, as well as to both spinal and hypothalamic autonomic regions (Shapiro *et al.*, 1984, Van der Kooy *et al.*, 1983). For example, evidence has implicated this structure as an essential "chemoreceptor trigger zone" in the control of emesis (Borison *et al.*, 1984); thus, circulating substances are believed to gain direct access to neural elements within this structure thereby initiating the neural processes which lead to vomiting. Indeed, it has been reported that systemic ET-1 infusion in dogs induces vomiting (Goetz *et al.*, 1988), although the specific locus at which ET-1 acted to produce this effect was not established. Further implication of the area postrema in cardiovascular control mechanisms is evidenced by the fact that destruction of the area postrema has been reported to result in hypertension (Yitalo *et al.*, 1974). Accordingly, Ferguson *et al* (1988) demonstrated that electrical stimulation of area postrema neurons in the rat resulted in rapid, reversible decreases in both blood pressure and HR. Alternatively, lower intensity stimulation in rabbit area postrema has been reported to decrease sympathetic postganglionic renal nerve activity (Hasser *et al.*, 1987).

As previously mentioned, AngII-hypertension is hemodynamically similar to endothelin-hypertension and AngII has been suggested to elicit its central effects on the cardiovascular system, at least in part, through actions within the area

postrema where electrolytic ablation of the area postrema significantly attenuates the neurogenic component associated with AngII-hypertension (Fink *et al.*, 1987, Casto *et al.*, 1984). These studies further help to support results from electrophysiological studies demonstrating that area postrema neurons are influenced by circulating AngII (Carpenter *et al.*, 1988, Papas *et al.*, 1990) as well as by changes in blood pressure (Papas *et al.*, 1990). If part of the hypertensive response to continuous ET-1 infusion was due to sympathetic stimulation via identified receptors in the area postrema, then APX, as in AngII hypertension, should have produced an alteration in the response to peripheral application of the peptide. In fact, this portion of the study demonstrates that APX does not alter the hypertensive response to a chronic elevation in circulating ET-1, suggesting that endothelin-hypertension occurs in the absence of chronic sympathetic stimulation through activation of receptors in the area postrema. Alternatively, a neurogenic component of endothelin-hypertension may arise from other central sites. Though a suggested contribution of an increased sympathetic nerve activity to endothelin-hypertension has not been ruled out, these data are further consistent with the hypothesis that the chronic blood pressure effects of circulating ET-1 are due to a direct effect of ET-1 on vascular smooth muscle.

D. The Renin-Angiotensin System and Endothelin-Hypertension

The data from this portion of the studies indicate that endothelin hypertension is prevented by the ACE inhibitor captopril. Earlier, we demonstrated that ET-1, when infused intravenously for seven days into conscious rats at 5.0

pmol·kg^{-1}·min^{-1}, produces a significant, sustained and reversible elevation in MAP when rats are maintained on a fixed salt intake of 6.0 mEq·day^{-1}. We also have demonstrated that this hypertension is attributable only to an increase in TPR since CO, SV, HR, water intake, urine output and sodium and potassium excretion were not changed. When this infusion protocol is combined with a concomitant infusion of captopril at a rate of 1.0 mg·kg^{-1}·hr^{-1}, i.v., the pressor response is inhibited with no observable variations in the measured hemodynamic or fluid/electrolyte balance parameters mentioned above.

Because of the various pharmacological properties of captopril, there are several potential mechanisms by which this agent could effectively prevent ET-1 hypertension. One possibility is the ability of captopril to inhibit ACE (Rubin *et al.*, 1978), thereby preventing the generation of AngII. The dose of captopril utilized in this investigation has been shown by other investigators to effectively inhibit AngII formation in normal rats (Wallace *et al.*, 1987). It also is well known that an increase in the circulating plasma level of AngII produces a sustained, salt-dependent increase in MAP (Pawloski *et al.*, 1990); this form of hypertension has a hemodynamic profile similar to that seen in ET-1 hypertension, i.e. a sustained elevation in TPR with little effect on other hemodynamic parameters. An interaction between ET-1 and the RAS has been suggested in a study where short-term infusion of ET-1 into intact animals increased PRA (Miller *et al.*, 1989, Goetz *et al.*, 1988). In addition, two recent investigations by Kawaguchi *et al* (1990, 1991) demonstrated that ET-1 is a potent stimulant of ACE in cultured bovine pulmonary artery endothelial cells; maximum stimulation (a 2-fold

increase) of ACE occurred at $1x10^{-8}$ M ET-1. Therefore, in this particular study, it is conceivable that ET-1 induced elevations in $[AngII]_P$ sufficient to produce hypertension; administration of captopril would then prevent hypertension by inhibiting an increase in $[AngII]_P$ concentrations. Direct measurements performed in the current study, however, indicate that ET-1 did not significantly increase $[AngII]_P$. These observations suggest that if AngII is involved in ET-1 hypertension, it is probably at a local tissue level. Such an interaction between the RAS and ET-1 at the endothelial level is supported by Dohi *et al* (1991, 1992) who recently demonstrated that AngII directly stimulates endothelial ET-1 production, *in situ*, through an increase in the expression and transcription of ET-1 mRNA; there is currently no evidence, though, that ET-1 increases local AngII formation by vascular tissue.

In addition to possible effects on the *vascular* RAS, the *renal* RAS could also be involved in the actions of ET-1. ET-1, like AngII, has been found to exert very potent effects on renal function, including reductions in renal blood flow (RBF), glomerular filtration rate (GFR) and urinary sodium excretion (Miller *et al.*, 1989, Goetz *et al.*, 1988, Banks, 1990). In addition, receptors for both peptides have been identified in the glomeruli, vasa recta and medulla (Wilkes *et al.*, 1991). Localized stimulation of renal AngII formation by ET-1 could therefore contribute to the observed hypertension, which would be reversed by an action of captopril on renal ACE. In support, one recent investigation performed in anesthetized dogs and rats demonstrated that captopril attenuated ET-1 induced decreases in RBF, $U_{Na}V$ and urine flow while having little effect on the short-term pressor response

to ET-1 (Banks, 1990). Daily measurements of urine volume and electrolyte excretion in the current study, however, did not reveal any significant actions of ET-1 on renal function.

Though it is generally accepted that the antihypertensive mechanism of captopril is through inhibition of AngII formation, several *in vivo* and *in vitro* studies suggest that captopril has vasodilator activity which is independent of the RAS and ACE-inhibition. Investigations have shown that captopril's unique sulfhydryl moiety (-SH) acts as a scavenger of free radicals such as superoxide anion (O_2^-); O_2^--mediated vasoconstriction has been shown to occur through inactivation of EDRF (Aruoma *et al.*, 1991, Goldschmidt *et al.*, 1991). Nagase *et al* (1990) and Haller *et al* (1991) recently demonstrated that ET-1 enhances the release of oxygen radicals *in vitro* and in vivo. Likewise, ET-1 has been shown to be a potent secretagogue for EDRF (Ohlstein *et al.*, 1990). Accordingly, it is possible that captopril administration, in this study, may have prevented ET-1 hypertension development via a scavenging action on ET-1-induced O_2^- generation allowing the vasodilator action of ET-1-induced EDRF release to antagonize any ET-1-induced vasoconstriction. This may not be a viable mechanism in this study, however, since the use of another ACE inhibitor, enalapril, also was able to effectively prevent endothelin-hypertension; enalapril does not possess a chemical sulfhydryl moiety and has not been shown to have superoxide scavenging properties (Sweet *et al.*, 1981 and 1983, Gross *et al.*, 1980, Humke *et al.*, 1991). Other potential mechanisms for captopril's antihypertensive effect in this model may be through the inhibition of kinin degradation (Rubin *et*

al., 1978) or through the release of vasodilator prostanoids (Swartz *et al.*, 1980). These endogenous vasodilators and others are known to antagonize the vasoconstrictor effect of ET-1 (Rubanyi *et al.*, 1991). The results of this portion of the study, therefore, suggested that endothelin-hypertension may be caused by tissue-specific, RAS activation and/or possible super-oxide generation since captopril effectively attenuates hypertension development. To further describe the potential interaction(s) between the RAS and ET-1 in endothelin-hypertension, losartan, an non-peptidic AT_1-specific receptor antagonist was used.

It has been recently demonstrated that there are at least two sub-types of AngII receptors. The AngII-receptor sub-type associated with vascular smooth muscle, AT_1 (Chiu *et al.*, 1989) is selectively blocked by compounds such as potassium 2-n-butyl-4-chloro-5-hydroxymethyl-1-[2-(1H-tetrazole-5-yl)bi-phenyl-4-yl-methyl]imidazole (*DuP753, losartan*, Chiu *et al.*, 1990, Wong *et al.*, 1990a). Losartan, a competitive antagonist, was utilized in the current study since it, unlike earlier peptidic AngII receptor antagonists, is totally lacking in agonist activity and has no influence on vascular responses to noradrenaline, vasopressin, bradykinin or ACE activity (Chiu *et al.*, 1990, Wong *et al.*, 1990a,b,c,d,e). In conscious, normotensive rats or in rats with DOCA-salt hypertension, losartan has no hypotensive effect alone, but in normotensive rats treated with furosemide, in rats with hypertension due to renal artery ligation, and in SHR, losartan has a distinct antihypertensive effect (Wong *et al.*, 1990a,b,c,d,e). Bolus administration of losartan in the current study revealed that the compound was able to produce significant reductions in MAP for periods up to 24-hrs after administration

during the ET-1 infusion period when MAP was significantly elevated. Again, as demonstrated by others, above, losartan had little effect on MAP during the control or recovery periods when animals were normotensive. MAP, however, was significantly elevated when measured during the next 24-hr period suggesting that the antihypertensive effects of losartan are quickly alleviated in this model of hypertension through unidentified mechanisms but may be related to its metabolism. This finding is discordant with the early descriptions of the actions and metabolic fate of losartan which suggest that losartan, as a parent compound, is quickly metabolized *in vivo* into a non-competitive antagonist of AT_1 receptors (Chiu *et al.*, 1990). The metabolite, EXP 3174, was calculated to be at least 41 times more potent than losartan and has an apparent half-life of ~2 days *in vivo* (unpublished observation). Therefore, if endothelin-hypertension is a result of an action of AngII on AT_1 receptors, then the antihypertensive effect of bolus losartan during endothelin-hypertension should have been maintained by the presence of the imidazole metabolite. Nevertheless, if metabolism of the compound was related to its acute activity, then chronic infusion of an effective dose of losartan should have produced a sustained inhibition of endothelin-hypertension. The data of the current study, however, demonstrated that continuous application of losartan by chronic i.v. infusion was ineffective at preventing endothelin-hypertension development or maintenance despite full and continuous blockade of AT_1 receptors (there was no blood pressure response to bolus pressor doses of AngII administered throughout the protocol). Similarly, stimulation of the renal RAS (and thereby an action of AngII on renal function)

also does not appear to play a role in endothelin-hypertension development or maintenance since losartan had no significant effect on urinary electrolyte or fluid balance throughout the protocol in any of the groups of animals. In conclusion, despite a short-term effect of losartan to lower MAP during endothelin-hypertension, this portion of the study suggests that the development and maintenance of endothelin-hypertension does not appear to require an action of AngII on AT_1 receptors.

CONCLUSIONS

The investigations of this dissertation were designed to address the hypotheses relating to the hemodynamic actions of endothelin and the mechanism(s) of endothelin-hypertension. First, acute bolus, short-term infusion and chronic infusion of ET-1 demonstrated that much of the peptide's pressor action is associated with insignificant increases in TPR with little action on other cardiovascular hemodynamics such as CO, HR, and SV. In addition, these initial studies demonstrated a significant difference in the potencies of the two endothelin isopeptides ET-1 and ET-3; ET-3 being significantly less potent than ET-1.

Second, renal actions of ET-1 seem to play no significant role in the sustained pressor response to chronically infused ET-1 since urinary sodium and water retention was not observed in any of the experiments presented. In contrast, endothelin-hypertension is distinctly dependent on sodium intake since it was observed that a three-fold increase in total daily salt intake significantly augmented the sustained pressor response to infused ET-1.

Third, endothelin-hypertension is not accompanied by significant alterations in circulating concentrations of ET-1 or AngII. These observations could be explained by the unique molecular kinetics of endothelin's interaction with its

receptor (Yanagisawa *et al.*, 1989b) as well as its rapid elimination from the circulation (Yanagisawa *et al.*, 1988b). Additionally, endothelin-hypertension may be associated with specific compartmental elevations in tissue ET-1 (Dohi *et al.*, 1991) and AngII (Dohi *et al.*, 1992) activity which were not analyzed in these studies.

Fourth, ET-1 interacts with the baroreceptor reflex in a unique fashion since it was observed that endothelin-hypertension, over a period of seven days, is significantly augmented by sino-aortic denervation. These observations are in accord with those of Hinojosa-Laborde *et al* (1989) describing a similar augmenting effect of sino-aortic denervation on the pressor response to acute application of ET-1.

Fifth, actions of ET-1 at the area postrema do not seem to play a role in the development or maintenance of endothelin-hypertension since the onset and level of hypertension obtained in area postrema ablated rats was not significantly different from that obtained in normal animals. This is surprising considering the data describing an extensive population of ET-1 receptors within this circumventricular organ (Ferguson *et al.*, 1991) and the peripheral responses elicited by microinjection of ET-1 into this medullary region (Ferguson *et al.*, 1990), although this disparity might be explained by the differences in the doses of ET-1 administered.

Sixth, an action of AngII at AT_1 receptors does not appear to be necessary in the development or maintenance of endothelin-hypertension since this condition is not alleviated by specific AngII-receptor antagonism. The finding that ACE

inhibition effectively prevents endothelin-hypertension development and maintenance suggests that alternate pathways in the renin-angiotensin, prostaglandin, and/or bradykinin systems may be important in the physiology of endothelin-hypertension. For instance, as suggested earlier, it has been shown that converting enzyme inhibition prevents kinin metabolism and thereby stimulates the relaxation response of bradykinin on smooth muscle; the effects of bradykinin have been demonstrated to effectively attenuate constrictor responses of endothelin on smooth muscle. Studies using available bradykinin receptor antagonists could further elucidate this possible mechanism of action. Additionally, recent studies (Santos *et al.*, 1992, Jaiswal *et al.*, 1992, and Diz *et al.*, 1992) suggest that converting enzyme inhibition may shunt the metabolism of AngI to alternate metabolites such as Ang 1-7 or Ang 3-8 which may have vasodilatory actions on vascular smooth muscle possibly through stimulation of prostaglandin production and the production of other endothelial-derived relaxing factors. Assays directed at the measurement of these alternate vasodilator metabolites will provide further insight into this possible mechanism of action. As well, currently available and specific ET-1 receptor antagonists should prove useful in describing a direct mechanism of action of ET-1 in endothelin hypertension.

Therefore, based on the findings of this dissertation and the proposals above, the following scheme (Figure 71), describing possible mechanistic pathways for the cardiovascular actions of continuously infused ET-1 is suggested:

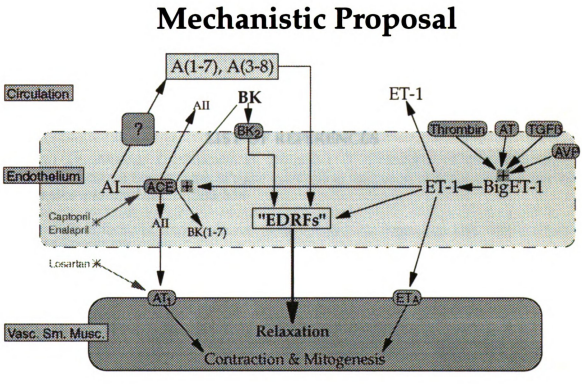

Figure 71: Mechanistic proposal for the actions of ET-1 during endothelin-hypertension.

LIST OF REFERENCES

LIST OF REFERENCES

ADVENIER C, SARRIA B, NALINE E, PUYBASSET L, LAGENTE V: Contractile activity of three endothelins (ET-1, ET-2 and ET-3) on the human isolated bronchus. *Br J Pharmacol* 1990;100:168-172

ANDO K, HIRATA Y, SHICHIRI M, EMORI T, MARUMO F: Presence of immunoreactive endothelin in human plasma. *FEBS Letters* 1989;245:164-166

ANDO K, HIRATA Y, TAKEI Y, KAWAKAMI M, MARUMO F: Endothelin-1-like immunoreactivity in human urine. *Nephron* 1991;57:36-39

ÄNGGÅRD E, GALTON S, RAE G, THOMAS R, MCLOUGHLIN L, DE NUCCI G, VANE JR: The fate of radioiodinated endothelin-1 and endothelin-3 in the rat. *J Cardiovasc Pharmacol* 1989;13(5):S46-S49

ARAI H, HORI S, ARAMORI I, OHKUBO H, NAKANISHI S: Cloning and expression of a cDNA encoding an endothelin receptor. *Nature* 1990;348:730-732

ARUOMA OI, AKANMU D, CECCHINI R, HALLIWELL B: Evaluation of the ability of the angiotensin-converting enzyme inhibitor captopril to scavenge reactive oxygen species. *Chem Biol Interact* 1991:77;303-314

BANKS RO: Effects of endothelin on renal function in dogs and rats. *Am J Physiol* 1990:258(27);F775-F780

BENHAM CD, TSIEN RW: A novel receptor-operated calcium-permeable channel activated by ATP in smooth muscle. *Nature* 1987;328:275-278

BENIGNI A, PERICO N, GASPARI F, ZOJA C, BELLIZZI L, GABANELLI M, REMUZZI G: Increased renal endothelin production in rats with reduced renal mass. *Am J Physiol* 1991;260(29):F331-F339

BEN-ISHAY D, SALITERNIK R, WELNER A: Separation of two strains of rats with inbred dissimilar sensitivity to DOCA-salt hypertension. *Experentia* 1972;28:1321-1322

BERBINSCHI A, KETELSLEGERS JM: Endothelin in urine. *Lancet* 1989; July 1:46

BIANCHI C, GUTKOWSKA J, BALLAK M, THIBAULT G, GARCIA R, GENEST J, CANTIN M: Radioautographic localization of ^{125}I-atrial natriuretic factor binding sites in the brain. *Neuroendocrin* 1986;44:365-372

BLACKBURN K, HIGHSMITH RF: Nickel inhibits endothelin-induced contractions of vascular smooth muscle. *Am J Physiol* 1990;258:C1025-C1030

BLAUSTEIN MP, HAMLYN JM: Pathogenesis of essential hypertension: a link between dietary salt and high blood pressure. *Hypertension* 1991;18(suppl 5):III-184-III-195

BORGES R, VON GRAFENSTEIN H, KNIGHT DE: Tissue selectivity of endothelin. *Eur J Pharmacol* 1989;165:223-230

BORISON HL, BORISON R, MCCARTHY LE: Role of the area postrema in vomiting and related functions. *Fed Proc* 1984;43:2955-2958

BOULANGER C, LÜSCHER TF: Release of endothelin from the porcine aorta. *J Clin Invest* 1990;85:587-590

BRAIN SD: The direct observation of arteriolar constriction induced by endothelin in vivo. *Eur J Pharmacol* 1989;160:401-403

BRAIN SD, TIPPINS JR, WILLIAMS TJ: Endothelin induces potent microvascular constriction. *Br J Pharmacol* 1988;95:1005-1007

BROWN AM, BIRNBAUMER L: Direct G-protein gating of ion channels. *Am J Physiol* 1988;254:H401-H410

CAO L, BANKS RO: Cardiovascular and renal actions of endothelin: effects of calcium-channel blockers. *Am J Physiol* 1990;258(27):F254-F258

CARPENTER DO, BRIGGS DB, KNOX AP, STROMINGER N: Excitation of area postrema neurons by transmitters, peptides, and cyclic nucleotides. *J Neurophysiol* 1988;59:358-369

CASTO R, PHILLIPS I: Cardiovascular actions of microinjections of angiotensin II in the brain stem of rats. *Am J Physiol* 1984;246:R811-R816

CAVELLI DECARVALHO MH, NIGRO D, SCIVOLETTO R, BARBEIRO HV, DEOLIVEIRA MA, DENUCCI G, FORTES ZB: Comparison of the effect of endothelin on microvessels and macrovessels in Goldblatt II and deoxycorticosterone acetate-salt hypertensive rats. *Hypertension* 1990;15(suppl I):I-68-I-71

CERNACEK P, STEWART D: Immunoreactive endothelin in human plasma: marked elevations in patients in cardiogenic shock. *Biochem Biophys Res Commun* 1989;161:562-567

CHAPLEAU MW, HAJDUCZOK G, ABBOUD FM: Dual effects of endothelin on baroreceptor activity. *Hypertension* 1990;Abstract #6269

CHAPLEAU MW, HAJDUCZOK G, ABBOUD FM: Endothelin suppresses baroreceptor activity: a new mechanism contributing to hypertension? *Hypertension* 1989;14(3)Abstract:336

CHIU AT, HERBLIN WF, MCCALL DE, ARDECKY RJ, CARINI DJ, DUNCIA JV, PEASE LJ, WONG PC, WEXLER RR, JOHNSON AL, TIMMERMANS PBMWM: Identification of angiotensin II receptor subtypes. *Biochem Biophys Res Commun* 1989;165:196-203

CHOU S-Y, DAHHAN A, PORUSH JG: Renal actions of endothelin: interaction with prostacyclin. *Am J Physiol* 1990;259(28):F645-F652

CLOZEL J-P, CLOZEL M: Effects of endothelin on the coronary vascular bed in open-chest dogs. *Circ Res* 1989;65:1193-1200

CORNET S, BRAILLON A, GUILMARD C, CHABRIER P-E, PIROTZKY E, BRAQUET P: Involvement of endothelin in renal processes. *Hypertension* 1990;15:724-728

COWLEY AW, DECLUE JW: Quantification of baroreceptor influence on arterial pressure changes seen in primary angiotensin-induced hypertension in dogs. *Circ Res* 1976;39(6):779-787

COWLEY AW, MCCAA R: Acute and chronic dose-response relationships for angiotensin, aldosterone, and arterial pressure at varying levels of sodium intake. *Circ Res* 1976;39:788-797

COZZA EN, GOMEZ-SANCHEZ CE, FOEKING MF, CHIOU S: Endothelin binding to cultured calf adrenal zona glomerulosa cells and stimulation of aldosterone secretion. *J Clin Invest* 1989;84:1032-1035

DAVENPORT AP, NUNEZ DJ, HALL JA, KAUMANN AJ, BROWN MJ: Autoradiographical localization of binding sites for porcine [^{125}I]endothelin-1 in humans, pigs and rats: functional relevance in humans. *J Cardiovasc Pharmacol* 1989;13(Suppl 5):S166-S170

DE NUCCI G, THOMAS R, D'ORLEANS-JUSTE P, ANTUNES E, WALDER C, WARNER TD, VANE JR: Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc Nat Acad Sci USA* 1988;85:9797-9800

DE WARDENER HE: The primary role of the kidney and salt intake in the aetiology of essential hypertension: part I. *Clin Sci* 1990;79:193-200

DE WARDENER HE: The primary role of the kidney and salt intake in the aetiology of essential hypertension: part II. *Clin Sci* 1990;79:289-297

DIZ DI, PIRRO NT: Differential actions of angiotensin II and angiotensin-(1-7) on transmitter release. *Hypertension* 1992;19:II-41-II-48

DOHI Y, HAHN AWA, BOULANGER CM, BÜHLER FR, LÜSCHER TF: Endothelin stimulated by angiotensin II augments contractility of spontaneously hypertensive rat resistance arteries. *Hypertension* 1992;19:131-137

DOHI Y, HAHN AWA, BÜHLER FR, LÜSCHER TF: Angiotensin II sensitizes hypertensive resistance arteries by stimulating vascular endothelin production. *Hypertension* 1991:18(3);390 (abstract)

DUBIN D, PRATT RE, COOKE JP, DZAU VJ: Endothelin, a potent vasoconstrictor, is a vascular smooth muscle mitogen. *J Vasc Med Biol* 1989;1(No. 3):150-154

EDWARDS RM, TRIZNA W, OHLSTEIN EH: Renal microvascular effects of endothelin. *Am J Physiol* 1990;259(28):F217-F221

FERGUSON AV: The area postrema: a cardiovascular control centre at the blood-brain interface? *Can J Physiol Pharmacol* 1990a;69:1026-1034

FERGUSON AV, MARCUS P: Area postrema stimulation induced cardiovascular changes in the rat. *Am J Physiol* 1988;255:R855-R860

FERGUSON AV, SMITH P: Cardiovascular responses induced by endothelin microinjection into the area postrema. *Regul Peptide* 1990b;27:75-85

FERGUSON AV, SMITH P: Circulating endothelin influences area postrema neurons. *Regulatory Peptides* 1991;32:11-21

FERGUSON AV, SMITH P: Electrophysiological and cardiovascular evidence for endothelin actions within the area postrema. *Soc Neurosci Absts* 1989;15:965 (#3856)

FINK GD, BRUNER C, MANGIAPANE MM: Area postrema is critical for angiotensin-induced hypertension in rats. *Hypertension* 1987;9:355-361

FOLTA A, LEE L, JOSHUA IG, WEBB RC: Dietary salt increases coronary vasoconstrictor sensitivity to endothelin-1. *Coronary Artery Dis* 1991;2:95-99

FRANCO-CERECEDA A: Endothelin- and neuropeptide Y-induced vasoconstriction of human epicardial coronary arteries in vitro. *Br J Pharmacol* 1989;97:968-972

FUKUDA N, IZUMI Y, SOMA M, WATANABE Y, WATANABE M, HATANO M, SAKUMA I, YASUDA H: L-N^G-Monomethyl arginine inhibits the vasodilating effects of low dose of endothelin-3 on rat mesenteric arteries. *Biochem Biophys Res Commun* 1990;167:739-745

FUKUDA Y, HIRATA Y, TAKETANI S, KOJIMA T, OIKAWA S, NAKAZATO H, KOBAYASHI Y: Endothelin stimulates accumulations of cellular atrial natriuretic peptide and its messenger RNA in rat cardiocytes *Biochem Biophys Res Commun* 1989;164(3):1431-4136

FUKUDA Y, HIRATA Y, YOSHIMI H, TAKATSUGU K, KOBAYASHI Y, YANAGISAWA M, MASAKI T: Endothelin is a potent secretagogue for atrial natriuretic peptide in cultured rat atrial myocytes. *Biochem Biophys Res Commun* 1988;155:167-172

FURCHGOTT RF, ZAWADSKI J: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;288:373-376

FYHRQUIST F, SAIJONMAA O, MATSÄRINNE K, TIKKANEN I, ROSENLÖF K, TIKKANEN T: Raised plasma endothelin-1 concentration following cold pressor test. *Biochem Biophys Res Commun* 1990;169:217-221

GALRON R, BDOLAH A, KLOOG Y, SOKOLOVSKY M: Endothelin/sarafotoxin receptor induced phosphoinositide turnover: Effects of pertussis and cholera toxins and of phorbol ester. *Biochem Biophys Res Commun* 1990;171:949-954

GARDINER SM, COMPTON AM, BENNETT T: Effects of indomethacin on the regional haemodynamic responses to low doses of endothelins and sarafotoxin. *Br J Pharmacol* 1990;100:158-162

GIAID A, GIBSON SJ, IBRAHIM NBN, LEGON S, BLOOM SR, YANAGISAWA M, MASAKI T, VARNDELL IM, POLAK JM: Endothelin 1, an endothelium-derived peptide, is expressed in neurons of the human spinal cord and dorsal root ganglia. *Proc Natl Acad Sci USA* 1989;86:7634-7638

GILLESPIE MN, OWASOYO JO, MCMURTRY IF, O'BRIEN RF: Sustained coronary vasoconstriction provoked by a peptidergic substance released from endothelial cells in culture. *J Pharmacol Exp Ther* 1985;236:339-343

GIVEN M, LOWE R, LIPPTON H, HYMAN A, SANDER G, GILES T: Hemodynamic actions of endothelin in conscious and anesthetized dogs. *Peptides* 1989;10:41-44

GOETZ KL, WANG BC, MADWED JB, ZHU JJ, LEADLEY RJ JR: Cardiovascular, renal, and endocrine responses to intravenous endothelin in conscious dogs. *Am J Physiol* 1988;255(6):R1064-R1068

GOLDSCHMIDT JE, TALLARIDA RJ: Pharmacological evidence that captopril possesses an endothelium-mediated component of vasodilation: effect of sulfhydryl groups on endothelium-derived relaxing factor. *J Pharmacol Exp Therap* 1991:257(3);1136-1145

GOTO K, KASUYA Y, MATSUKI N, TAKUWA Y, KURIHARA H, ISHIKAWAV T, KIMURA S, YANAGISAWA M, MASAKI T: Endothelin activates dihydropyridine-sensitive, voltage-dependent calcium channels in vascular smooth muscle. *Proc Natl Acad Sci USA* 1989;86:3915-3918

GROSS DM, SWEET CS, ULM EH, BACKLUND EP, MORRIS AA, WEITZ D, BOHN DL, WENGER HC, VASSIL TC, STONE CA: Effect of N-[(S)-1-carboxy-3-phenylpropyl]-L-Ala-L-Pro and its ethyl ester (MK-421) on angiotensin converting enzyme in vitro and angiotensin I pressor response in vivo. *J Pharmacol Exp Therap* 1980;216:552-557

GRUBER KA, WHITAKER JM, BUCKALEW VM: Endogenous digitalis-like substance in plasma of volume-expanded dogs. *Nature* 1980;287:743-745

GULATI A, REBELLO S: Down-regulation of endothelin receptors in the ventrolateral medulla of spontaneously hypertensive rats. *Life Sci* 1991;48:1207-1215

GUYTON AC, COLEMAN TG, COWLEY, JR AW, SCHEEL KW, MANNING RD, NORMAN, JR RA: Arterial pressure regulation: overriding dominance of the kidneys in long-term regulation and in hypertension. *Am J Med* 1972;52:584-594

GUYTON AC, COLEMAN TG: Quantitative analysis of the pathophysiology of hypertension. *Circ Res* 1969;24/25(Suppl I):I1-I19

HADDY FJ: Digitalis-like circulating factor in hypertension: potential messenger between salt balance and intracellular sodium. *Cardiovasc Drugs Ther* 1990;4:343-349

HAN SP, TRAPANI AJ, FOK KF, WESTFALL TC, KNUEPFER MM: Effects of endothelin on regional hemodynamics in conscious rats. *Eur J Pharmacol* 1989;159:303-305

HASSER EM, NELSON DO, HAYWOOD JR, BISHOP VS: Inhibition of renal sympathetic neurvous activity by area postrema stimulation in rabbits. *Am J Physiol* 1987;253:H91-H99

HAYWOOD JR, SHAFFER RA, FASTENOW C, FINK GD, BRODY MJ: Regional blood flow measurement with pulsed Doppler flowmeter in conscious rat. *Am J Physiol* 1981;241(10):H273-H278

HINOJOSA-LABORDE C, OSBORN J, COWLEY A JR: Hemodynamic effects of endothelin in conscious rats. *Am J Physiol* 1989;256:H1742-1746

HIRAI M, MIYABO S, OOYA E, MIYANAGA K, AOYAGI N, KIMURA K, KISHIDA S, NAKAI T: Endothelin-3 stimulates the hypothalamic-pituitary-adrenal axis. *Life Sci* 1991;48:2359-2363

HIRATA Y, MATSUNAGA T, ANDO K, FURUKAWA T, TSUKAGOSHI H, MARUMO F: Presence of endothelin-1-like immunoreactivity in human cerebrospinal fluid. *Biocehm Biophys Res Commun* 1990;166:1274-1278

HIRATA Y, YOSHIMI H, EMORI T, SCHICHIRI M, MARUMO F, WATANABE TX, KUMAGAE S, NAKAJIMA K, KIMURA T, SAKAIBARA S: Receptor binding activity and cytosolic free calcium response by synthetic endothelin analogues in cultured rat vascular smooth muscle cells. *Biochem Biophys Res Commun* 1989;160:228-234

HIRATA Y, YOSHIMI H, TAKAICHI S, YANAGISAWA M, MASAKI T: Binding and receptor down-regulation of a novel vasoconstrictor endothelin in cultured rat vascular smooth muscle cells. *FEBS Letters* 1988;239:13-17

HIRATA Y, YOSHIMI H, TAKATA S, WATANABE TX, KUMAGAI S, NAKAJIMA N, SAKAKIBARA S: Cellular mechanism of action by a novel vasoconstrictor endothelin in cultured rat vascular smooth muscle cells. *Biochem Biophys Res Comm* 154;1988:868-875

HOFFMAN A, KEISER HR, GROSSMAN E, GOLDSTEIN DS, GOLD PW, KLING M: Endothelin concentrations in cerebrospinal fluid in depressive patients. *Lancet* 1989;2:1519

HOYER D, WAEBER C, PALACIOS, JM: [^{125}I]Endothelin-1 binding sites: autoradiographic studies in the brain and periphery of various species including humans. *J Cardiovasc Pharmacol* 1989;13(Suppl 5):S162-S165

HU JR, HARSDORF RV, LANG RE: Endothelin has potent inotropic effects in rat atria. *Eur J Pharmacol* 1988;158:275-278

HUMKE U, LEVENS N, WOOD J, HOFBAUER K: The acute renal actions of angiotensin converting enzyme inhibitors in the sodium-depleted conscious primate are mediated by inhibition of the renin-angiotensin system. *J Pharmacol Exp Therap* 1991;260:29-35.

INOUE A, YANGISAWA M, KIMURA S, KASUYA Y, MIYAUCHI T, GOTO K, MASAKI T: The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Natl Acad Sci* 1989;86:2863-2867,

INOUE A, YANAGISAWA M, TAKUWA Y, MITSUI Y, KOBAYASHI M, MASAKI T: The human preproendothelin-1 gene. *J Biol Chem* 1989;264:14954-14959

INOUE Y, OIKE M, NAKAO K, KITAMURA K, KURIYAMA H: Endothelin augments unitary calcium channel currents on the smooth muscle cell membrane of guinea pig portal vein. *J Physiol* 1990;423:171-191

ISHIKAWA T, YANAGISAWA M, KIMURA S, GOTO K, MASAKI T: Positive chronotropic effects of endothelin, a novel endothelium-derived vasoconstrictor peptide. *Pflugers Arch* 1988;413:108-110

ISHIKAWA T, YANAGISAWA M, KIMURA S, GOTO K, MASAKI T: Positive inotropic action of a novel endothelium-derived vasoconstrictor peptide, endothelin, on guinea pig atria. *Am J Physiol* 1988;255:H970-H973

ITOH S, VAN DEN BUSSE M: Sensitization of baroreceptor reflex by central endothelin in conscious rats. *Am J Physiol* 1991;260:H1106-H1112

ITOH Y, YANAGISAWA M, OHKUBO S, KIMURA C, KOSAKA T, INOUE A, ISHIDA N, MITSUI Y, ONDA H, FUJINO M, MASAKI T: Cloning and sequence of cDNA encoding the precursor of a human endothelium-derived vasoconstrictor peptide, endothelin: Identity of human and porcine endothelin. *FEBS Lett* 1988;231:440-444

IVES HE: Ion transport defects and hypertension: Where is the link? *Hypertension* 1989;14(6):590-597

JAISWAL N, DIZ DI, CHAPPELL MC, KHOSLA MC, FERRARIO CM: Stimulation of endothelial cell prostaglandin production by angiotensin peptides. *Hypertension* 1992;19:II-49-II-55

JONES CR, KILEY CR, PELTON JT, MOHR M: Autoradiographic localisation of the binding sites for [^{125}I]endothelin in the rat and human brain. *Neurosci Lett* 1989;97:276-279

JOSHUA IG: Endothelin-induced vasoconstriction of small resistance vessels in the microcirculation of the rat cremaster muscle. *Microvasc Res* 1990;40:191-198

KAI H, KANAIDE H, NAKAMURA M: Endothelin-sensitive intracellular calcium store overlaps with caffeine-sensitive one in rat aortic smooth muscle cells in primary culture. *Biochem Biophys Res Commun* 1989;158:235-243

KANAGY NL, PAWLOSKI CM, FINK GD: Role of aldosterone in angiotensin II-induced hypertension in rats. *Am J Physiol* 1990;259:R102-R109

KATAOKA Y, KOIZUMI S, KUMAKURA K, KURIHARA M, NIWA M, UEKI S: Endothelin-triggered brain damage under hypoglycemia evidenced by real-time monitoring of dopamine release from rat striatal slices. *Neurosci Lett* 1989;107:75-80

KATOH T, CHANG H, UCHIDA S, OKUDA T, KUROKAWA K: Direct effects of endothelin in the rat kidney. *Am J Hypertension* 1990;258(27):F397-F402

KAWAGUCHI H, SAWA H, YASUDA H: Effect of endothelin on angiotensin converting enzyme activity in cultured pulmonary artery endothelial cells. *J Hypertens* 1991:9;171-174

KAWAGUCHI H, SAWA H, YASUDA H: Endothelin stimulates angiotensin I to angiotensin II conversion in cultured pulmonary artery endothelial cells. *J Mol Cell Cardiol* 1990:22;839-842

KIMURA S, KASUYA Y, SAWAMURA T, SHINMI O, SUGITA Y, YANAGISAWA M, GOTO K, MASAKI T: Structure-activity relationships of endothelin: importance of the c-terminal moiety. *Biochem Biophys Res Comm* 1988;156:1182-1186

KING AJ, BRENNER BM, ANDERSON S: Endothelin: a potent renal and systemic vasoconstrictor peptide. *Am J Physiol* 1989;256(25):F1051-F1058

KING BD, SACK D, KICHUK MR, HINTZE TH: Absence of hypertension despite chronic marked elevations in plasma norepinephrine in conscious dogs. *Hypertension* 1987;9:582-590

KING G, JOHNSON A: Receptor-mediated transport of insulin across endothelial cells. *Science* 1985;227:1583-1586

KITAMURA K, YUKAWA T, MORITA S, ICHIKI Y, ETO T, TANAKA T: Distribution and molecular form of immunoreactive big endothelin-1 in porcine tissue. *Biochem Biophys Res Commun* 1990;170:497-503

KNUEPFER MM, HAN SP, TRAPANI AJ, FOK KF, WESTFALL TC: Regional hemodynamic and baroreflex effects of endothelin in rats. *Am J Physiol* 1989;257(26):H918-H926

KOHNO M, MURAKAWA K, HORIO T, KURIHARA N, YOKOKAWA K, YASUNARI K, FUKUI T, TAKEDA T: Endothelin stimulates release of atrial natriuretic factor in anesthetized rats. *Metabolism* 1990;39(6):557-559

KOMANICKY P, MELBY JC: Hypertensinogenic potencies of aldosterone and deoxycorticosterone in the rat. *Hypertension* 1982;4:140-145

KOMURO, I, H KURIHARA, T SUGIYAMA, F TAKAKU AND Y YAZAKI: Endothelin stimulates *c-fos* and *c-myc* expression and proliferation of vascular smooth muscle cells. *FEBS Lett* 1988;238:249-252

KON V, YOSHIOKA T, FOGO A, ICHIKAWA I: Glomerular actions of endothelin *in vivo*. *J Clin Invest* 1989;83:1762-1767

KOSEKI C, IMAI M, HIRATA Y, YANAGISAWA M, MASAKI T: Binding sites for endothelin-1 in rat tissues: An autoradiographic study. *J Cardiovasc Pharmacol* 1989;13:S153-S154

KOSEKI C, IMAI M, HIRATA Y, YANAGISAWA M, MASAKI, T: Autoradiographic distribution in rat tissues of binding sites for endothelin: a neuropeptide? *Am J Physiol* 1989;256(25):R858-R866

KOSEKI C, IMAI M, HIRATA M, YANAGISAWA M, MASAKI T: Autoradiographic distribution of binding site for [^{125}I]-endothelin in rat tissues. In: *Abstracts of International Symposium on Biosignaling in Cardiac and Vascular Systems* p37, 1988

KOSEKI C, IMAI M, HIRATA Y, YANAGISAWA M, MASAKI, T: Autoradiographic localization of [^{125}I]-endothelin-1 binding sites in rat brain. *Neurosci Res* 1989;6:581-585

KOZUKA M, ITO T, HIROSE S, TAKAHASHI K, HAGIWARA H: Endothelin induces two types of contractions of rat uterus: Phasic contractions by way of voltage-dependent calcium channels and developing contractions through a second type of calcium channels. *Biochem Biophys Res Commun* 1989;159:317-323

KRÄMER BK, SMITH TW, KELLY RA: Endothelin and increased contractility in adult rat ventricular myocytes. *Circ Res* 1991;68:269-279

KRIEGER JE, ROMAN RJ, COWLEY AW: Hemodynamics and blood volume in angiotensin II salt-dependent hypertension in dogs. *Am J Physiol* 1989;257(26):H1402-H1412

KUWAKI T, KOSHIYA N, CAO W, TAKAHASHI H, TERUI N, KUMADA M: Modulatory effects of endothelin-1 on central cardiovascular control in rats. *Jap J Physiol* 1990;40:827-841

LE MONNIER DE GOUVILLE AC, MONDOT S, LIPPTON H, HYMAN H, CAVERO I: Hemodynamic and pharmacological evaluation of the vasodilator and vasoconstrictor effects of endothelin-1 in rats. *J Pharmacol Exp Ther* 1990;252:300-311

LEE M-E, DE LA MONTE SM, NG S-C: Expression of the potent vasoconstrictor endothelin in the human central nervous system. *J Clin Invest* 1990;86:141-147

LERMAN A, CLICK RL, NARR BJ, WIESNER RH, KROM RAF, TEXTOR SC, BURNETT JC: Elevation of plasma endothelin associated with systemic hypertension in humans following orthotopic liver transplantation. *Transplant* 1991;51(3):646-650

LERMAN A, HILDEBRAND FL, AARHUS LL, BURNETT JC: Endothelin has biological actions at pathophysiological concentrations. *Circulation* 1991;83:1808-1814

LIPPTON, H, J GOFF AND A HYMAN: Effects of endothelin in the systemic and renal vascular beds *in vivo*. *Eur J Pharmacol* 1988;155:197-199

LIU J, CHEN R, CASLEY DJ, NAYLER, WG: Ischemia and reperfusion increase ^{125}I-labeled endothelin-1 binding in rat cardiac membranes. *Am J Physiol* 1990;258(27):H829-H835

LÓPEZ-FARRÉ A, MONTAÑÉS I, MILLÁS I, LÓPEZ-NOVOA JM: Effect of endothelin on renal function in rats. *Eur J Pharmacol* 1989;163:187-189

LUFT FC, WILCOX CS, UNGER T, KUHN R, DEMMERT G, ROHMEISS P, GANTEN D, STERZEL RB: Angiotensin-induced hypertension in the rat. Sympathetic nerve activity and prostaglandins. *Hypertension* 1989;14:396-403

LÜSCHER TF, YANG Z, DIEDERICH D, BÜHLER FR: Endothelium-derived vasoactive substances: potential role in hypertension, atherosclerosis, and vascular occlusion. *J Cardiovasc Pharmacol* 1989;14(Suppl 6):S63-S69

MACCUMBER MW, ROSS CA, GLASER BM, SNYDER SH: Endothelin: visualization of mRNAs by in situ hybridization provides evidence for local action. *Proc Natl Acad Sci USA* 1989;86:7285-7289

MACQUIN-MAVIER I, LEVAME M, ISTIN N, HARF A: Mechanisms of endothelin-mediated bronchoconstriction in the guinea pig. *J Pharmacol Exp Ther* 1989;250(2):740-745

MAGGI CA, GIULAIANI S, PARACCHINI R, SANTICIOLI P, ROVERO P, GIACHETTI A, MELI A: The C-terminal hexapeptide, endothelin-(16-21), discriminates between different endothelin receptors. *Eur J Pharmacol* 1989;166:121-122

MARGULIES KB, HILDEBRAND FL, LERMAN A, PERRELLA MA, BURNETT JC: Increased endothelin in experimental heart failure. *Circulation* 1990;82:2226-2230

MARTIN ER, BRENNER BM, BALLERMANN BJ: Heterogeneity of cell surface endothelin receptors. *J Biol Chem* 1990;265:14044-14049

MASAKI T, YANAGISAWA M, GOTO K, KIMURA S: Role of endothelin in mechanisms of local blood pressure control. *J Hypertens* 1990;8(suppl):S107-S112

MASAOKA H, SUZUKI R, HIRATA Y, EMORI T, MARUMO F, HIRAKAWA K: Raised plasma endothelin in aneurysmal subarachnoid haemorrhage. *Lancet* 1989;1402

MASUDA Y, MIYAZAKI H, KONDOH M, WATANABE H, YANAGISAWA M, MASAKI T, MURAKAMI K: Two different forms of endothelin receptors in rat lung. *FEBS Lett* 1989;257(2):208-210

MATSUMOTO H, SUZUKI N, ONDA H, FUJINO M: Abundance of endothelin-3 in rat intestine, pituitary gland and brain. *Biochem Biophys Res Commun* 1989;164:74-80

MATSUMURA Y, NAKASE K, IKEGAWA R, HAYASHI K, OHYAMA T, MORIMOTO S: The endothelium-derived vasoconstrictor peptide endothelin inhibits renin release in vitro. *Life Sci* 1989;44:149-157

MAZZOCCHI G, REBUFFAT P, MENEGHELLI V, MALENDOWICZ LK, KASPRZAK A, NUSSDORFER GG: Effects of prolonged infusion with endothelin-1 on the function and morphology of rat adrenal cortex. *Peptides* 1990;11:767-772

MENDELSOHN FAO, QUIRION R, SAAVEDRA JM, AGUILERA G: Autoradiographic localization of angiotensin II receptors in rat brain. *Proc Natl Acad Sci USA* 1984;81:1575-1579

MIASIRO N, YAMAMOTO H, KANAIDE H, NAKAMURA M: Does endothelin mobilize calcium from intracellular store sites in rat aortic vascular smooth muscle cells in primary culture? *Biochem Biophys Res Commun* 1988;156:312-317

MILLER WL, REDFIELD MM, BURNETT, JR JC: Integrated cardiac, renal and endocrine actions of endothelin. *J Clin Invest* 1989;83:317-320

MINKES RK, COY DH, MURPHY WA, MCNAMARA DB, KADOWITZ PJ: Effects of porcine and rat endothelin and an analog on blood pressure in the anesthetized cat. *Eur J Pharmacol* 1989;164:571-575

MITCHELL J, BOHR DF: Experimental hypertension in the pig. In: *Experimental genetic models of hypertension* Edited by W DeJong New York: Elsevier, 1984:147

MIYAUCHI T, YANAGISAWA M, TOMIZAWA T, SUGISHATA Y, SUZUKI N, FUJINO M, AJISAKA P, GOTO K, MASAKI T: Increased plasma concentrations of endothelin-1 and big endothelin-1 in acute myocardial infarction. *Lancet* 1989;2:53-54

MOSER PC, PELTON JT: Behavioural effects of centrally administered endothelin in the rat. *Br J Pharmacol* 1989;96:347P

NAGASE T, FUKUCHI Y, JO C, TERAMOTO S, UEJIMA Y, ISHIDA K, SHIMIZU T, ORIMO H: Endothelin-1 stimulates arachidonate 15-lipoxygenase activity and oxygen radical formation in the rat distal lung. *Biochem Biophys Res Commun* 1990:168(2);485-489

NAKAJIMA K, KUBO S, KUMAGAYE S, NISHIO H, TSUNEMI M, INUI T, KURODA H, CHINO N, WATANABE TX, KIMURA T, SAKAKIBARA S: Structure-activity relationship of endothelin: Importance of charged groups. *Biochem Biophys Res Commun* 1989;163:424-429

NISHIO I, SHIMA H, TSUDA K, HANO T, MASUYAMA Y: Relationship between the sympathetic nervous system and sodium potassium adenosine triphosphatase inhibitor in salt-sensitive patients with essential hypertension. *J Hypertens* 1988;6(4):S216-S218

O'BRIEN RF, ROBBINS RJ, MCMURTRY IF: Endothelial cells in culture produce a vasoconstrictor substance. *J Cell Physiol* 1987;132:263-270

ÖHLEN A, RAUD J, HEDQVIST P, WIKLUND NP: Microvascular effects of endothelin in the rabbit tenissimus muscle and hamster cheek pouch. *Microvasc Res* 1989;37:115-118

OHLSTEIN EH, HOROHONICH S, HAY DWP: Cellular mechanisms of endothelin in rabbit aorta. *J Pharmacol Exp Ther* 1989;250(2):548-555

OHLSTEIN EH, VICKERY L, SAUERMELCH C, WILLETTE RN: Vasodilation induced by endothelin: role of EDRF and prostanoids in rat hindquarters. *Am J Physiol* 1990:259(28);H1835-H1841

ÖHMAN KP, HOFFMAN A, KEISER HR: Endothelin-induced vasoconstriction and release of atrial natriuretic peptides in the rat. *Acta Physiol Scand* 1990;138:549-556

OKADA K, MIYAZAKI Y, TAKADA J, MATSUYAMA K, YAMAKI T, YANO M: Conversion of big endothelin-1 by membrane-bound metalloendopeptidase in cultured bovine endothelial cells. *Biochem Biophys Res Commun* 1990;171:1192-1198

OLSEN M, HALL J, MONTANI J-P, GUYTON A, LANGFORD H, CORNELL J: Mechanisms of AngII natriuresis and antinatriuresis. *Am J Physiol* 1985;249:F299-307

OUCHI Y, KIM S, SOUZA AC, IIJIMA S, HATTORI A, ORIMO H, YOSHIZUMI M, KURIHARA H, YAZAKI Y: Central effect of endothelin on blood pressure in conscious rats. *Am J Physiol* 1989;256(25):H1747-H1751

PAPAS S, SMITH P, FERGUSON AV: Electrophysiological evidence that systemic angiotensin influences rat area postrema neurons. *Am J Physiol* 1990;258:R70-R76

PAWLOSKI C: Angiotensin II and arterial pressure regulation in hypertension. Dissertation 1990; Michigan State University

PAWLOSKI C, EICKER N, BALL L, MANGIAPANE M, FINK M: Effect of circulating vasopressin on arterial pressure regulation in rats. *Am J Physiol* 1989;257:H209-218

PAWLOSKI CM, FINK GD: Circulating angiotensin II and drinking behavior in rats. *Am J Physiol* 1990:259(28);R531-R538

RAKUGI H, NAKAMURA M, SAITO H, HIGAKI J, OGIWARA T: Endothelin inhibits renin release from isolated rat glomeruli. *Biochem Biophys Res Comm* 1988;155:1244-1247

RAKUGI H, NAKAMARU M, TABUCHI Y, NAGANO M, MIKAMI H, OGIHARA, T: Endothelin stimulates the release of prostacyclin from rat mesenteric arteries. *Biochem Biophys Res Commun* 1989;160(2):924-928

REID AF, PARKES DG, COGHLAN JP, SCOGGINS BA, WHITWORTH JA: Haemodynamic effects of long-term endothelin infusion in conscious sheep. *Clin Exper Pharmacol Physiol* 1990;17:241-245

REISER G, DONIE F: Endothelin induces a rise of inositol 1,4,5-trisphosphate, inositol 1,3,4,5 tetrakisphosphate levels and of cytosolic calcium activity in neural cell line. *Eur J Neurosci* 1989;25:221-225

RESNIK TJ, SCOTT-BURDEN T, BÜHLER FR: Activation of phospholipase A2 by endothelin in cultured vascular smooth muscle cells. *Biochem Biophys Res Commun* 1989;158:279-286

ROUBERT P, GILLARD V, PLAS P, GUILLON JM, CHABRIER PE, BRAQUET P: Angiotensin II and phorbol-esters potently down-regulate endothelin (ET-1) binding sites in vascular smooth muscle cells. *Biochem Biophys Res Commun* 1989;164:809-815

RUBANYI GM: Endothelium-derived vasoconstrictor factors. In: *Endothelial Cells* (Ed. Ryan US), Vol. 3, pp. 61-74. CRC Press, Boca Raton, FL, 1988.

RUBANYI GM: Maintenance of "basal" vascular tone may represent a physiological role for endothelin. *J Vasc Med Biol* 1989:1;315-316

RUBANYI GM, PARKER BOTELHO LH: Endothelins *FASEB J* 1991:5(12);2713-2720

RUBIN B, LAFFAN RJ, KOTLER DG, O'KEEFE EH, DEMAIO DA, GOLDBERG ME: SQ 14,225 (D-3-mercapto-2-methylpropranoyl-1-proline), a novel orally active inhibitor of angiotensin I converting enzyme. *J Pharmacol Exp Ther* 1978:204;271-280

SAAVEDRA J, ISRAEL A, PLUNKETT L, KURIHARA M, SHIGEMATSU K, COREA M: Quantitative distribution of AngII binding sites in rat brain by autoradiography. *Peptides* 1986;7:679-687

SAITO T, YANAGISAWA M, MIYAUCHI T, SUZUKI N, MATSUMOTO H, FUJINO M, MASAKI T: Endothelin in human circulating blood: effects of major surgical stress (abstract). *Jpn J Pharmacol* 1989;49(Suppl):215P

SAITO Y, NAKAO K, ITOH H, YAMADA T, MUKOYAMA M, ARIA H, HOSODA K, SHIRAKAMI G, SUGA S, JOUGASAKI M, MORICHIKA S, IMURA H: Endothelin in human plasma and culture medium of aortic endothelial cells-detection and characterization with radioimmunoassay using monoclonal antibody. *Biochem Biophys Res Comm* 1989;161:320-326

SAITO Y, NAKAO K, MUKOYAMA M, IMURA H: Increased plasma endothelin level in patients with essential hypertension. *N Engl J Med* 1990;322:205

SAKURAI T, YANAGISAWA M, TAKUWA Y, MIYAZAKI H, KIMURA S, GOTO K, MASAKI T: Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. *Nature* 1990;348:732-735

SANTOS RAS, BROSNIHAN KB, JACOBSEN DW, DICORLETO PE, FERRARIO CM: Production of angiotensin-(1-7) by human vascular endothelium. *Hypertension* 1992;19:II-56-II-61

SAWAMURA T, KIMURA S, SHINMI O, SUGITA Y, YANAGISAWA M, MASAKI T: Analysis of endothelin related peptides in culture supernatant of porcine aortic endothelial cells: Evidence for biosynthetic pathway of endothelin-1. *Biochem Biophys Res Commun* 1989;162:1287-1294

SCHIFFRIN EL, THIBAULT G: Plasma endothelin in human essential hypertension. *Am J Hypertens* 1991;4:303-308

SCHVARTZ I, ITTOOP O, HAZUM E: Bovine cerebellum endothelin receptor: solubilization and identification. *Endocrinology* 1990;126:3218-3222

SECREST RJ, COHEN ML: Endothelin: differential effects in vascular and nonvascular smooth muscle. *Life Sci* 1989;45:1365-1372

SELYE H, HALL CE, ROWLEY EM: Malignant hypertension produced by treatment with deoxycorticosterone acetate and sodium chloride. *Can Med Assoc J* 1943;49:88-92

SERRADEIL-LE GAL C, JOUNEAUX C, SANCHEZ-BUENO A, RAUFASTE D, ROCHE B, PRÉAUX AM, MAFFRAND JP, COBBOLD PH, HANOUNE J, LOTERSZTAJN, S: Endothelin action in rat liver. *J Clin Invest* 1991;87:133-138

SETO S, AKAHOSHI M, OZEKI S, NAGAO S, KUSANO S, ODA S, HASHIBA K: Pressor effect of centrally administered endothelin and its possible relation to the calcium-calmodulin system. *Circulation 80* 1989;Suppl. II:II-203

SHAH A, LEWIS M, HENDERSON A: Inotropic effects of endothelin in ferret ventricular myocardium. *Eur J Pharmacol* 1989;163:365-367

SHAPIRO RE, MISELIS RR: The central neural connections of the area postrema of the rat. The area postrema in the rat. *J Comp Neurol* 1984;234:344-364

SHIBA R, YANAGISAWA M, MIYAUCHI T, ISHII Y, KIMURA S, UCHIYAMA Y, MASAKI T, GOTO K: Elimination of intravenously injected endothelin-1 from the circulation of the rat. *J Cardiovasc Pharmacol* 1989;13(suppl 5):S98-S101

SHICHIRI M, HIRATA Y, KANNO K, OHTA K, EMORI T, MARUMO J: Effect of endothelin-1 on release of arginine vasopressin from perfused rat hypothalamus. *Biochem Biophys Res Commun* 1989;163:1332-1337

SHICHIRI M, HIRATA Y, ANDO K, EMORI T, OHTA K, KIMOTO S, OGURA M, INOUE A, MARUMO F: Plasma endothelin levels in hypertension and chronic renal failure. *Hypertension* 1990;15:493-496

SHIGENO T, MIMA T, TAKAKURA K: Endothelin-1 acts in cerebral arteries from the adventitial but not from the luminal side. *J Cardiovasc Pharmacol* 1989;13(suppl 5):S174-176

SHINMI O, KIMURA S, SAWAMURA T, SUGITA Y, YOSHIZAWA T, UCHIYAMA Y, YANAGISAWA M, GOTO K, MASAKI T, KANAZAWA I: Endothelin-3 is a novel neuropeptide: isolation and sequence determination of endothelin-1 and endothelin-3 in porcine brain. *Biochem Biophys Res Commun* 1989;164:587-593

SHINMI O, KIMURA S, YOSHIZAWA T, SAWAMURA T, UCHIYAMA Y, SUGITA Y, KANAZAWA I, YANAGISAWA M, GOTO K, MASAKI T: Presence of endothelin-1 in porcine spinal cord: isolation and sequence determination. *Biochem Biophys Res Commun* 1989;162(1):340-346

SPOKES RA, GHATEI MA, BLOOM SR: Studies with Endothelin-3 and Endothelin-1 on rat blood pressure and isolated tissues: Evidence for multiple endothelin receptor subtypes. *J Card Pharmacol* 1989;13(Suppl 5):S191-192

SPORN MB, ROBERTS AB: Peptide growth factors are multifunctional. *Nature* 1988;332:217-219

STEWART DJ, KUBAC G, COSTELLO KB, CERNACEK P: Increased plasma endothelin-1 in the early hours of acute myocardial infarction. *J Am Coll Cardiol* 1991;18(1):38-43

STEWART DJ, LANGLEBEN D, CERNACEK P, CIANFLONE K: Endothelin release is inhibited by coculture of endothelial cells with cells of vascular media. *Am J Physiol* 1990;259:H1928-H1932

STEWART DJ, LEVY RD, CERNACEK P, LANGLEBEN D: Increased plasma endothelin-1 in pulmonary hypertension: marker or mediator of disease? *Ann Intern Med* 1991;114(6):464-469

SUZUKI N, MIYAUCHI T, TOMOBE Y, MATSUMOTO H, GOTO K, MASAKI T, FUJINO M: Plasma concentrations of endothelin-1 in spontaneously hypertensive rats and DOCA-salt hypertensive rats. *Biochem Biophys Res Commun* 1990;167(3):941-947

SWALES JD, TANGE JD: The influence of acute sodium depletion on experimental hypertension in the rat. *J Lab Clin Med* 1971;78:369-379

SWARTZ SL, WILLIAMS GH, HOLLENBERG NK, CRANTZ FR, LEVINE L, MOORE TJ, DLUHY RG: Increase in prostaglandins during converting enzyme inhibition. *Clin Sci* 1980;59:133S-135S

SWEET CS, GROSS DM, ARBEGAST PT, GAUL SL, BRITT PM, LUDDEN CT, WEITZ D, STONE CA: Antihypertensive activity of N-[(S)-1-(ethoxycarbonyl)-3-phenylpropyl]-L-Ala-L-Pro (MK-421), an orally active converting enzyme inhibitor. *J Pharmacol Exp Therap* 1980;216:558-566

SWEET CS: Pharmacological properties of the converting enzyme inhibitor, enalapril maleate (MK-421). *Federation Proc* 1983;42:167-170

TABUCHI Y, NAKAMARU M, RAKUGI H, NAGANO M, HIGASHIMORI K, MIKAMI H, OGIHARA T: Effects of endothelin on neuroeffector junction in mesenteric arteries of hypertensive rats. *Hypertension* 1990;15:739-743

TAKAGI M, MATSUOKA H, ATARASHI K, YAGI S: Endothelin: a new inhibitor of renin release. *Biochem Biophys Res Commun* 1988;157(3):1164-1168

TAKAHASHI H, MATSUZAWA M, IKEGAKI I: Effect of a vasoconstrictive peptide, endothelin, on blood pressure and sympathetic nerve activity. *Jpn J Hyper* 1988;11:25

TAKASAKI C, TAMIYA N, BDOLAH A, WOLLBERG Z, KOCHVA E: Sarafotoxins S6: several isotoxins from *Atractaspis engaddensis* (burrowing asp) venom that affect the heart. *Toxicon* 1988;26:543-548

TAKUWA N, TAKUWA Y, YANAGISAWA M, YAMASHITA M, MASAKI T: A novel vasoactive peptide endothelin stimulates mitogenesis through inositol lipid turnover in Swiss 3T3 fibroblasts. *J Biol Chem* in press

TAKUWA Y, KASUYA Y, TAKUWA N, KUDO M, YANAGISAWA M, GOTO K, MASAKI T, YAMASHITA K: Endothelin receptor is coupled to phospholipase C via a pertussis toxin-insensitive guanine-nucleotide binding regulatory protein in vascular smooth muscle cells. *J Clin Invest* 1990;85:653-658

THIEMERMANN C, LIDBURY P, THOMAS R, VANE JR: Endothelin inhibits ex vivo platelet aggregation in the rabbit. *Eur J Pharmacol* 1988;158:181-182

THOMPSON RM, LERMAN A, AARHUS LL, BURNETT JC: The effect of dietary sodium on the pressor and endocrine action of endothelin. *JACC* 1991;17(2):238A(abstract)

TOFOVIC SP, PONG AS, JACKSON EK: Effects of angiotensin subtype 1 and subtype 2 receptor antagonists in normotensive versus hypertensive rats. *Hypertension* 1991;18:774-782

TOMOBE Y, ISHIKAWA T, YANAGISAWA M, KIMURA S, MASAKI T, GOTO K: Mechanisms of altered sensitivity to endothelin-1 between aortic smooth muscles of spontaneously hypertensive and Wistar-Kyoto rats. *J Pharmacol Exp Ther* 1991;257(2):555-561

TOMOBE Y, MIYAUCHI T, SAITO A: Effects of endothelin on the renal artery from spontaneously hypertensive and Wistar-Kyoto rats. *Eur J Pharmacol* 1988;152:373-374

UCHIDA Y, NINOMIYA H, SAOTOME M, NOMURA A, OHTSUKA M, YANAGISAWA M, GOTO K, MASAKI T, HASEGAWA S: Endothelin, a novel vasoconstrictor peptide, as potent bronchoconstrictor. *Eur J Pharmacol* 1988;154:227-228

VAN DER KOOY D, KODA LY: Organization of the projections of a circumventricular organ: The area postrema in the rat. *J Comp Neurol* 1983;219:328-338

VAN RENTERGHEM, C, P VIGNE, J BARHANIN, A SCHMID-ALLIANA, C FRELIN, LAZDUNSKI M: Molecular mechanism of action of the vasoconstrictor peptide endothelin. *Biochem Biophys Res Commun* 1988;157:977-985

VIERHAPPER H, WAGNER O, NOWOTNY P, WALDHÄUSEL W: Effect of endothelin-1 in man. *Circulation* 1990;81:1415-1418

VIJAYARAGHAVAN J, GUILLERMO-SCICLI A, CARRETERO OA, SLANGHTER C, MOOMAW C, HERSH LB: The hydrolysis of endothelins by neutral endopeptidase 24.11 (enkephalinase). *J Biol Chem* 1990;265:14150-14155

WALLACE ECH, BALMFORTH AJ, MORTON JJ: Enhanced diurnal variation of blood pressure in the renal hypertensive rat: effect of angiotensin II suppression. *Clin Sci* 1987:73;271-275

WANNER TD, MITCHELL JA, DENUCCI G, VANE JR: Endothelin-1 and endothelin-3 release EDRF from isolated perfused arterial vessels of the rat and rabbit. *J Cardiovasc Pharmacol* 1989;13(suppl 5):S85-S88

WATANABE T, KUSUMOTO K, KITAYOSHI T, SHIMAMOTO N: Positive inotropic and vasoconstrictive effects of endothelin-1 in in vivo and in vitro experiments: characteristics and the role of L-type calcium channels. *J Cardiovasc Pharmacol* 1989;13(5):S108-S111

WATANABE H, MIYAZAKI H, KONDOH M, MASUDA Y, KIMURA S, YANAGISAWA M, MASAKI T, MURAKAMI T: Two distinct types of endothelin receptors are present on chick cardiac membranes. *Biochem and Biophys Res Comm* 1989;161:1252-1259

WIKLUND NP, ÖHLÉN A, CEDERQVIST B: Adrenergic neuromodulation by endothelin in guinea pig pulmonary artery. *Neurosci Lett* 1989;101:269-273

WIKLUND NP, ÖHLÉN A, CEDERQVIST B: Inhibition of adrenergic neuroeffector transmission by endothelin in the guinea-pig femoral artery. *Acta Physiol Scand* 1988;134:311-312

WILKES BM, SUSIN M, MENTO PF, MACICA CM, GIRARDI EP, BOSS E, NORD EP: Localization of endothelin-like immunoreactivity in rat kidneys. *Am J Physiol* 1991:260(29);F913-F920

WINQUIST RJ, SCOTT AL, VLASUK GP: Enhanced release of atrial natriuretic factor by endothelin in atria from hypertensive rats. *Hypertension* 1989;14:111-114

WONG PC, HART SD, ZASPEL AM, CHIU AT, ARDECKY RJ, SMITH RD, TIMMERMANS PBMWM: Functional studies of nonpeptide angiotensin II receptor subtype-specific ligans: DuP 753 (AII-1) and PD 123177 (AII-2). *J Pharmacol Exp Therap* 1990a;255:584-592

WONG PC, PRICE WA, CHIU AT, DUNCIA JV, CARINI DJ, WEXLER RR, JOHNSON AL, TIMMERMANS PBMWM: Non-peptide angiotensin II receptor antagonists. VIII. Characterization of functional antagonism displayed by DuP 753, an orally active antihypertensive agent. *J Pharmacol Exp Therap* 1990b;252:719-725

WONG PC, PRICE WA, CHIU AT, DUNCIA JV, CARINI DJ, WEXLER RR, JOHNSON AL, TIMMERMANS PBMWM: Non-peptide angiotensin II receptor antagonists. IX. Antihypertensive activity in rats of DuP 753, an orally active antihypertensive agent. *J Pharmacol Exp Therap* 1990c;252:726-732

WONG PC, PRICE WA, CHIU AT, DUNCIA JV, CARINI DJ, WEXLER RR, JOHNSON AL, TIMMERMANS PBMWM: Hypotensive action of DuP 753, an angiotensin II antagonist, in spontaneously hypertensive rats. *Hypertension* 1990d;15:459-468

WONG PC, PRICE WA, CHIU AT, DUNCIA JV, CARINI DJ, WEXLER RR, JOHNSON AL, TIMMERMANS PBMWM: Non-peptide angiotensin II receptor antagonists. XI. Pharmacology of EXP 3174: an active metabolite of DuP 753, an orally active antihypertensive agent. *J Pharmacol Exp Therap* 1990e;255:211-217

WRIGHT CE, FOZARD JR: Differences in regional vascular sensitivity to endothelin-1 between spontaneously hypertensive and normotensive Wistar-Kyoto rats. *Br J Pharmacol* 1990;100:107-113

WRIGHT CE, FOZARD JR: Regional vasodilation is a prominent feature of the haemodynamic response to endothelin in anaesthetized spontaneously hypertensive rats. *Eur J Pharmacol* 1988;155:201-203

WU C-C, BOHR DF: Role of endothelium in the response to endothelin in hypertension *Hypertension.* 1990;16:677-681

XIE P, MCDOWELL TS, CHAPLEAU MW, HAJDUCZOK G, ABBOUD FM: Rapid baroreceptor resetting in chronic hypertension: implications for normalization of arterial pressure. *Hypertension* 1991;17:72-79

YAMADA K, YOSHIDA S: Role of endogenous endothelin on renal functions in rats. *Am J Physiol* 1991;260(29):F34-F38

YANAGISAWA M, INOUE A, ISHIDAWA T, KASUYA Y, KIMURA S, KUMAGAYE S, NAKAJIMA K, WATANABE T, SAKAKIBARA S, GOTO K, MASAKI T: Primary structure, synthesis, and biological activity of rat endothelin, an endothelium-derived vasoconstrictor peptide. *Proc Natl Acad Sci* 1988a;85:6964-6967

YANAGISAWA M, INOUE A, TAKUWA Y, MITSUI Y, KOBAYASHI M, MASAKI T: The human preproendothelin-1 gene: Possible regulation by endothelial phosphoinositide turnover signaling. *J Cardiovasc Pharmacol* 1989a;13(suppl 5):S13-S17

YANAGISAWA M, KURIHARA H, KIMURA S, TOMOBE T, KOBAYASHI M, MITSUI Y, YAZAKI Y, GOTO K, MASAKI T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988b;332:411-415

YANAGISAWA M, MASAKI T: Endothelin, a novel endothelium-derived peptide. *Biochem Pharmacol* 1989b;38:1877-1883

YANAGISAWA M, MASAKI T: Molecular biology and biochemistry of the endothelins. *Trends Pharmacol Sci* 1989c;10:374-378

YITALO P, KARPPANEN H, PAASONEN MK: Is the area postrema a control centre of blood pressure? *Nature* 1974;274:58-59

YOKOKAWA K, KOHNO M, MURAKAWA K, YASUNARI K, TAKEDA T: Effect of endothelin-1 on cytosolic calcium ions in cultured human endothelial cells. *J Hypertens* 1990;8:843-849

YOKOKAWA K, TAHARA H, KOHNO M, MURAKAWA K, YASUNARI K, NAKAGAWA K, HAMADA T, OTANI S, YANAGISAWA M, TAKEDA T: Hypertension associated with endothelin-secreting malignant hemangioendothelioma. *Ann Intern Med* 1991;114(3):213-215

YOSHIHIKO S, NAKAO K, YAMADA T, ITOH H, MUKOYAMA M, ARAI H, HOSODA K, SHIRAKAMI G, SUGA S-I, JOUGASAKI M, OGAWA Y, IMURA H: Plasma endothelin-1-like immunoreactivity

level in healthy subjects and patients with hypertension. *43rd Coun High Blood Press Res Absts*, 1989

YOSHIZAWA T, KIMURA S, KANAZAWA I, UCHIYAMA Y, YANAGISAWA M, MASAKI T: Endothelin localizes in the dorsal horn and acts on the spinal neurones: possible involvement of dihydropyridine-sensitive calcium channels and substance P release. *Neurosci Lett* 1989;102:179-184

YOSHIZAWA T, SHINMI O, GIAID A, YANAGISAWA M, GIBSON SJ, KIMURA S, UCHIYAMA Y, POLAK JM, MASAKI T, KANAZAWA I: Endothelin: a novel peptide in the posterior pituitary system. *Science Wash DC* 1990;247:462-464

YSUCHIYA K, NARUSE M, SANAKA T, NARUSE K, ZENG ZP, NITTA K, DEMURA H, SHIZUME K, SUGINO N: Renal and hemodynamic effects of endothelin in anesthetized dogs. *Am J Hypertens* 1990;3:792-795