Endothelin and ET$_A$ receptors in long-term arterial pressure homeostasis in conscious nonhuman primates

GLENN A. REINHART, LEE C. PREUSSER, TERRY J. OPGENORTH, CRAIG D. WEGNER, AND BRYAN F. COX
Pharmaceutical Discovery, Abbott Laboratories, Abbott Park, Illinois 60064-6119

Received 3 May 2000; accepted in final form 13 July 2000

Reinhart, Glenn A., Lee C. Preussner, Terry J. Opgenorth, Craig D. Wegner, and Bryan F. Cox. Endothelin and ET$_A$ receptors in long-term arterial pressure homeostasis in conscious nonhuman primates. Am J Physiol Regulatory Integrative Comp Physiol 279: R1701–R1706, 2000.—This study was designed to quantify the long-term contribution of endogenous endothelin-1 (ET-1) and ET$_A$ receptors to the regulation of arterial pressure under normal conditions in nonhuman primates. Therefore, mean arterial pressure (MAP) and heart rate were measured 24 h/day with the use of telemetry techniques in conscious cynomolgus monkeys under control conditions, during administration of an ET$_A$ selective receptor antagonist (ABT-627; 5 mg/kg, 2 times a day by mouth, 4 days), and a 6-day posttreatment period. Systemic ET$_A$ blockade reduced MAP (24 h) from $89 \pm 3$ to $82 \pm 2$ and $79 \pm 2$ mmHg on days 1 and 4, respectively. Subsequently, MAP remained suppressed for 3 days posttreatment. Heart rate increased from $111 \pm 5$ to $122 \pm 4$ and $128 \pm 6$ beats/min on days 1 and 4 of ABT-627, respectively, and remained above control for 3 days posttreatment. Plasma ET-1 concentration increased from $1.0 \pm 0.3$ to $1.9 \pm 0.4$ pg/ml in response to ABT-627 (day 4) but decreased to control values 4 days posttreatment. These data demonstrate a physiologically important role for endogenous ET-1 and ET$_A$ receptors in the long-term regulation of arterial pressure and plasma ET-1 levels in the conscious nonhuman primate.

**THE ENDOTHELINS** comprise a family of small structurally related peptides that exert potent vasoconstrictor, renal, and mitogenic effects through activation of ligand-specific receptors. Endothelin-1 (ET-1), the most physiologically relevant ET isoform, is synthesized by numerous cell types including those of the vascular endothelium and vascular smooth muscle cells (17, 22, 23, 28). The potent vascular effects of ET-1 have led many investigators to propose a role for the ET system in the modulation of blood pressure and vascular tone. Additionally, ET has been implicated in the pathogenesis of some forms of hypertension as well as cardiovascular and renal diseases associated with endothelial dysfunction (17, 23). Although the potential for the ET system to impact arterial pressure regulation is widely recognized, the quantitative role of endogenous ET-1 and ET$_A$ receptors in long-term arterial pressure homeostasis under normal physiological conditions has not been completely defined.

The physiological effects of ET-1 are mediated by two distinct, differentially expressed receptor subtypes designated ET$_A$ and ET$_B$ (22). In the peripheral vasculature, ET$_A$ receptors are expressed primarily on the surface membrane of vascular smooth muscle cells and mediate in large part the potent, characteristically sustained vasoconstrictor response associated with administration of exogenous ET peptides. ET$_B$ receptors also occur on vascular smooth muscle cells, albeit less densely than ET$_A$ receptors, and mediate (at least in part) ET-induced vasoconstriction in many vascular beds (17, 22, 23). Also, and in contrast to ET$_A$ receptors, ET$_B$ receptors are highly expressed on vascular endothelial cells. ET$_B$ receptors mediate the initial transient vasodilatation produced by systemic injection of exogenous ET, a response that is followed immediately by a potent, long-lasting ET$_A$-mediated vasoconstriction. In addition, studies suggest that the vasodilatory response associated with ET$_B$-receptor activation may be mediated by increased release of nitric oxide and prostanooid-like substances (17, 22, 23). Although the physiological role of ET$_B$ receptors (and their disparate effects on vascular tone) is not completely defined, these receptors (especially those of the pulmonary epithelium) may serve an important clearance function by eliminating circulating ET-1 via receptor-mediated endocytosis (11, 13).

The ability of nonselective ET$_{AB}$- and selective ET$_A$-receptor blockade to reduce blood pressure has been assessed in experimental animals (primarily rodents with the use of indirect blood pressure measurement techniques) under mostly hypertensive conditions. In the spontaneously hypertensive rat (SHR), acute or chronic administration of ET$_A$ or ET$_{AB}$ antagonists produced either no effect (18–20, 27) or only modest reductions in blood pressure (2, 4, 9). In the normotensive Wistar-Kyoto rat, a genetic control for the SHR, blood pressure was typically unaffected by systemic ET blockade of various duration (2, 4, 18–20, 27), suggest-

**Address for reprint requests and other correspondence:** G. A. Reinhart, Dept. 46-R Bldg. AP-9, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6119 (E-mail: glenn.reinhart@abbott.com).

http://www.ajpregu.org 0363-6119/00 $5.00 Copyright © 2000 the American Physiological Society

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
ing a minimal role for endogenous ET-1 in maintaining normal blood pressure levels in this strain of rat. Also, systemic ET-1-receptor blockade failed to reduce hypertension induced by chronic inhibition of nitric oxide synthase in rats (31). In contrast, arterial pressure was decreased significantly by ET_A or ET_A/B blockade in rats fed a high-fructose diet (but not in normotensive controls) (29) and in rats with DOCA-salt hypertension (1, 4, 12, 27). Similarly, acute administration of bosentan, a nonselective ET_A/B antagonist, markedly reduced arterial pressure in anesthetized dogs with renovascular hypertension, a response that was attenuated in anesthetized normotensive control animals (8). A more recent study in conscious dogs demonstrated a modest dose-dependent decrease in mean arterial pressure in anesthetized dogs with renovascular hypertension, a response that was induced by administration of an orally active, nonselective ETA antagonist, a nonselective ETA/B antagonist, markedly reduced arterial pressure in anesthetized dogs with renovascular hypertension, a response that was attenuated in anesthetized normotensive control animals (8). A more recent study in conscious dogs demonstrated a modest dose-dependent decrease in mean arterial pressure (MAP) in response to selective, systemic ET_A blockade (24). When viewed collectively, these studies support a role for the endogenous ET system in maintaining blood pressure in some experimental models of hypertension (albeit with marked inconsistencies), but they provide little insight into the role of ET-1 in long-term blood pressure regulation under normal conditions.

Thus the quantitative contribution of endogenous ET-1 to long-term arterial pressure homeostasis under normal physiological conditions has not been fully delineated. Moreover, the relative contribution of ET_A (or ET_B) receptors in mediating any influence that endogenous ET might exert in long-term blood pressure regulation also remains to be defined. Therefore, the present study was designed to assess quantitatively the contribution of basal ET-1 levels and the selective role of ET_A receptors in long-term arterial pressure homeostasis in conscious nonhuman primates. To accomplish this objective, MAP and heart rate were recorded 24 h/day from conscious cynomolgus monkeys chronically instrumented with indwelling arterial telemetry transmitters. Systemic ET_A-receptor blockade was induced by administration of an orally active, highly selective ET_A-receptor antagonist ABT-627. Conscious animals were monitored continuously while residing in their home cages during a 4-day control period followed by 4 days of systemic ET_A-receptor blockade and, subsequently, a 6-day posttreatment recovery period.

METHODS

Animal preparation. Six male cynomolgus primates weighing 6.6–7.9 kg were used in this study. All procedures were approved by Abbott Laboratories' Institutional Animal Care and Use Committee and carried out in American Association for Accreditation of Laboratory Animal Care-accredited facilities. For surgical implantation of the telemetry transmitter, anesthesia was induced with ketamine (10 mg/kg im) followed by isofluorane (1–1.5%). With the use of aseptic techniques, a ventral midline incision was made, and a telemetry transmitter (model TL10M2-D70-PC or D70-PCT, Data Sciences International) was anchored in the abdominal cavity with nonabsorbable suture. A gel-filled catheter connected to the transmitter was tunneled to the femoral area. Via a small femoral incision, the catheter was inserted into a femoral artery, advanced into the abdominal aorta and secured, and the femoral incision was closed. The abdominal incision was closed in layers. In addition, a venous vascular access port (Access Technologies) was implanted subcutaneously and contralateral to the telemetry transmitter; the connecting catheter was secured in the femoral vein in a manner similar to the arterial catheter. Preoperatively, the animals were treated with K_+_penicillin (20,000 units/kg iv) and gentamycin (2 mg/kg iv). Postoperatively, buprenorphine (0.01 mg/kg im, 2 times a day) was given to provide analgesia. Amoxicillin (5 mg/kg) and Tribriissen (trimethoprim and sulfadiazine; 30 mg/kg) were given postoperatively for a period of 5–7 days. The vascular access ports were flushed with sterile saline biweekly or after each use and filled with a sterile solution of saline containing heparin (1,000 units/ml) and gentamycin (3.2 mg/ml).

After several weeks, the animals were housed in individual cages in a room maintained at 22 ± 1°C, with a 14:10-h light-dark cycle. They were fed a standard primate diet (Purina Primate Diet 5038), and water was provided ad libitum. Fresh fruit (banana) was given twice daily throughout the entire study as part of the dosing regimen.

To allow continuous measurement of MAP and heart rate while the animals moved freely in their home cages, the telemetry receivers were mounted to the front of each cage. The connecting wires extended through a wall port to a data-collection computer (Compaq DeskPro/133M) located in an adjoining room. MAP and heart rate data were sampled continuously from each animal in 10-s bursts at 2-min intervals for 24 h/day. Daily values for MAP and heart rate were determined for each animal for each 24-h period beginning at 7:30 AM, excluding the 2.5-h feeding and maintenance period (10:30 AM to 1:00 PM). Thus each daily average was calculated from the 645 data points collected during a 21.5-h period.

Experimental protocol. A 4-day control period was carried out to establish baseline values for MAP, heart rate, hematocrit, and plasma concentrations of ET-1 and electrolytes. Throughout the entire 14-day protocol, the animals were fed bananas (or bananas containing an active drug) twice daily at 12-h intervals (7:30 AM, 7:30 PM). To establish systemic ET_A-receptor blockade, the orally active ET_A selective receptor antagonist ABT-627 (5 mg/kg, twice a day) was added to cored bananas for 4 days. After 4 days of ET_A-receptor blockade, treatment with ABT-627 was discontinued, and a 6-day recovery period ensued. On the first and fourth day of the control, treatment, and recovery periods, the animals were removed from their home cages and placed in minimally restraining chairs beginning at 10:30 AM, 3 h after administration of the morning banana (or banana + ABT-627). Venous blood (10 ml) was collected via the vascular access port into chilled tubes (Vacutainer, Beckton Dickson) containing EDTA (for determination of plasma concentrations of ET-1 and ABT-627) or lithium heparin (for plasma electrolyte concentrations), and the animals were returned to their home cages. Blood samples were stored on ice until centrifuged under refrigeration. Aliquots of EDTA-plasma were stored frozen (−80°C) until assayed for plasma ET-1 and ABT-627 concentrations.

Analytic methods. Plasma concentrations of sodium and potassium were measured in fresh heparinized plasma with the use of a Synchron El-Ise Electrolyte System (Beckman). Hematocrit was determined with the use of a micromethod. Plasma concentrations of ET-1 were determined by ELISA (QuantiGlo, R&D Systems, Minneapolis, MN). Plasma concentrations of ABT-627 were measured by reverse-phase high-performance liquid chromatography with ultraviolet detection (25).
Statistical analysis. Results are expressed as the group mean ± SE. Experimental and recovery data were compared with control data with the use of ANOVA with Dunnett’s t-test for repeated measures (10). Control values were averaged to calculate a single control value for statistical comparisons.

RESULTS

Changes in 24-h MAP and heart rate during 4 days of oral administration of the ET\textsubscript{A} antagonist ABT-627 are summarized in Fig. 1. Although MAP failed to decrease during the first 3 h of ET\textsubscript{A} blockade (data not shown), decreases in MAP occurred later in the day as evidenced by the decrease in the 24-h mean. On day 1, MAP decreased from a control value of 89 ± 3 to 82 ± 2 mmHg; on day 4 of systemic ET\textsubscript{A} receptor blockade, MAP fell to 79 ± 2 mmHg. Once administration of the ET\textsubscript{A} antagonist was terminated, MAP slowly increased toward baseline values. By the fourth day of the recovery period (and thereafter), MAP was not significantly different from pretreatment values.

The prolonged hypotension induced by systemic ET\textsubscript{A} blockade was associated with a pronounced, sustained tachycardia; heart rate increased from a control value of 111 ± 5 to 122 ± 4 beats/min on the first day of ET\textsubscript{A} blockade. Heart rate increased to 128 ± 6 beats/min on day 4 of the ABT-627 treatment period. After treatment with the ET\textsubscript{A}-receptor antagonist was terminated, heart rate declined steadily toward control values. By the fourth day of the posttreatment period, heart rate was not significantly different from pretreatment control values.

Plasma sodium concentration decreased modestly from a control value of 145.1 ± 1.6 to 142.5 ± 0.4 mM (P, 0.05) after 4 days of ABT-627 and to 142.0 ± 1.1 mM (P < 0.05) the first day after administration of ABT-627 was terminated. By the fourth day of the posttreatment period, plasma sodium concentration was not significantly different from pretreatment control values. The plasma concentration of potassium (control: 3.8 ± 0.1 mM) was not significantly affected by systemic ET\textsubscript{A} blockade. Similar to plasma sodium concentration, the hematocrit decreased modestly from a control value of 44 ± 1 to 42 ± 1% (P < 0.05) after 4 days of ABT-627 and to 41 ± 1% (P < 0.05) after administration of ABT-627 was terminated. By the fourth day of the posttreatment period, hematocrit values increased to 43 ± 1% and were not significantly different from control values.

The effects of subacute systemic ET\textsubscript{A}-receptor blockade on the plasma concentration of ET-1 are summarized in Fig. 2. Plasma ET-1 concentration was not different from control on the first day of ETA blockade (3 h after administration of the first dose of ABT-627). In contrast, and despite the sustained decrease in MAP caused by ABT-627, plasma ET-1 concentration increased nearly twofold from a control value of 1.0 ± 0.3 to 1.9 ± 0.4 pg/ml (P < 0.05) after 4 days of continuous ET\textsubscript{A}-receptor blockade. Subsequently, plasma ET-1 levels declined toward control values after administration of ABT-627 was terminated. Plasma ET-1 concentrations were not different from control values by the fourth day after treatment was terminated.

Oral administration of ABT-627 (5 mg/kg, 2 times a day) produced plasma concentrations of 89 ± 17 ng/ml 3 h after administration of the first dose and 132 ± 37 ng/ml on day 4 of ABT-627 (a range of 174–258 nM). Plasma concentrations of ABT-627 declined to 68 ± 12 ng/ml 15 h after the final dose (day 1 of recovery) and to 19 ± 2 ng/ml 4 days after administration of ABT-627.
was terminated (when MAP was not significantly different from pretreatment control values).

Regarding the efficacy of the ETA blockade, it should be noted that ABT-627 has a $K_i$ at the ETA receptor of 34 pM (25). On the basis of the concentrations of ABT-627 achieved and assuming a similar degree (98%) of plasma protein binding for cynomolgus monkey plasma as in that of rat and human plasma (33), the expected free fraction of ABT-627 available to the ETA receptor is 60-100-fold above the $K_i$. Thus given the 1,000-fold selectivity of ABT-627 for the ETA versus the ETB receptor, the plasma concentrations obtained during the treatment period would be expected to provide a complete and selective ETA blockade.

DISCUSSION

The present study defines the physiological and quantitative importance of endogenous ET-1 and basal activation of ETA receptors in long-term blood pressure homeostasis in the conscious nonhuman primate. These results suggest that under normal conditions, ET-1, through direct activation of ETA receptors, contributes significantly to the long-term regulation of arterial pressure. Furthermore, these results highlight the importance of the vascular endothelium, which, through elaboration of vasodilator (e.g., nitric oxide) or vasoconstrictor (e.g., ET-1) substances, appears to play a vital regulatory role in the long-term control of arterial pressure. Finally, because of the importance of the kidney in long-term blood pressure homeostasis (7, 15, 16), the present findings are consistent with the hypothesis that endogenous synthesis (and release) of ET-1 and subsequent activation of intrarenal ETA receptors exert a continual antinatriuretic tone that significantly impacts arterial pressure levels long term.

Although the acute pressor and chronic hypertensive effects of ET-1 challenge and sustained ET activation are well documented (17, 29, 32), very few studies have assessed the role of endogenous ET-1 or the specific role of ETA receptors in the long-term regulation of blood pressure under normal physiological conditions.

In the present study, subacute administration of an orally active ETA selective receptor antagonist significantly reduced 24-h MAP values for a period of 7 days in the conscious primate. We hypothesize that this sustained hypotension produced by systemic ETA blockade is mediated through a peripheral vasodilatory effect (elicited by an attenuated ET-1- and ETA-dependent peripheral vasoconstriction) in conjunction with a concomitant increase in renal excretory function that allows MAP to remain suppressed below baseline values. Although the modest decrease in the plasma sodium concentration and hematocrit induced by systemic ETA blockade appears to contradict an increase in renal excretory function per se, the steady-state hypotensive response (days 3 and 4) suggests that retention of fluid and electrolytes was insufficient to return MAP toward normal values. Thus it is likely that ETA blockade produced an increase in renal excretory function sufficient to overwhelm any compensatory antinatriuretic responses elicited by ETA blockade-induced hypotension. Therefore, we hypothesize that ETA blockade appears to contradict an increase in renal excretory function per se, the steady-state hypotensive response (days 3 and 4) suggests that retention of fluid and electrolytes was insufficient to return MAP toward normal values. Thus it is likely that ETA blockade produced an increase in renal excretory function sufficient to overwhelm any compensatory antinatriuretic responses elicited by ETA blockade-induced hypotension. Therefore, we hypothesize that basal synthesis (and release) of ET-1 and subsequent activation of intrarenal ETA receptors exert a continual antinatriuretic tone (through hemodynamic or direct tubular mechanisms) that impacts significantly long-term arterial pressure levels.

In consideration of other mechanisms by which systemic ETA blockade would produce sustained hypotension, it is worth noting that selective activation of intrarenal ETB receptors has been associated with increased renal medullary blood flow and decreased tubular reabsorption of sodium (14). If sustained over time, these renal effects would be expected to reduce the extracellular fluid volume and decrease arterial pressure. Therefore, it could be hypothesized that in the present study, systemic ETA blockade (in concert with elevated plasma ET-1 concentrations) may lead to increased activation of intrarenal ETB receptors thereby producing an ETB-dependent natriuresis that would lead to decreases in arterial pressure. However, a recent study from our laboratory has demonstrated that prolonged systemic, selective ETB blockade in-
creases rather than decreases arterial pressure in the nonhuman primate, a response that is abolished by blockade of ETA receptors (26). Thus it seems unlikely that the hypotension induced by ETA blockade in the present study is the result of indirect activation of intrarenal ETB receptors.

Previous studies by other investigators employing various experimental models of hypertension have defined an inconsistent role for endogenous ET-1 in maintaining blood pressure in experimental hypertension (1, 4, 9, 12, 18–20, 27, 31). Moreover, studies of sustained ET-1-receptor blockade in normotensive rats suggest a minimal role for endogenous ET-1 in the maintenance of baseline blood pressure (2, 4, 9, 27, 29). The present results provide novel quantitative insight regarding the contribution of endogenous ETA and ETB receptors to the long-term regulation of blood pressure under normal physiological conditions in the conscious nonhuman primate. Furthermore, the present data extend the functional role of basal ET-1 synthesis and endogenous activation of ETA receptors beyond the acute modulation of vascular tone to that of a potent long-term determinant (regulator?) of arterial pressure.

In addition to prolonged decreases in MAP, systemic ETA blockade produced a pronounced, sustained tachycardia. Heart rate remained elevated above control values throughout the period of ETA blockade and the first 3 days of the posttreatment recovery period, a response that was inversely parallel to reductions in MAP. Presumably, the increase in heart rate induced by administration of ABT-627 reflects a sustained reflex-compensatory response to the marked hypotension induced by ETA-receptor blockade. However, a direct chronotropic effect of ETA-receptor blockade cannot be ruled out. Activation of ETA receptors has been associated with a negative chronotropic effect (23), thus blockade of ETA receptors might be expected to increase heart rate independent of changes in MAP. To date, few data exist in the literature regarding the effects of ETA blockade on heart rate in conscious animals. However, recent studies demonstrate that systemic administration of an ETA antagonist at doses that increased renal blood flow but only tended to decrease arterial pressure produced modest acute increases in heart rate in conscious dogs (3). Such a response is consistent with a normal reflex-compensatory response to a modest vasodilatory challenge.

Subacute administration of the ETA selective antagonist ABT-627 produced a modest twofold increase in plasma concentrations of ET-1 in conscious nonhuman primates. Historically, the effects of ETA blockade on plasma ET-1 concentrations have been negligible (21), but in more recent studies, ETA-receptor blockade in experimental animals has been associated with modest elevations of plasma ET-1 levels (1, 5). The twofold increase in plasma concentrations of ET-1 induced by ETA blockade in the present study (and others) may reflect an upregulation of ET-1 synthetic and secretory pathways leading to increased spillover of ET-1 into the plasma. Alternatively, the elevated plasma ET-1 concentrations induced by ETA blockade may reflect the inhibition of a modest ETA-mediated clearance of ET-1. Because ET-1 and ETA ligand-receptor complexes can undergo cellular internalization subsequent to the binding of ET-1 to the receptor (6), it is conceivable that a systemic sustained blockade of ETA receptors may reduce the elimination of ET-1 from the paracrine environment allowing additional ET-1 to diffuse into the plasma compartment.

In summary, the present data demonstrate a physiologically significant contribution by endogenous ET-1 and ETA receptors to the maintenance of normal levels of arterial pressure in the conscious nonhuman primate. These data suggest that endogenous synthesis and release of ET-1 and subsequent activation of ETA receptors play an important role in the long-term regulation of arterial pressure homeostasis under normal physiological conditions.

**Perspectives**

The present study defines a clear quantitatively important contribution of endogenous ET-1 and ETA receptors to the maintenance of arterial pressure in the chronically instrumented nonhuman primate. Under a primary theory of long-term blood pressure regulation, the renal body fluid-feedback mechanism, the kidney, is considered to play a dominant role in the long-term regulation of arterial pressure (7, 15, 16). Because ETA blockade produced sustained reductions in arterial pressure, the present data suggest an important role for endogenous basal ET-1 synthesis (and ETA-receptor activation) in the regulation of normal renal excretory function and arterial pressure. In addition, these data strengthen an already existing rationale for the application of selective ETA blockade to chronic circulatory disorders such as hypertension or congestive heart failure. Finally, the present study also highlights the importance of elucidating the physiological role of biological mediators such as ET-1 under normal basal conditions. Although studies of disease-state models are critical, a clear understanding of normal physiological processes provides a valuable physiological perspective because it is difficult, if not impossible, to predict a priori which models may (or may not) mask a specific biological response.

In addition, the significant contribution of endogenous ET-1 and ETA receptors to baseline arterial pressure levels in the conscious nonhuman primate underscores a growing appreciation for the importance of the vascular endothelium in the regulation of circulatory homeostasis. Vascular endothelial cells are capable of synthesizing both nitric oxide and ET-1, two potent vasoactive agents that exert generally opposite effects. It is well established that chronic inhibition of nitric oxide synthesis leads to a sustained hypertension in experimental animals. Thus the impact of basal nitric oxide formation on arterial pressure homeostasis is clear. However, despite the many studies that have served to characterize various aspects of the ET system, very few studies to date have quantified the ef-
Effects of ET₄-receptor blockade on blood pressure homeostasis under normal baseline conditions. Thus the physiological importance of the ET system (and the vascular endothelium) in mediating long-term blood pressure homeostasis under physiological conditions may not be fully appreciated.

The authors gratefully acknowledge the diligent efforts of Richard Nelson for careful maintenance of the primate colony. We also gratefully acknowledge Dr. Carmine Lanni and Kathleen Hamel for measuring plasma ET-1 concentrations, Ma. Bach-Nga Nguyen for determination of plasma concentrations of ABT-627, and Dr. M. K. McKay for the figures. We are also pleased to acknowledge the many Abbott scientists involved in the discovery and characterization of ABT-627.

REFERENCES


