Blood pressure regulation by ETₐ and ETₐ receptors in conscious, telemetry-instrumented mice and role of ETₐ in hypertension produced by selective ETₐ blockade

Ryan M. Fryer, Pamela A. Rakestraw, Patricia N. Banfor, Bryan F. Cox, Terry J. Opgenorth, and Glenn A. Reinhart. Blood pressure regulation by ETₐ and ETₐ receptors in conscious, telemetry-instrumented mice and role of ETₐ in hypertension produced by selective ETₐ blockade. Am J Physiol Heart Circ Physiol 290: H2554–H2559, 2006. First published January 6, 2006; doi:10.1152/ajpheart.01221.2005.—The net contribution of endothelin type A (ETA) and type B (ETB) receptors to blood pressure regulation in humans and experimental animals, including the conscious mouse, remains undefined. Thus we assessed the role of ETA and ETB receptors in the control of basal blood pressure and also the role of ETA receptors in maintaining the hypertensive effects of systemic ETB blockade in telemetry-instrumented mice. Mean arterial pressure (MAP) and heart rate were recorded continuously from the carotid artery and daily (24 h) values determined. At baseline, MAP ranged from 99 ± 1 to 101 ± 1 mmHg and heart rate ranged between 547 ± 15 and 567 ± 19 beats/min (n = 6). Daily oral administration of the ETB selective antagonist A-192621 [10 mg/kg twice daily] increased MAP to 108 ± 1 and 112 ± 2 mmHg on days 1 and 5, respectively. Subsequent coadministration of the ETA selective antagonist atrasentan (5 mg/kg twice daily) in conjunction with A-192621 (10 mg/kg twice daily) decreased MAP to baseline values on day 6 (99 ± 2 mmHg) and to below baseline on day 8 (89 ± 3 mmHg). In a separate group of mice (n = 6) in which the treatment was reversed, systemic blockade of ETB receptors produced no hypertension in animals pretreated with atrasentan, underscoring the importance of ETA receptors to maintain the hypertension produced by ETB blockade. In a third group of mice (n = 10), ETA blockade alone (atrasentan; 5 mg/kg twice daily) produced an immediate and sustained decrease in MAP to values below baseline (baseline values = 101 ± 2 to 103 ± 2 mmHg; atrasentan decreased pressure to 95 ± 2 mmHg). Thus these data suggest that ETA and ETB receptors play a physiologically relevant role in the regulation of basal blood pressure in normal, conscious mice. Furthermore, systemic ETB receptor blockade produces sustained hypertension in conscious telemetry-instrumented mice that is absent in mice pretreated with an ETA antagonist, suggesting that ETA receptors maintain the hypertension produced by ETB blockade.

AT AS THE UTILITY of the genetically altered mouse as a model of cardiovascular function continues to increase, it has become increasingly obvious that neurohumoral control of hemodynamic function and blood pressure in the “normal” mouse is not always similar to other mammalian species such as rat or dog. Recent studies have demonstrated that functional responses in the mouse aorta in the presence of a number of constrictor [phenylephrine, ANG II, urotensin II, and endothelin (ET)-1 (4, 8, 34)] and dilator [acetylcholine, adenosine, and histamine (4, 34)] substances are markedly different from responses observed in rats and other species. Moreover, blockade of the renin-angiotensin system produces greater reductions in arterial pressure in C57BL/6J mice than observed in rats, suggesting that blood pressure values in the mouse are more dependent on the endogenous renin-angiotensin system than in the rat (4). The endothelin system is important in blood pressure regulation in the rat (9, 30), dog (27), and primate (31, 33). Given that the ET and renin-angiotensin systems closely interact with regard to renin release and the regulation of vascular resistance (3) and the fact that the vascular reactivity in the mouse to ET-1 is markedly different from rats and humans (34, 41), we hypothesized that the overall contribution of ET and type A (ETA) and type B (ETB) receptors to blood pressure regulation in the mouse may be different from other animals.

ET-1 is a potent vasoconstrictor capable of exerting an array of physiological effects, including the potential to alter arterial pressure and circulatory function through interactions with ETA and ETB receptors (21, 25). As such, ET receptors may play an important role in the pathophysiology of cardiac, vascular, and renal diseases associated with regional and systemic vasoconstriction (15, 37).

The ETA and ETB receptors are each coded by a specific gene and possess some overlap in their tissue distribution (2, 16, 35). In the peripheral vasculature, ETA receptors are expressed primarily on the surface membrane of vascular smooth muscle cells where they mediate, in large part, the potent and characteristically sustained vasoconstrictor response associated with administration of exogenous ET peptides (32). Administration of exogenous ET-1 to an intact animal produces a classic, transient hypotension and vasoconstriction that is mediated via ETB receptors through enhanced generation of nitric oxide and prostaglandin-related substances, a response that precedes ETA-mediated vasoconstriction (14, 38, 40). Other experimental data suggest that ETB receptors may mediate a portion of ET-induced vasoconstriction in some vascular beds, a response...
that may be mediated by ETβ receptors expressed on vascular smooth muscle cells (18, 24, 26).

We have demonstrated in conscious primates that sustained hypertension produced by systemic ETβ receptor blockade is mediated by ETα receptors (32), suggesting that ETβ receptors are part of an important clearance mechanism in primates that limits circulating ET-1 stimulation of ETα receptors, thereby maintaining blood pressure at normal levels. Similar results were demonstrated by other investigators in the rat (9, 31) but were not observed in ETβ-deficient mice that were administered an early generation peptidic ETα antagonist BQ-123 (28). Moreover, the role of ET and systemic and localized ET receptor subtypes in the control of basal blood pressure in mice remains controversial (13, 22, 42). It has been demonstrated that collecting duct-derived ET-1 is an important physiological regulator of systemic blood pressure in the mouse but that the collecting duct ETα receptor does not appear to modulate blood pressure under physiological conditions (1, 13). Additionally, other studies have failed to definitively demonstrate a critical role for systemic ETα receptors in basal blood pressure regulation in the mouse or have obtained only a weak, nonsignificant depressor response in the face of ETα blockade (22, 42).

Thus the present study characterizes the effects of sustained systemic ETβ receptor blockade on arterial pressure homeostasis in normal and unrestrained conscious C57Bl/6 mice by using quantitative radiotelemetry techniques. Subsequently, we tested the hypothesis that the increase in arterial pressure produced by ETβ receptor blockade is indirectly mediated by activation of the ETα receptor by using two unique experimental protocols. In a final study we also delineate the quantitative contribution of systemic ETα receptors in basal blood pressure homeostasis in the normal and unrestrained conscious mouse.

METHODS

Telemetry Transmitter Implantation

C57Bl/6 mice (25–30 g) were instrumented with telemetry transmitters (PA-C20, Data Sciences International; St. Paul, MN). Briefly, mice were anesthetized via intraperitoneal injection of ketamine and xylazine (100 mg/kg, 2.0 ml; and 15 mg/kg, 0.3 ml, respectively; groups 1 and 2) or via induction and maintenance of anesthesia with volatile isoflurane (2.0%; group 3). A midline incision was made through the skin, the right common carotid artery was isolated under a dissecting microscope, and the catheter was threaded into the aortic arch. Through the same incision, the radiotransmitter was tucked under the skin and sutured into place with 7-0 silk (Ethicon; Somerville, NJ). Mice were allowed to recover for at least 1 wk. Subsequently, arterial pressure and heart rate were continuously recorded and are reported as 24-h means. 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.

Dosing Groups and Drug Preparation

We used a dosing regimen similar to that employed in a previous study in primates to define the role of ETα receptors in ETβ-induced hypertension whereby we demonstrated that 10 mg/kg, but not 0.1 or 1.0 mg/kg, of A-192621 twice daily produced hypertension in conscious primates (an effect that was completely abolished by atrasentan) (32). Atrasentan (ETα antagonist) and/or A-192621 (ETβ antagonist) (39) were synthesized at Abbott Laboratories and administered as a suspension via oral gavage in 0.2% hydroxypropyl methylcellulose twice daily at 0.02 ml/g body wt.

Group 1. The effects of selective ETβ blockade with or without coblockade of ETα receptors were delineated (Fig. 1A). After 5 days of baseline telemetry recording, A-192621 (10 mg/kg twice daily) was administered for 8 days (n = 6). Atrasentan (5 mg/kg twice daily) was administered concomitantly with A-192621 during the last 3 days of A-192621 treatment. Subsequently, animals were allowed to recover for 3 days (Fig. 1A).

Group 2. A separate group of animals (n = 6) were implanted with telemetry transmitters to determine if prior blockade of ETα receptors would prevent hypertension produced by ETβ blockade (Fig. 2A). After 5 days of baseline telemetry recording, atrasentan (5 mg/kg twice daily) was administered for 3 days alone before coadministration with A-192621 (10 mg/kg twice daily) for 3 days.

Group 3. In a final group of animals (n = 10) and after 5 days of baseline telemetry recording, the contribution of ETα receptors to basal blood pressure was assessed via administration of atrasentan alone for 5 days (5 mg/kg twice daily; Fig. 3A).

Statistical Analysis

Changes in mean arterial pressure (MAP) and heart rate from mean baseline during administration of atrasentan and/or A-192621 were determined with repeated-measures ANOVA with Dunnett’s t-test (Prism 4.03), and statistical significance was determined at P < 0.05 and P < 0.01.

Fig. 1. Effect of endothelin type B receptor (ETβ) blockade with A-192621 in the absence or presence of endothelin type A receptor (ETα) blockade with atrasentan after 5 days of baseline recording (A) on mean arterial pressure (MAP; B) and heart rate (C). *P < 0.05. **P < 0.01.
RESULTS

Group 1

Baseline (days −5 to −1) 24-h MAP values ranged between 99 ± 1 and 101 ± 1 mmHg (Fig. 1B). Selective ET<sub>B</sub> blockade with A-192621 produced an immediate and graded increase in MAP (to 112 ± 1 mmHg on days 3 and 5) throughout the 5-day treatment period. Hypertension produced by ET<sub>B</sub> blockade was abolished by simultaneous blockade of ET<sub>A</sub> receptors with atrasentan, suggesting the importance of ET<sub>A</sub> receptors in the genesis of hypertension produced by ET<sub>B</sub> blockade. Baseline heart rate ranged between 570 ± 14 and 584 ± 12 beats/min (Fig. 2C). Although ET<sub>A</sub> blockade with atrasentan produced no statistically significant effects on heart rate relative to baseline, heart rate transiently increased after atrasentan administration (to 593 ± 8 beats/min). Subsequently, heart rate trended downward (to 554 ± 9 beats/min). Coadministration of A-192621 after 3 days of atrasentan treatment produced a significant reduction in heart rate (to 522 ± 14 beats/min; n = 6 per 24-h group mean).

Group 2

Selective ET<sub>A</sub> blockade with atrasentan produced an immediate and stable reduction in MAP (to 93 ± 3 mmHg) that was maintained for 3 days, suggesting an important role of ET<sub>A</sub> receptors in basal blood pressure homeostasis; baseline MAP ranged between 99 ± 4 and 101 ± 4 mmHg (Fig. 2B). Simultaneous administration of atrasentan and A-192621 produced no change in MAP (range = 93 ± 4 to 95 ± 4 mmHg), definitively demonstrating the importance of ET<sub>A</sub> receptors in the genesis of hypertension produced by ET<sub>B</sub> blockade. Baseline heart rate ranged between 570 ± 14 and 584 ± 12 beats/min (Fig. 2C). Although ET<sub>A</sub> blockade with atrasentan produced no statistically significant effects on heart rate relative to baseline, heart rate transiently increased after atrasentan administration (to 593 ± 8 beats/min). Subsequently, heart rate trended downward (to 554 ± 9 beats/min). Coadministration of A-192621 after 3 days of atrasentan treatment produced a significant reduction in heart rate (to 522 ± 14 beats/min; n = 6 per 24-h group mean).
Selective ET_{A} blockade with atrasentan produced an immediate and stable reduction in MAP (to 95 ± 2 mmHg) that was maintained for 5 days, demonstrating the essential role of ET_{A} receptors in the regulation of normal blood pressure values; baseline MAP ranged between 101 ± 2 and 103 ± 2 mmHg (Fig. 3B). Baseline heart rate ranged between 555 ± 11 and 577 ± 13 beats/min (Fig. 3C). Although ET_{A} blockade with atrasentan produced no statistically significant effects on heart rate relative to baseline, heart rate transiently increased after atrasentan administration (to 582 ± 15 beats/min). Subsequently, heart rate was modestly reduced on days 3–5 (to 534 ± 9 beats/min; n = 10 per 24-h group mean).

**DISCUSSION**

We demonstrate in the conscious and unrestrained telemetry-instrumented mouse that systemic, selective blockade of ET_{B} receptors produces sustained hypertension (average increase in blood pressure 10 ± 1 mmHg above baseline) that is maintained by ET_{A} receptor stimulation (Figs. 1B and 2B). Furthermore, these data suggest that ET_{B} receptors also play an important role in basal blood pressure regulation (Fig. 1B). Finally, we demonstrate that ET_{A} receptors contribute to basal blood pressure values in normal, conscious mice because atrasentan, a selective ET_{A} antagonist, produced sustained reductions in blood pressure to values significantly below baseline (average decrease = 7 ± 1 mmHg; Figs. 2B and 3B).

A clear understanding of the individual role of ET receptor subtypes in the control of blood pressure in the mouse is important given the recent finding that vascular constriction in the mouse in response to ET-1 is markedly different from that observed in rats and humans (41). Moreover, Russell and Watts (34) have demonstrated that the mouse vasculature responds differently not only from ET-1 but also from ANG II, an effect that was further substantiated in vivo by Cholewa and colleagues (4), who demonstrated distinct differences between mice and rats regarding blood pressure regulation by the endogenous renin-angiotensin system. Specifically, they demonstrated that infusion of captopril or losartan resulted in a 55–90% greater fall in blood pressure in mice compared with rats, suggesting that blood pressure regulation in the mouse is more dependent on the renin-angiotensin system than it is in rats.

Regarding the contribution of the endogenous endothelin system in the control of blood pressure in mice, Ohuchi and colleagues (28) have demonstrated that mice with the piebald (s) mutation of the ET_{B} gene and reduced ET_{B} receptor expression have elevated blood pressure relative to wild-type mice, even though plasma levels of immunoreactive ET-1 were not increased. Further, intra-arterial administration of BQ-788, an early-generation peptidic ET_{B} receptor antagonist, increased aortic blood pressure acutely in wild-type mice but not in ET_{B}−/− mice; whether plasma ET-1 levels were increased in response to acute ET_{B} blockade was not tested. Similarly, acute administration of the ET_{A} antagonist BQ-123 did not markedly alter blood pressure in hypertensive ET_{B}−/− deficient animals. Although the apparent hypertension is consistent with the effects of ET_{B} deficiency or ET_{B} blockade in other species, it is not clear why the effects of acute receptor blockade in piebald mice differ from the present results. It is possible, however, that the disparate results are due to differences in experimental design (acute vs. chronic; n = 4), the pharmacological agents employed, or differences in mouse strain (C57Bl/6 mice in the present study vs. genetically modified mice on a 129/SvEv background).

In studies performed in rats, Gariepy and colleagues (12) have demonstrated that spotting-lethal (sl) rats, which carry a naturally occurring ET_{B} gene deletion (11), are hypertensive and exhibit severe salt-sensitive hypertension (increase in MAP of 41 mmHg vs. sodium-deficient diet). Elmarakby et al. (9) have demonstrated that ET_{A} blockade with atrasentan in these rats reduced systolic pressure to values similar to wild-type rats. In addition, ET_{A} blockade with atrasentan at doses similar to those used in the present study attenuated salt-dependent increases in blood pressure to near normal levels, suggesting that ET_{A} receptors play a physiologically significant role in maintaining the salt-dependent hypertension produced by ET_{B} deficiency. Thus ET_{B} receptor deficiency in mice and rats causes hypertension that is dependent, in large part, on activation of ET_{A} receptors.

The underlying mechanism of hypertension produced by ET_{B} receptor antagonism has been debated. Because ET_{B} receptor stimulation can produce transient dilation of some vascular beds and transient hypotension in vivo (7, 23), it has been postulated that a loss of ET_{B}-dependent vasodilation (resulting from ET_{B} blockade) might increase vascular tone, accounting for elevations in blood pressure. This hypothesis, however, is not supported in the present study. Although it is possible that a loss of ET_{B}-mediated vasodilatory tone may contribute to an acute circulatory response to systemic ET_{B} blockade, the consistent ability of ET_{A} blockade to abolish ET_{B}-dependent hypertension in the present study in mice (whether administered before or after A-192621) and other studies in rats and primates (9, 32) suggests that indirect activation of ET_{A} receptors is a major mechanism by which systemic ET_{B} blockade produces hypertension. Moreover, this is the first study to demonstrate that reversal of the treatments (ET_{A} blockade before ET_{B} blockade) prevents the hypertension produced by ET_{B} blockade alone (Fig. 2B), thereby demonstrating the essential role of ET_{A} receptors in maintaining the sustained hypertension elicited by ET_{B} blockade.

Finally, given the dominant role of the kidney in regulating arterial pressure, the renal effects of altered ET_{B} activity must also be considered. Studies in the anesthetized rat (17, 19), the anesthetized dog (6), and conscious dog (36) demonstrate that low-level ET-1 or ET_{B} receptor activation produces natriuretic and diuretic effects. Studies performed in isolated rat nephron segments are consistent with these in vivo observations (10, 29). Thus it has been hypothesized that at normal levels of endogenous activation, ET_{B} receptors exert a natriuretic tone such that loss of this tone through ET_{B} suppression or ET_{B} blockade may produce antinatriuretic effects and therefore cause hypertension (20). Consistent with this hypothesis, recent elegant studies performed in the mouse have demonstrated that collecting duct-specific knockout of ET-1 causes hypertension (1), whereas collecting duct-specific suppression of ET_{A} receptors has no effect on blood pressure (13). These studies suggest that collecting duct ET_{A} receptors do not influence blood pressure in the mouse and may reflect the presence of another intrarenal population of ET_{A} receptors that serve to modulate fluid and electrolyte balance (and blood
pressure) independent of the collecting duct and that are antagonized by systemic exposure to an ET\textsubscript{A}-selective antagonist. In addition, other indirect mechanisms, such as changes in renal sympathetic tone or other neurohumoral factors, cannot be ruled out as potential mediators of hypotension produced by sustained ET\textsubscript{A} blockade.

The doses of A-192621 and atrasentan employed in the present study have been shown to produce similar effects on basal blood pressure in primates (32). Indeed, ET\textsubscript{A} receptor blockade with atrasentan (5 mg/kg twice daily) in telemetry-instrumented conscious primates immediately reduced MAP by \(\sim 10\) mmHg, an effect that is sustained through 4 days of treatment, suggesting that ET\textsubscript{A} receptors play an important role in normal primate blood pressure homeostasis (33). However, until the present study the role of ET\textsubscript{A} receptors in the maintenance of blood pressure in the mouse was less clear. Kuwaki and colleagues (22) suggested that ET\textsubscript{A} receptors do not play an important role in basal blood pressure homeostasis in mice because blood pressure in ET\textsubscript{A} receptor-deficient infant mice, ET\textsubscript{A}\(-/-\) was not different from homozygous wild-type mice, ET\textsubscript{A}\(+/+\) (as measured by using the servo-null micropressure technique under halothane anesthesia). However, the results of the present study, in normal unrestrained conscious mice by using telemetry to capture 24-h blood pressure values, clearly demonstrate that ET\textsubscript{A} receptors contribute to the regulation of basal blood pressure in the intact mouse, an observation also consistent with studies performed in conscious primates (33) and dogs (27).

Studies performed in other species consistently demonstrate that plasma ET-1 increases dose dependently in the presence of ET\textsubscript{B} blockade and is exacerbated in the presence of concomitant ET\textsubscript{A} blockade in primates (32, 33) and rats (9). ET\textsubscript{A} blockade alone also increases ET-1 in conscious primates (33) and in rats (9) because of the functional blockade of the cellular internalization of the ET\textsubscript{A}-ET-1 complex (5). Although we were unable to assess changes in ET-1 levels in mice in the present study, the fact that ET\textsubscript{A} blockade abolished hypertension produced by inhibition of ET\textsubscript{B} receptors implies that circulating ET-1 likely increased in response to ET\textsubscript{B} blockade.

In the present study, ET\textsubscript{B} blockade with A-192621 produced sustained hypertension in mice concomitant with decreases in heart rate. Although the increase in blood pressure is likely a direct effect of reduced ET-1 clearance and increased ET\textsubscript{A} activation, A-192621-induced bradycardia likely reflects a compensatory reduction in heart rate to maintain blood pressure homeostasis. It is interesting to note, however, that during concomitant ET\textsubscript{A}/ET\textsubscript{B} blockade (days 2–3; Fig. 1C), heart rate decreased to values below baseline despite significant reductions in blood pressure. Thus, although heart rate fell in response to hypertension, the expected converse response did not occur as blood pressure was reduced by systemic ET\textsubscript{A} blockade. Curiously, heart rate also trended downward in mice after multiple days of selective ET\textsubscript{A} blockade with atrasentan (Figs. 2C and 3C) despite significant reductions in blood pressure. These data imply that ET\textsubscript{A} receptors may modulate heart rate via a currently undefined mechanism refractory to baroreflex control.

In summary, we report several novel findings related to the cardiovascular physiology of the mouse and the role of the ET system in blood pressure control. We demonstrate in the unrestrained conscious C57Bl/6 mouse that selective systemic inhibition of ET\textsubscript{B} receptors produces a sustained hypertension that is maintained by ET\textsubscript{A} receptor activation. Furthermore, the present data demonstrate that ET\textsubscript{A} and ET\textsubscript{B} receptors contribute to basal blood pressure homeostasis in conscious, normal mice, thereby highlighting the importance of the ET\textsubscript{A} and ET\textsubscript{B} receptor systems in blood pressure regulation in the mouse.

ACKNOWLEDGMENTS

We gratefully acknowledge the expertise of William T. Noonan in the continued development of the mouse telemetry model.

REFERENCES


