Elevation of blood pressure by genetic and pharmacological disruption of the ETB receptor in mice

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Ohuchi, Takashi, Tomoyuki Kuwaki, Guang-Yi Ling, Damiane Dewit, Ki-Hwan Ju, Makoto Onodera, Wei-Hua Cao, Masashi Yanagisawa, and Mamoru Kumada. Elevation of blood pressure by genetic and pharmacological disruption of the ETB receptor in mice. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1071–R1077, 1999.—Exogenously administered endothelin (ET) elicits both pressor and depressor responses through the ETA and/or the ETB receptor on vascular smooth muscle cells and ETB on endothelial cells. To test whether ETB has pressor or depressor effects under basal physiological conditions, we determined arterial blood pressure (BP) in ETB-deficient mice obtained by crossing inbred mice homozygous for targeted disruption of the ETB gene with mice homozygous for the piebald (s) mutation of the ETB gene (ETBs/s). F1 ETB+/s and ETB+/+ progeny share an identical genetic background but have ETB levels that are ~3/4 and ~1/2 respectively, of wild-type mice (ETB+/+). BP in ETB+/s mice was significantly higher, by ~20 mmHg, than that in ETB+/s or ETB+/+ mice. Immunoreactive ET-1 concentration in plasma as well as respiratory parameters was not different between ETB+/s and ETB+/+ mice. A selective ETB antagonist, BQ-788, increased BP in ETB+/s and ETB+/+ but not in ETB+/+ mice. Pretreatment with indomethacin, but not with Nω-monomethyl-L-arginine, can attenuate the observed pressor response to BQ-788. The selective ETA antagonist, BO-123 did not ameliorate the increased BP in ETB+/s mice. Moreover, BP in mice heterozygous for targeted disruption of the ETA gene was not different from that in wild-type controls. These results suggest that endogenous ET elicits a depressor effect through ETA under basal conditions, in part through tonic production of prostanoids, and not through secondary mechanisms involving respiratory control or clearance of circulating ET.

endothelin; hypertension; endothelin receptors; gene targeting; indomethacin

ENDOTHELINS (ETs), a family of 21-amino acid peptides (31), are potent vasoconstrictors that have been implicated in the pathogenesis of several diseases with abnormalities in vascular tone (10). ETs act on two subtypes of G protein-coupled heptahelical receptors named the ETA and ETB receptors (1, 26). Vascular smooth muscle cells express ETA and/or ETB that directly mediates vasoconstriction by ETs (27). In contrast, endothelial cells express ETB that mediates endothelium-dependent vasodilatation via nitric oxide and prostaicyclin formation and likely counteracts the predominating pressor effect of pharmacological doses of ETs (4). ETB can also act as a “clearance” receptor for circulating ETs to help limit the plasma level of these potentially toxic peptides (5). Consequently, the physiological role of endogenous ETs in the regulation of vascular tone is likely complex. Elevation of arterial blood pressure (BP) was recently reported in mice heterozygous for targeted disruption of the ET1 gene (ET-1−/−) (16). This observation suggests that ET-1 may act as a depressor rather than a pressor agent in normal states, although the exact mechanism is unknown.

Neonatal or juvenile lethality is a significant problem for cardiovascular research using “knockout” mice. For example, a null mutation created by the targeted disruption of the mouse ETB gene produces a recessive phenotype of white-spotted coat and aganglionic megacolon, the latter leading to juvenile death (11). A naturally occurring mutant ETB allele, piebald (s), has a retroposon inserted within intron 1 of the ETB gene (Yanagisawa, unpublished data), resulting in about one-fourth the normal level of expression of structurally intact ETB mRNA (11) and an approximately fourfold reduction of tissue ETB density in homozygotes. These mice exhibit reduced coat color spotting and rarely manifest megacolon. Using the piebald allele, we obtained healthy adult mice with systemic ETB deficiency. An identical genetic background between control and experimental groups was maintained by crossing the inbred ETB+/+ mice on the SSL background with ETB+/− mice on an inbred 129/SvEv background. Using this strategy, we were able to examine the role(s) of ETB in the regulation of BP in adult animals.

ET and ET receptors are also expressed in the brain, where their participation in the central control of cardiorespiratory function has been proposed (18). For example, ET-1−/−, ET-1−/− (17), and ETA−/− mice (3) have impaired ventilatory responses to hypoxia and hypercapnia. Altered interaction between the cardiovascular and respiratory centers in the central nervous system may explain some of the hypertension in ET-1−/− mice (16, 17, 19). Therefore, we also investigated whether hypertensive ETB-deficient mice have ventilatory abnormalities.

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Three specific questions were addressed in this study. 1) Do mice deficient in ET\textsubscript{B} have abnormal basal BP? 2) If so, can we reproduce the abnormality by pharmacological disruption of ET\textsubscript{B}? 3) What are the possible mechanisms of the ET\textsubscript{B}-mediated BP alteration? To investigate the latter, we looked for changes in immunoreactive ET-1 concentration in plasma, respiratory parameters, and responsiveness to ET\textsubscript{A} and ET\textsubscript{B} antagonists.

**MATERIALS AND METHODS**

Adult mice. Inbred piebald homozygotes were purchased from the Jackson Laboratory. ET\textsubscript{B}\textsuperscript{−/−} mice on an inbred 129/SvEv background were obtained as previously described (11). By crossing inbred (SSL) ET\textsubscript{B}\textsuperscript{+/+} homozygotes with ET\textsubscript{B}\textsuperscript{−/−} mice (Fig. 1A), we obtained F\textsubscript{1} ET\textsubscript{B}\textsuperscript{−/−} and ET\textsubscript{B}\textsuperscript{+/+} mice genetically identical except for the ET\textsubscript{B} gene, with a systemic expression dosage of ~\%/ and % of wild-type mice, respectively. They were easily identified by the coat color (11): ET\textsubscript{B}\textsuperscript{−/−} mice exhibit white spotting over 40–50% of body surface area, whereas all ET\textsubscript{B}\textsuperscript{+/+} mice have a homogenous agouti coat. A small number of ET\textsubscript{B}\textsuperscript{−/−} mice (7 of 37) manifested megacolon; data obtained from these animals were excluded. Inbred 129/SvEv wild-type (ET\textsubscript{B}\textsuperscript{+/+}) mice were used as controls.

To examine the relative importance of ET\textsubscript{B} and ET\textsubscript{A} in BP regulation, we also used ET\textsubscript{A}\textsuperscript{−/−} mice on an inbred 129/SvEv background (3). Only ET\textsubscript{A}\textsuperscript{−/−} mice and their wild-type littermates are useful for physiological studies, because ET\textsubscript{A}\textsuperscript{−/−} mice die soon after birth. There is no known hypomorphic allele of the ET\textsubscript{A} gene. Genotype of these animals was determined by PCR on DNA extracted from a tail cut biopsy (3).

All experiments were performed in male mice to avoid possible differences related to menstrual cycling in females. All animal procedures conformed to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences recommended by the Physiological Society of Japan.

Newborn mice. Although BP measurement in newborn mice is difficult at present, we developed a reliable method to measure ventilation in newborn mice (17). Using this method (see below), we measured ventilation in inbred ET\textsubscript{B}\textsuperscript{−/−} mice of both sexes obtained by crossing inbred ET\textsubscript{B}\textsuperscript{−/−} mice (11). They are easily identified by coat color 2–3 days after birth in a manner similar to that in ET\textsubscript{A}\textsuperscript{−/−} mice. Three- to five-day-old ET\textsubscript{B}\textsuperscript{−/−} pups with a mean body weight of 2.08 ± 0.10 g (n = 10, mean ± SE) were used. At this age, ET\textsubscript{B}\textsuperscript{−/−} pups did not show any signs of megacolon. They all survived at least 6 days after the experiments.

Measurement of BP, plasma concentration of immunoreactive ET-1, arterial blood gas, and pH. Four series of experiments were performed using different sets of the animals. First, baseline BP was determined in experimental groups of mice and their controls: 17- to 28-wk-old ET\textsubscript{B}\textsuperscript{−/−} (n = 9), 17- to 28-wk-old ET\textsubscript{B}\textsuperscript{−/−} (n = 9), 17- to 28-wk-old ET\textsubscript{B}\textsuperscript{−/−} (n = 7), 34- to 44-wk-old ET\textsubscript{A}\textsuperscript{−/−} heterozygotes (n = 8), and 34- to 44-wk-old wild-type mice (n = 7). Arterial BP was measured by cannulation of the femoral artery with polyethylene tubing under halothane anesthesia. The following day, pulsatile BP was measured continuously for 2 h under conscious and unrestrained conditions in a quiet environment after at least 30 min of acclimatization (16). Heart rate (HR) was calculated by a tachometer monitoring BP signals. After completion of the BP measurements, arterial blood was collected from the femoral catheter. An aliquot (40–50 μl) of blood was used for arterial blood gas measurement (17), and plasma from the remainder was prepared for other studies.

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In another series of experiments, nine 129/SvEv wild-type, four ET\textsubscript{B}\textsuperscript{−/−}, and five ET\textsubscript{B}\textsuperscript{+/+} male mice were cannulated,
under halothane anesthesia, with polyethylene tubing into the right femoral and left carotid arteries for measurement of BP and injection of agents, respectively. The carotid cannula was advanced so that the tip lay near the aortic arch. The following day, pulsatile BP was measured while mice were under conscious and unrestrained conditions, in the manner described above. BQ-788, a highly selective ET\(_B\) antagonist (14), was administered at a constant volume of 0.5 \(\mu\)l/g body wt to deliver doses of 0, 0.1, 0.3, 1, and 3 mg/kg. Possible volume effect from repeated injections in individual animals was negligible because repeated administration of vehicle five times did not affect BP in preliminary experiments. BQ-788 stock solution (6 mg/ml) was dissolved in 0.6% Na\(_2\)CO\(_3\), and subsequent dilutions were made with saline. BQ-123, a selective ET\(_\Lambda\) antagonist (13), was dissolved in saline and injected intra-arterially as a single dose in another group of four ET\(_B\)/-/- mice. The dose of BQ-123 (1 mg/kg) was selected so that it could completely inhibit the pressor response induced by a subsequent injection of 1 nmol/kg of ET-1 (13).

In the next group of experiments, the effects of intra-arterial injections of BQ-788 on BP were studied in six wild-type mice under ketamine (100 mg/kg ip) and xylazine (0.5 mg/kg ip) anesthesia to exclude secondary influences such as emotional stress from higher brain centers (9). Rectal temperature was monitored by a thermistor and maintained between 36 and 37°C (ATB-1100; Nihon Kohden, Tokyo, Japan).

Finally, pretreatment with indomethacin (0, 14, and 28 \(\mu\)mol/kg ia; \(n = 5, 6, \) and 5, respectively), an inhibitor of prostaglandin production, or N\(^\circ\)-monomethyl-L-arginine (L-NMMA; 250 \(\mu\)mol/kg ip, \(n = 5\)), an inhibitor of nitric oxide synthase, on the pressor response to acute ET\(_B\) blockade was examined in wild-type mice. After recovery from the transient pressor response to the injection of each drug, BQ-788 was administered in the manner described above. Indomethacin was dissolved in 95% ethanol, and L-NMMA was dissolved in saline. Doses of indomethacin and L-NMMA were chosen according to doses reported in previous investigations (4, 16).

Measurement of ventilation. Ventilation in adult (17- to 28-wk old) ET\(_B\)/-/- and ET\(_B\)/+/- mice and newborn (3- to 5-day old) ET\(_B\)/-/- and ET\(_B\)/+/- mice was measured according to the method described previously (3, 17, 24). In brief, respiratory cycle time and tidal volume were determined for every breath by whole body plethysmography, with respiratory frequency and minute volume calculated over a 1- to 2-min measurement period. In each animal, measurement was repeated four times in each experimental gas condition: room air (control for hypoxia), hypoxic (1:1 room air-N\(_2\)) gas mixtures, 100% O\(_2\) (control for hypercapnia), and hypercapnic (5% CO\(_2\)-95% O\(_2\)) gas mixture. Changes in respiratory frequency and minute volume in response to hypoxia and hypercapnia were calculated. Special attention was paid to maintaining a constant body temperature in studies with newborn mice (3, 17, 24).

Statistical analysis. According to the data structure, statistical analysis of the results was carried out with either the Student's t-test (paired and unpaired) or Dunnett's test for multiple comparison. Statistical analysis was performed with a statistics package program (SuperANOVA; Abacus Concepts, Berkeley, CA). Differences were considered to be significant at \(P < 0.05\). Results are expressed as means ± SE.

RESULTS

BP, HR, arterial blood gas and pH, and plasma concentration of immunoreactive ET-1. We measured BP of the ET\(_B\)-deficient and control animals under conscious and unrestrained conditions via an indwell-

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mediated signaling in mice. In contrast, BP in ETB−/− mice is not affected (change in BP = 2 ± 2 mmHg, n = 4) by intra-arterial injection of the ETA-specific antagonist BQ-123, indicating that the elevation of basal BP in ETB−/− mice is unlikely to be due to stimulation of pressor ETA.

Effect of intra-arterial injections of BQ-788 in anesthetized mice. A similar dose-dependent pressor effect was seen in mice anesthetized with ketamine and xylazine, indicating that the effect of BQ-788 was probably not due to antagonism of ETB in the central nervous system (Fig. 2C).

Effect of pretreatment with indomethacin or L-NMMA on pressor response to acute ETB blockade. Activation of endothelial ETB is known to induce the production of the depressor agents prostacyclin and nitric oxide (4). We found that pretreatment with indomethacin dose-dependently and significantly attenuates the pressor response to acute ETB blockade with BQ-788 in wild-type mice (Fig. 3). In contrast, L-NMMA increases basal BP by ~20 mmHg for >30 min, yet has no effect on the response to BQ-788 (Fig. 3). These findings suggest that the increase in BP in response to BQ-788 in wild-type mice may be mediated, at least in part, by inhibition of tonic prostaglandin (probably prostacyclin) production.

Ventilation. Abnormal central cardiorespiratory control is speculated to be one of the mechanisms of hypertension in ET-1-deficient mice (16, 17, 19). To determine whether a similar mechanism might contribute to the hypertension in ETB-deficient mice, we examined basal ventilation and changes in response to hypoxia and hypercapnia using whole body plethysmography. No difference in any studied ventilatory parameters between ETB−/− and ETB+/− adult mice was observed under basal or stimulated conditions (Table 2). Residual ETB in ETB−/− mice is unlikely to have influ-

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**Fig. 2.** Pressor response to acute ETB blockade. A: representative polygraph tracings illustrating responses of BP and HR to an administration of 3.0 mg/kg of BQ-788 in a wild-type mouse (left) and an ETB−/− mouse (right). BQ-788 was injected intra-arterially at moment shown by arrows. B: dose-response relationship of changes in mean BP in response to BQ-788 in awake 129/SvEv wild-type, ETB−/−, and ETB+/− mice. Difference between peak values and preinjection values was plotted as means ± SE. Dose-response curve for ETB−/− was significantly more blunted than that for ETB+/− or ETB+/− (P < 0.05). Baseline BPs were 115 ± 8 mmHg in ETB−/−, 137 ± 7 mmHg in ETB−/−, and 119 ± 7 mmHg in ETB+/−. C: dose-response relationship of changes in BP in response to BQ-788 in ketamine-xylazine anesthetized wild-type mice. Baseline BP was 84 ± 12 mmHg. *P < 0.05; **P < 0.01 vs. vehicle.

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**Fig. 3.** Effect of pretreatment with vehicle, indomethacin, or Nω-monomethyl-L-arginine (L-NMMA) on pressor response to acute ETB blockade in wild-type mice. Indomethacin transiently induced a pressor response of ~30 mmHg, but BP returned to baseline within 15 min. L-NMMA initially induced a pressor response that peaked ~25 mmHg over baseline. A sustained increase ~20 mmHg over baseline continued during the following entire experimental period. Note that slope of dose-response curve was similar in L-NMMA-pretreated and vehicle-pretreated groups, whereas it was smaller in indomethacin-pretreated group (P < 0.01). Baseline BPs before pretreatment were 108 ± 7 mmHg in vehicle pretreatment, 109 ± 11 mmHg in L-NMMA pretreatment, 108 ± 6 mmHg in indomethacin (14 μmol/kg) pretreatment, and 111 ± 4 mmHg in indomethacin (28 μmol/kg) pretreatment. *P < 0.05; **P < 0.01 vs. dose 0 of BQ-788.
Table 2. Comparison of $V$ and $f$ and their changes in response to hypoxia and hypercapnia between ET$_B^{-/-}$ and ET$_B^{+/+}$ adult mice and between ET$_B^{-/-}$ and ET$_B^{+/+}$ mice in an awake condition

<table>
<thead>
<tr>
<th></th>
<th>Adult</th>
<th>Newborn</th>
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<tr>
<td></td>
<td>ET$_B^{-/-}$</td>
<td>ET$_B^{+/+}$</td>
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<tr>
<td>n</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>29.1 ± 1.1</td>
<td>30.8 ± 0.9</td>
</tr>
<tr>
<td>Room air</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V$, ml·min$^{-1}$</td>
<td>100 g$^{-1}$</td>
<td>100 g$^{-1}$</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>115 ± 6</td>
<td>118 ± 7</td>
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<tr>
<td></td>
<td>291 ± 9</td>
<td>283 ± 5</td>
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<tr>
<td>Hypoxia</td>
<td></td>
<td></td>
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<tr>
<td>$V$, ml·min$^{-1}$</td>
<td>100 g$^{-1}$</td>
<td>100 g$^{-1}$</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>124 ± 10</td>
<td>124 ± 8</td>
</tr>
<tr>
<td>$\Delta V$, %</td>
<td>6.9 ± 4.2</td>
<td>5.0 ± 4.8</td>
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<tr>
<td>$\Delta f$, %</td>
<td>15.4 ± 2.1*</td>
<td>14.0 ± 2.4*</td>
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<tr>
<td>100% $O_2$</td>
<td></td>
<td></td>
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<tr>
<td>$V$, ml·min$^{-1}$</td>
<td>100 g$^{-1}$</td>
<td>100 g$^{-1}$</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>107 ± 6</td>
<td>113 ± 6</td>
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<tr>
<td>Hypercapnia</td>
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<tr>
<td>$V$, ml·min$^{-1}$</td>
<td>100 g$^{-1}$</td>
<td>100 g$^{-1}$</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>124 ± 7</td>
<td>146 ± 9</td>
</tr>
<tr>
<td>$\Delta V$, %</td>
<td>34.6 ± 4.5*</td>
<td>28.7 ± 4.9*</td>
</tr>
<tr>
<td>$\Delta f$, %</td>
<td>4.4 ± 2.5</td>
<td>6.4 ± 1.7*</td>
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Values are means ± SE. $V$, respiratory minute volume; $f$, respiratory frequency; $\Delta$, change. Significantly different from corresponding value in control condition (room air for hypoxia and 100% $O_2$ for hypercapnia, respectively); *$P < 0.05$ (paired t-test). Note that there is no difference in any variables between ET$_B^{-/-}$ and ET$_B^{+/+}$ adult mice and between ET$_B^{-/-}$ and wild-type (ET$_B^{+/+}$) newborn mice (t-test).

end this study because no abnormality was detected in ET$_B$-null newborn mice (Table 2).

**DISCUSSION**

The present study, using ET$_B$-deficient mice that are healthy into adulthood, suggests that ET$_B$ not only counteracts the potent pressor effect of elevated levels of ETs in pharmacological (and possibly pathophysiological) settings (10), but also maintains BP at a relatively lower level when circulating ETs are within the normal range. We hypothesize that endogenous ET-1 acts as a peripheral depressor agent at least in part through activation of the endothelial ET$_B$. Alternatively, mice deficient in ET$_B$ might have abnormal central cardiovascular control, with impaired chemoreceptor reflexes and abnormally high sympathetic outflow like that seen in ET-1-deficient mice (15, 17–19). However, this seems unlikely, because arterial blood pH, $P_{CO_2}$, $P_{O_2}$ (Table 1), ventilatory frequency and volume, and the magnitude of the ventilatory response to hypoxia and hypercapnia are not different between ET$_B^{-/-}$ and ET$_B^{+/+}$ mice (Table 2). In our preliminary experiments, power spectral analysis of fluctuations in the R-R intervals recorded by electrocardiogram (15) revealed that cardiac sympathetic and vagal activities were not different between ET$_B^{-/-}$ and ET$_B^{+/+}$ mice. Therefore, the mechanism(s) of the hypertension in ET$_B$-deficient mice and that in ET-1 deficient mice (16, 17, 19) appear to be different.

It is also possible that the elevated BP in ET$_B^{-/-}$ mice might be a result of developmental rather than homeostatic effects, in light of the fact that null mutations of ET-1 (16), ET-3 (2), ET$_A$ (3), ECE-1 (30), or ET$_B$ genes (11) result in congenital malformations. However, our pharmacological data demonstrating an elevation of BP in ET$_B^{-/+}$ mice elicited by the acute administration of an ET$_B$ antagonist to levels comparable to those in ET$_B^{-/-}$ mice strongly suggest that the elevated BP in ET$_B^{-/-}$ mice reflects a disruption of homeostatic function of the ET$_B$. Genetic evidence presented in this study is further supported by preliminary experiments using ET$_B^{-/-}$ mice that are rescued from the lethal megacolon phenotype by a tissue-specific transgene that expresses ET$_B$ in enteric neurons (23). These mice, which are healthy into adulthood, lack vascular ET$_B$ and exhibit hypertension of similar magnitudes (=20 mmHg) to that seen in ET$_B^{-/-}$ mice. These results also support the view that the endothelial ET$_B$ plays an essential depressor role to maintain BP in the normal range. Recently, Giller et al. (8) reported hypertension in piebald-lethal (sl) mice, another naturally occurring mutant with ET$_B$ deletion; however, this observation might be the result of circulatory distress in the intestine associated with megacolon.

It has been suggested that ET$_B$ is possibly a clearance receptor for circulating ETs (5). Our study found that plasma concentrations of immunoreactive ET-1 in ET$_B^{-/-}$ and ET$_B^{+/+}$ mice (both ~17 pg/ml) are significantly higher than those in ET$_A^{-/-}$ mice and wild-type mice (~11 pg/ml). The elevated BP in ET$_B^{-/-}$ mice may be a consequence of reduced clearance of circulating ETs, with increased levels of ETs available at ET$_A$ and subsequent vasoconstriction. However, this possibility seems unlikely for the following four reasons. First, the apparent discrepancy in plasma concentrations of immunoreactive ET-1 may only reflect the difference in genetic background. Specifically, ET$_B^{-/-}$ and ET$_B^{+/+}$ are F$_1$ of ET$_B^{-/-}$ and ET$_B^{+/+}$ mice, whereas ET$_A^{-/-}$ and wild-type mice have a pure 129/SvEv background. Moreover, the rank order of BP (ET$_B^{-/-}$ > ET$_B^{+/+}$ = ET$_B^{+/+}$) cannot be explained by the plasma concentrations of immunoreactive ET-1 (ET$_B^{-/-}$ = ET$_B^{+/+}$ > ET$_B^{+/+}$). Furthermore, there is no significant correlation between BP and the ET-1 levels in plasma collected immediately after the BP measurement (data not shown). Second, BP in ET$_B^{-/-}$ mice is not affected by intra-arterial injection of BQ-123, a selective ET$_A$ antagonist (13). Third, the fact that BP is indistinguishable between ET$_A^{-/-}$ mice and wild-type littersmates suggests that a small change in the ratio of ET$_B$ to ET-1 may be readily compensated by other vasoregulatory mechanisms. Finally, it is difficult to explain the short duration of the pressor response to BQ-788 in the present study by the clearance hypothesis, because ET-1 characteristically evokes a long-lasting vasoconstriction that is extremely resistant to washout (31). Therefore, the role of ET$_B$ as a clearance receptor, if any,
BLOOD PRESSURE IN ET\textsubscript{B} RECEPTOR-DEFICIENT MICE

is unlikely to account for the observed elevation of BP in ET\textsubscript{B}\textsuperscript{−/−} mice.

Pharmacological studies suggest that under physiological conditions, endogenous ETs stimulate a sustained release of ET\textsubscript{B}-mediated dilator substance(s) produced by vascular endothelial cells, one of which is prostacyclin. Although BQ-788 antagonizes the effect of subsequent administration of an exogenous ET\textsubscript{B} agonist, some papers (14), although not all (22), report that it has no detectable effect on basal BP. This inconsistency may result from route of administration, choice of anesthetic, and/or species-specific differences. Our preliminary experiments using intravenous administration of BQ-788 resulted in much smaller pressor responses than when it was delivered intra-arterially. Because the ET\textsubscript{B} is abundantly expressed in the lung (5), it is possible that a large fraction of intravenously administered BQ-788 is sequestered in the lung and not delivered to the systemic circulation. Recently, Verhaar et al. (29) reported that ET\textsubscript{B} antagonism by BQ-788 causes local vasoconstriction in human forearm blood vessels. Thus, in both humans and mice, vascular ET\textsubscript{B} appears to tonically mediate vasodilation.

The importance of prostaglandins in systemic BP regulation has been controversial (7, 20); however, this study supports a potential role. It is unclear which product of cyclooxygenase is responsible for the present observation, because indomethacin is not a specific inhibitor for prostacyclin synthesis. Recently, normotension in prostacyclin receptor-deficient mice was reported (21). This observation does not necessarily contradict the possible involvement of prostacyclin in physiological BP regulation, because compensatory mechanisms such as an increase in nitric oxide cannot be excluded in these mutant mice. Lack of an inhibition by pretreatment with L-NMMA, on the other hand, is not likely to be secondary to insufficient dosing of the drug, because the pressor effect of L-NMMA lasts for >30 min and encompasses all of the resting experimental period with BQ-788. Nevertheless, the residual pressor response to BQ-788 after pretreatment with indomethacin remains to be explained.

Perspectives

Although the pharmacological experiments in this study strongly suggest that deficiency of vascular ET\textsubscript{B} is the cause of hypertension in ET\textsubscript{B}\textsuperscript{−/−} mice, we do not exclude the possibility that deficiency of renal ET\textsubscript{B} also contributes to the elevation of BP. ET\textsubscript{B} is richly distributed in glomerular endothelial cells and vasa recta bundles as well as tubular epithelial cells in the kidney, and it is proposed to be involved in water and sodium metabolism (10). We are currently investigating this possibility using transgenically “rescued” ET\textsubscript{B}\textsuperscript{−/−} mice that do not develop megacolon. In this model, the dopamine \textbeta\textsubscript{2}-hydroxylase gene promoter transgenically directs expression of ET\textsubscript{B} in enteric neuroblasts during embryonic development (6, 23). These animals completely lack ET\textsubscript{B} in vascular endothelium and kidney. However, the possibility of ectopic and/or exaggerated expression of the ET\textsubscript{B} in adrenal medulla or other catecholamine-synthesizing cells cannot be excluded at present. We hope that after the complete biochemical and physiological characterization of cardiovascular and renal function in these transgenic animals, combined with this present report, we will be able to come to a conclusion on the roles of vascular and renal ET\textsubscript{B} in BP regulation.

Development of clinically useful ET receptor antagonists is expected to be beneficial in treating certain cardiovascular conditions accompanied by an elevation in ET levels, such as congestive heart failure and essential hypertension (12, 25). These endeavors are consistent with the original view of ET as a potent vasoconstriction factor (31). The present results indicate that ET may also play a role as a vasodilator in physiological states. Thus we must carefully consider the potential multifaceted effects of ET\textsubscript{B} antagonism in the treatment of these disorders.

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