Endothelin in the splanchnic vascular bed of DOCA-salt hypertensive rats

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Wang, Hong, Alex F. Chen, Stephanie W. Watts, James J. Galligan, and Gregory D. Fink. Endothelin in the splanchnic vascular bed of DOCA-salt hypertensive rats. Am J Physiol Heart Circ Physiol 288: H729–H736, 2005. First published October 7, 2004; doi:10.1152/ajpheart.00388.2004.—Vascular capacitance is reduced by endothelin-1 (ET-1) in deoxycorticosterone (DOCA)-salt hypertensive rats. This may contribute to hypertension development. Because the splanchnic blood vessels (especially veins) are important in determining vascular capacitance, we tested the hypothesis that ET-1 levels in the splanchnic vasculature are elevated in hypertensive DOCA-salt compared with normotensive rats. Tissue ET-1 content was measured by ELISA in aorta, vena cava, superior mesenteric artery and vein, and small mesenteric arteries and veins from normotensive sham-operated (sham) and 4-wk DOCA-salt rats. We also determined ET-1 concentration in aortic and portal venous blood (draining the nonhepatic splanchnic organs) in anesthetized and conscious sham and DOCA-salt rats before and after acute blockade of ETB receptor-mediated plasma clearance of ET-1. Results showed a higher ET-1 content in veins than in arteries of similar size. However, ET-1 content was similar in vessels from sham and DOCA-salt rats, except in aorta and superior mesenteric artery, where ET-1 content was greater in DOCA-salt rats. ET-1 concentration was significantly higher in portal venous than in aortic blood, indicating net nonhepatic splanchnic release (nNHSR) of ET-1. However, nNHSR of ET-1 was higher in portal venous than in aortic blood, indicating net nonhepatic splanchnic release (nNHSR) of ET-1. Although nNHSR of ET-1 increased significantly after ETB receptor blockade in sham rats, it was completely unchanged in DOCA-salt rats. These data suggest that, despite the absence of ETB receptor-mediated plasma clearance of ET-1, neither the venous peptide content nor the net release of ET-1 is increased in the splanchnic vasculature of DOCA-salt rats. These results argue against the hypothesis that increased venomotor tone in DOCA-salt hypertension is caused by increased ET-1 concentration around splanchnic venous smooth muscle cells.

endothelin-1; splanchnic vasculature; vascular capacitance; veins; deoxycorticosterone

THE ENDOTHELIUM REGULATES local vascular tone through release of vasodilator and vasoconstrictor substances (21). Endothelin-1 (ET-1), synthesized by endothelial cells, is a very potent vasoconstrictor. It has been implicated in the pathogenesis of hypertension in humans and experimental animals (4, 7, 17, 18). ET-1 has an especially prominent role in salt-sensitive forms of hypertension, such as deoxycorticosterone (DOCA)-salt hypertension (31). One important effect of ET-1 in DOCA-salt hypertension is to increase total peripheral resistance by contracting arteries and arterioles (44). Most studies have shown, however, that arterial plasma levels of ET-1 are not increased in DOCA-salt rats (25, 26, 32, 36, 40). Nonetheless, prepro-ET-1 mRNA expression and immunoreactive ET-1 content of aorta and mesenteric arteries are increased in DOCA-salt rats (8, 19, 20, 32), suggesting that one mechanism of ET-1-induced arterial constriction in hypertension is increased level of the peptide around arterial smooth muscle. A caveat to that conclusion is raised by findings that contractions to ET-1 are significantly reduced in arteries of DOCA-salt compared with sham rats (15). Thus increased local production of ET-1 by arteries may be countered by decreased arterial responsiveness, and it remains unclear how ET-1 increases vascular resistance in DOCA-salt hypertension.

Hypertensive humans and experimental animals also exhibit decreased systemic vascular capacitance (28, 30). This is primarily the result of changes in the structure or vasoconstrictor activity of extrathoracic veins, especially those in the splanchnic bed (30). Reduced vascular capacitance contributes to the hemodynamics of hypertension by contributing, along with blood volume, to maintenance of “effective blood volume,” a critical determinant of venous return of blood to the heart and, thus, cardiac output (30). We recently reported evidence for increased venoconstriction in conscious DOCA-salt rats using repeated measurements of blood volume and mean circulatory filling pressure (MCFP), an in vivo index of venous smooth muscle contractile activity (12). Endogenous ET-1 appears at least partly responsible for that augmented venoconstriction, because we also showed that ET receptor antagonists lower MCFP in hypertensive DOCA-salt, but not normotensive, rats (16).

As with vascular resistance, two mechanisms could explain increased ET-1-mediated venoconstriction: 1) higher levels of the peptide around venous smooth muscle cells and/or 2) enhanced responsiveness of venous smooth muscle to ET-1. We previously showed that vena cava and small mesenteric veins from DOCA-salt rats do not exhibit increased reactivity to ET-1 in vitro (15, 41). Therefore, it was logical to conclude that higher ET-1-mediated venomotor tone in DOCA-salt rats is caused by increased ET-1 concentrations around venous smooth muscle. Measurement of prepro-ET-1 gene expression, as an index of ET-1 synthesis, in small veins, however, revealed no differences between sham and DOCA-salt rats (15). That finding does not support the hypothesis that increased venomotor activity in DOCA-salt rats is caused by local overproduction of ET-1 by veins.

Veins in DOCA-salt rats could be exposed to higher concentrations of ET-1 by mechanisms other than increased venous endothelial cell synthesis. For example, increased production and release into blood of ET-1 from upstream arterial endothelial cells could affect venous smooth muscle cell activity directly or provide “excess” ET-1 to be taken up into venous endothelial cells for subsequent release. To test these possibilities, we made two kinds of measurements to estimate ET-1 concentrations in the biophase around venous smooth
muscle cells: 1) total vascular ET-1 content and 2) venous plasma ET-1 concentration. Measurements were made in the splanchnic vascular bed, because it represents the most important capacitance bed in the circulation. We also estimated net nonhepatic splanchnic release (nNHSR) of ET-1 by determining the difference between ET-1 concentrations in inflowing arterial and outflowing portal venous blood. The liver was excluded from these experiments because of the difficulty of sampling blood from the hepatic venous drainage in the rat. This also prevents the interference of hepatic ET-1 extraction with the measurement of splanchnic release.

Initial experiments were performed in anesthetized rats. Additional studies were performed in conscious rats, because anesthesia and acute surgery can affect hormone synthesis and release.

Plasma ET-1 level is determined by a combination of factors, including production rate, metabolism, and clearance. Previous studies indicate that clearance by endothelial ET_B receptors is the most important mechanism for removing ET-1 from blood (3, 29, 34, 35). To test the possibility that a functional alteration in ET_B receptors might affect the venous plasma ET-1 concentration in DOCA-salt hypertension, we measured the portal venous-aortic plasma ET-1 difference after selective ET_B receptor blockade in anesthetized and conscious rats.

MATERIALS AND METHODS

Animals. The protocols were approved by the Michigan State University Committee on Animal Use and Care. Male Sprague-Dawley rats (Charles River Laboratories, Portage, MI; 175–225 g body wt) were housed two to three per cage under conditions of constant humidity and temperature and subjected to 12:12-h light-dark cycles. After 2–3 days of habituation, rats were uninephrectomized, DOCA pellets (200 mg/kg; Sigma) were implanted subcutaneously, and rats were offered drinking water that contained 1% NaCl and 0.2% KCl. Sham-operated rats were uninephrectomized and drank tap water. All rats were fed standard pellet rat chow (8640 Rodent Diet, Harlan/Teklad, Madison, WI). At 3 wk after surgery, systolic blood pressure was measured using the tail-cuff method (pneumatic transducer, Narco). Rats with a tail-cuff blood pressure of >150 mmHg were considered hypertensive.

Vascular ET-1 content measurement. At ~4 wk after DOCA implantation or sham surgery, rats were euthanized by pentobarbital sodium overdose (100 mg/kg ip; Sigma), and segments of thoracic aorta, thoracic vena cava, and abdominal vena cava were removed and dissected free of fat. The complete mesenteric vascular bed was removed and placed in a petri dish full of ice-cold Krebs solution. The small intestine was stretched gently and pinned flat. Superior mesenteric artery and vein and small mesenteric arteries and veins (150–250 μm diameter) were dissected free of fat and collected separately. The tissues were snap frozen in liquid nitrogen and stored at ~80°C until extraction of ET-1 was performed. On the day of extraction, tissues were homogenized with a Polytron in 1 ml of 1 M acetic acid containing 10 μg/ml peptatin A (ICN Pharmaceuticals, Costa Mesa, CA) and immediately heated to 100°C for 10 min. The homogenate was chilled and then centrifuged at 14,000 g for 30 min at 4°C. The supernatant was dried in a Speed-Vac and then reconstituted in 30 μl of calibrator diluent (R & D Systems, Minneapolis, MN). The reconstituted sample was stored at ~80°C until measurement of ET-1 was performed.

Catheterization and sampling of blood. At ~3.5 wk after DOCA implantation or sham surgery, vascular catheters were placed in each rat. Anesthesia was produced with pentobarbital sodium (30–50 mg/kg ip), and atropine (0.2 mg/kg ip; Sigma) was administered to decrease bronchial secretion. A silicone rubber catheter was inserted into the abdominal aorta via a femoral artery for blood sampling and blood pressure recording. Through a midline laparotomy, another catheter was inserted into the portal vein via a small branch of the portal vein for venous blood sampling. Because ET-1 concentration in portal venous blood is derived from inflowing arterial blood and in part from peptide released from splanchnic tissues (mainly endothelial cells) themselves, the difference (“step-up”) between arterial and portal venous plasma ET-1 concentrations was used as an index of nNHSR of ET-1. We excluded liver from the experiment to prevent the interference of hepatic ET-1 extraction with measurement of splanchnic release by portal venous, instead of hepatic venous, sampling. A third catheter was placed into the abdominal vena cava through a femoral vein for drug administration. In the anesthetized group, rats were allowed to recover from surgery on a heated pad for 30 min before blood sampling (see below). A-192621 (12 mg/kg iv; Abbott Laboratories), a selective ET_B receptor antagonist (42), was then given, and blood samples were collected again 30 min later. It has been shown by previous studies in our laboratory (16) that this dose of A-192621 is effective at blocking ET_B receptor antagonism and causes a significant increase in mean arterial pressure (MAP) in DOCA-salt rats. The increase reaches maximum at 30 min after A-192621 administration.

An additional experiment was performed in anesthetized rats to test the potential role of ET_A receptors in the plasma clearance and nNHSR of ET-1. A-182086 (12 mg/kg iv; Abbott Laboratories), a mixed ET_A–ET_B receptor antagonist, was given to rats 30 min after recovery from catheterization as described above. Aortic and portal venous blood samples were collected before and 1 h after drug treatment. The dose of A-182086 was shown to be efficacious in preliminary studies in which antagonist was tested against acute pressor actions of ET-1 (data not shown). Published studies also indicate that this dose is effective in blocking both receptor subtypes in rats (42). The nNHSR of ET-1 after A-182086 treatment was compared with that after treatment with the selective ET_B receptor antagonist A-192621. The difference was used as an index of the amount of ET-1 bound to ET_B receptors in the splanchnic vasculature.

In rats to be studied while conscious, the ends of all three catheters were tunneled subcutaneously to the head, where they exited the rat inside a stainless steel spring secured to the skull with jeweler’s screws and cyanoacrylate glue. Injections of enrofloxacin (5 mg/kg iv; Bayer) were given for bacterial prophylaxis. Rats were allowed to recover consciously on a heated pad under constant observation. They were then placed in stainless steel metabolism cages under loose tethering, allowing continuous access to all catheters without handling or otherwise disturbing the rat. Enrofloxacin (5 mg/kg iv) was administered to all rats for 3 days after surgery. Vascular catheters were filled with heparin saline solutions when not in use and flushed daily. Experiments were started after >4 days of surgical recovery, inasmuch as previous work in our laboratory indicated that normal food and saline intake in DOCA-salt rats typically did not recover to presurgery level for 4 days. Blood samples (with and without A-192621 treatment) were taken with ≥1 day separating experiments. In anesthetized and conscious groups, 0.5-ml blood samples were collected simultaneously from arterial and portal venous catheters into ice-chilled plastic syringes and transferred to ice-chilled plastic tubes containing EDTA. Blood samples were centrifuged at 10,000 g for 5 min at 4°C. Plasma was separated and stored at ~80°C until assayed.

Hemodynamic measurements. Arterial pressure of anesthetized rats was determined by connecting the arterial catheter to a transducer linked to a pen chart recorder (Kent Scientific, Torrington, CT). In the conscious group, arterial pressure was determined by connecting the arterial catheter to a low-volume-displacement pressure transducer (model TXD-300, Micro-Med, Louisville, KY) linked to a digital pressure monitor (model BPA-200, Micro-Med), which derives systolic, mean, and diastolic pressures and heart rate every 0.5 s (sampling rate = 1,000 Hz). All values were averaged minute-by-minute.
and saved using a computerized data acquisition system (model DMSI-200/4, Micro-Med).

Measurement of ET-1. Vascular ET-1 content and plasma ET-1 concentration were measured by quantitative sandwich enzyme immunoassay using the ET-1 Quantiglo chemiluminescent assay kit (R & D Systems, Minneapolis, MN). The minimum detectable concentration of ET-1 is \( \sim 0.16 \text{ pg/ml} \). Some cross-reactivity and interference were observed with human Big ET-2, porcine Big ET-39, human Big ET-38, bovine Big ET-39, rat Big ET-39, sarafotoxin, human ET-3, and human ET-2. Inter- and intra-assay coefficients of variation are 7.1 and 2.1%, respectively.

**Data analysis.** Values are means ± SE. Comparisons of vascular ET-1 content and plasma ET-1 concentration between DOCA-salt and sham rats were made using Student’s *t*-tests for paired or independent samples where appropriate. Bonferroni’s correction for multiple comparisons was applied where appropriate. Differences were considered significant at *P* < 0.05.

**RESULTS**

**MAP.** Average MAP recorded from conscious, freely moving DOCA-salt rats was significantly greater than the average value recorded from conscious sham rats. In anesthetized animals, the average MAP recorded from DOCA-salt rats was the same as that recorded from sham rats. The average MAP was significantly greater in conscious than in anesthetized DOCA-salt rats (Fig. 1).

**Vascular ET-1 content.** ET-1 content (normalized by tissue wet weight) in thoracic aorta, thoracic vena cava, abdominal vena cava, superior mesenteric artery and vein, and small mesenteric arteries and veins from sham and DOCA-salt rats was measured using ELISA after acid extraction (Fig. 2). On the basis of tissue weight, ET-1 content was greater in veins than in arteries of similar size. ET-1 content was higher in small than in larger vessels. ET-1 content was significantly higher in aorta and superior mesenteric artery from DOCA-salt than from sham rats. There was no difference in ET-1 content in other vessels between sham and DOCA-salt rats.

**Plasma concentrations of ET-1.** Plasma ET-1 concentration was significantly higher in portal vein than in aorta in anesthetized sham and DOCA-salt rats, indicating nNHSR of ET-1. However, there was no difference in portal venous plasma ET-1 level between sham and DOCA-salt rats or in aortic plasma ET-1 levels between the two groups (Fig. 3B). The aortic and portal venous plasma ET-1 concentrations were significantly lower in conscious than in anesthetized sham rats. The portal venous plasma ET-1 concentration was significantly lower in conscious than in anesthetized DOCA-salt rats (Fig. 3). The aortic plasma ET-1 concentration was lower in conscious than in anesthetized DOCA-salt rats, but the difference was not quite significant (*P* = 0.066).

**Total ETB receptor-mediated plasma clearance of ET-1.** Administration of the ETB receptor antagonist A-192621 caused a significant increase in MAP in anesthetized DOCA-salt and sham rats (Fig. 4A). A-192621 also caused a significant

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**Fig. 1.** Mean arterial pressure of anesthetized sham and deoxycorticosterone (DOCA)-salt hypertensive rats. Values are means; error bars represent SE. *P* < 0.05 vs. conscious sham. *P* < 0.05 vs. anesthetized DOCA.

**Fig. 2.** Endothelin-1 (ET-1) content in blood vessels from sham and DOCA-salt rats. Values are means; error bars represent SE. *P* < 0.05 vs. sham.

**Fig. 3.** Aortic and portal venous plasma ET-1 concentrations in anesthetized (A) and conscious (B) sham and DOCA-salt rats. Values are means; error bars represent SE. *P* < 0.05 vs. aorta. *P* < 0.05 vs. anesthetized rats.
increase in MAP in conscious sham, but not conscious DOCA-salt, rats (Fig. 4B).

A-192621 caused a significant increase in aortic plasma ET-1 concentration in anesthetized (Fig. 5A) and conscious (Fig. 5B) sham and DOCA-salt rats. There was no difference in aortic plasma ET-1 concentrations between sham and DOCA-salt rats after A-192621 treatment under anesthesia or in the conscious state. The change in aortic plasma levels of ET-1 before and after treatment also was not different between sham and DOCA-salt rats.

At 30 min after A-192621 treatment, portal venous plasma ET-1 concentration was significantly and similarly increased in anesthetized and conscious sham and DOCA-salt rats (Fig. 6). ETB receptor-mediated plasma clearance of ET-1 in nonhepatic splanchnic vasculature. The nNHSR of ET-1 was the same in anesthetized sham and DOCA-salt rats. After A-192621 treatment, there was a significant increase in nNHSR of ET-1 in sham, but not DOCA-salt, rats (Fig. 7A).

In conscious animals, nNHSR of ET-1 also was the same in sham and DOCA-salt rats. After ETB receptor antagonist treatment, nNHSR of ET-1 was significantly increased in sham rats but remained unchanged in DOCA-salt rats (Fig. 7B). The basal nNHSR of ET-1 was significantly lower in conscious than in anesthetized sham rats. The nNHSR of ET-1 was lower in conscious than in anesthetized DOCA-salt rats, but the difference was not significant (P = 0.057).

Potential ETA receptor-mediated plasma clearance of ET-1 in nonhepatic splanchnic vasculature. A-182086 treatment did not cause a significant change in MAP in DOCA-salt or sham rats. A-182086 significantly increased aortic and portal venous plasma ET-1 concentrations in both groups: from 2.3 ± 0.3 to 64.7 ± 5.7 pg/ml in aortic blood and from 3.5 ± 0.3 to 78.0 ± 8.5 pg/ml in portal venous blood of sham rats and from 1.1 ± 0.1 to 46.0 ± 8.7 pg/ml in aortic blood and from 2.1 ± 0.2 to 62.3 ± 8.0 pg/ml in portal venous blood of DOCA-salt rats. The nNHSR of ET-1 of both groups was significantly increased (Fig. 8). By comparing those values with data from A-192621-treated rats, the effect of ETA receptors on nNHSR of ET-1 was estimated. This effect was not significantly different in DOCA-salt and sham rats (14.4 ± 5.6 vs. 8.7 ± 4.6 pg/ml).

DISCUSSION

ET-1 content of mesenteric arteries and veins. Our studies focused on the splanchnic circulation, because the veins of the splanchnic vasculature hold the largest amount of blood within the venous system (~40% of total blood volume), so systemic vascular capacitance depends largely on veins (especially small mesenteric veins) in this region. Structural or neurohumoral changes in these vessels can lead to a reduction in vascular capacitance. This would increase cardiac filling pressure as blood is translocated toward the heart. The resulting elevation of cardiac output could be a factor in the development and maintenance of hypertension.

ET-1 content of blood vessels is determined by local vascular production and reuptake and storage of the peptide. These processes occur predominantly, but not exclusively, in endothelial cells. Total vascular content of ET-1 thus represents a rough index of local concentrations of the peptide available to cause vasoconstriction in vivo. Measurements of splanchnic vascular ET-1 content in this study produced novel findings. I)
On the basis of tissue weight, ET-1 content is higher in veins than in arteries. This may reflect a higher ratio of endothelial cells to other cell types in veins than in arteries or a greater ET-1 synthetic capacity of venous than of arterial endothelial cells.

2) ET-1 content was higher in smaller than in larger vessels. This may reflect the relative amount of endothelial cells in our samples (especially in arteries) but also is consistent with an important role for ET-1 in control of vascular resistance and capacitance, because these physiological functions are mainly served by smaller vessels in the intact circulation.

3) We found that vascular ET-1 content was significantly greater in DOCA-salt rats only in the aorta and superior mesenteric artery, and not in veins of any size. This suggests that production of ET-1 by venous endothelial cells is not increased in DOCA-salt rats and is consistent with our previous finding of unchanged prepro-ET-1 mRNA level in mesenteric veins in DOCA-salt rats (15).

Steady-state vascular content of ET-1, however, may not be an accurate indicator of local formation of ET-1 around venous capacitance vessels, because released ET-1 in vivo is subjected to rapid tissue binding, reuptake, and metabolism. The kinetics of ET-1 disposition are only beginning to be understood (9, 10).

ET-1 level in venous blood and nNHSR of ET-1. We conducted additional experiments designed to evaluate the rate of ET-1 release by the nonhepatic splanchnic vasculature of normotensive and hypertensive DOCA-salt rats in vivo. Although ET-1 release by endothelial cells is reported to be polarized toward vascular smooth muscle (80%) compared with blood (20%), we reasoned that venous blood (portal vein) draining the splanchnic bed (except the liver) would yield a reliable estimate of differences in ET-1 production and release by splanchnic vessels. Because part of portal venous plasma ET-1 is derived from arterial blood entering the splanchnic bed, it was also necessary to measure arterial plasma ET-1 levels. Thus, in analogy to other studies (1, 6), we used the difference between arterial and portal venous ET-1 plasma concentrations as an index of nNHSR of ET-1. Previous studies demonstrated that arterial plasma levels of ET-1 are not increased in DOCA-salt rats. Our results confirmed this finding in anesthetized and conscious DOCA-salt rats (Fig. 3). No studies have been reported on splanchnic venous plasma ET-1 level or nNHSR in this model of hypertension. We hypothesized that portal venous plasma ET-1 concentration and nNHSR of ET-1 would be higher in DOCA-salt than in sham rats. This would indicate that the endothelial cells in the splanchnic vasculature of DOCA-salt rats release more ET-1 in vivo and that venous smooth muscle cells of the splanchnic bed are exposed to higher ET-1 concentrations.

Data reported here show that portal venous plasma ET-1 level is significantly higher than aortic plasma ET-1 level in anesthetized hypertensive DOCA-salt and normotensive sham rats, indicating nNHSR of ET-1 in both groups of animals. However, there was no difference in portal venous plasma ET-1 level or in nNHSR of ET-1 between the two groups. This provides the first in vivo evidence that endothelial cells of the splanchnic vasculature do not produce more ET-1 in DOCA-salt than in sham rats. However, as mentioned above, plasma ET-1 level is determined by a combination of factors, including production rate, metabolism, clearance, and regional blood flow. Therefore, these results do not indicate that endothelial cells of the splanchnic vasculature of DOCA-salt rats do not produce more ET-1 than those of sham rats. However, arterial plasma ET-1 levels are not increased in DOCA-salt rats and are consistent with our previous finding of unchanged prepro-ET-1 mRNA level in mesenteric veins in DOCA-salt rats (15).

Fig. 6. Portal venous plasma ET-1 concentration before and after drug treatment in anesthetized (A) and conscious (B) sham and DOCA-salt rats. Values are means; error bars represent SE. *P < 0.05 vs. control. #P < 0.05 vs. anesthetized rats.

Fig. 7. Net nonhepatic splanchnic release of ET-1 before and after drug treatment in anesthetized (A) and conscious (B) sham and DOCA-salt rats. Values are means; error bars represent SE. *P < 0.05 vs. control. #P < 0.05 vs. anesthetized rats.
flow. Blood flows to the stomach and small and large intestines are not affected by DOCA-salt treatment (14, 43), so disparities in regional blood flow are not likely to contribute to our findings. Another mechanism that could influence venous plasma ET-1 level and nNHSR of ET-1 in DOCA-salt rats is altered ETB receptor-mediated uptake activity. For example, enhanced endothelial cell production and release of ET-1 in the splanchnic vasculature of DOCA-salt rats might not be apparent with our in vivo measurements if there is also increased removal of ET-1 from plasma by reuptake into endothelial cells.

ET-1 clearance by ETB receptors. ET-1 is rapidly removed from circulating blood, and current evidence suggests that the ETB receptor is very important for that process. ET-1 binds almost irreversibly to the ETB receptor under physiological conditions (38). Direct transport of ETB receptor-associated ET-1 to lysosomes for degradation may serve as the main mechanism for clearance of plasma ET-1 (2). To determine whether altered endothelial ETB receptor-mediated uptake activity contributes to venous plasma ET-1 level and nNHSR of ET-1, we measured the portal venous plasma ET-1 concentration and portal venous-aortic plasma ET-1 difference in the presence of the selective ETB receptor antagonist A-192621. We found a significant increase in portal venous-aortic plasma ET-1 difference in sham, but not DOCA-salt, rats after acute blockade of ETB receptors. Thus, in sham rats, there is significant clearance of ET-1 from the splanchnic circulation through uptake mediated by ETB receptors. The uptake activity of endothelial ETB receptors in the splanchnic vasculature of DOCA-salt rats, however, was significantly decreased (or even absent). However, the whole body clearance of ET-1 mediated by the ETB receptor (predominantly pulmonary), which is reflected by the aortic plasma ET-1 concentration before and after A-192621 treatment, was unchanged in DOCA-salt rats. These novel results reveal a regionally specific defect in ETB receptor-mediated plasma clearance of ET-1 in the splanchnic vasculature of DOCA-salt rats.

Mechanism and impact of reduced ETB-mediated plasma clearance of ET-1. Possible mechanisms underlying the decreased uptake activity of ETB receptor specifically in the splanchnic vasculature of DOCA-salt rats include 1) a decrease in overall endothelial cell function, 2) a decreased density of endothelial ETB receptors, 3) a lower binding affinity of ETB receptors for ET-1, or 4) altered postreceptor ET-1 disposition mechanisms. Available data do not allow a clear distinction between these possibilities, although functional responses of small mesenteric veins to ETB receptor activation did not differ in sham and DOCA-salt rats (15). Previous results indicate that immunoreactive ETB receptor number tends to be higher in the splanchnic vasculature of sham vs. DOCA-salt rats (41). However, that study did not distinguish between endothelial and smooth muscle ETB receptors; these probably differ in their ability to clear ET-1 from the circulation. Possible differences between large and small veins also were not considered. In future studies, we will use immunohistochemistry and binding studies to compare the density of endothelial ETB receptors in the mesenteric veins of sham and DOCA-salt rats. In any case, the physiological significance of reduced splanchnic ET-1 plasma clearance by ETB receptors is not known; i.e., it is uncertain whether the decreased uptake activity of ETB receptor in the splanchnic vasculature affects the ability of ET-1 to control vascular capacitance in DOCA-salt rats. Further work is required to address that question.

Potential role of ETA receptors in the plasma clearance of ET-1 in the splanchnic vasculature. Binding of ET-1 to ETA receptors on vascular smooth muscle cells may also influence circulating ET-1 level, because in contrast to other hormones, there appears to be a small amount of ET peptide in relation to the concentration of ET receptors (13). Besides, the binding of ET-1 to its receptors is particularly tight, and ET-1 remains bound to ETA receptors, even after their internalization (4). To test the possibility that ETA receptor binding contributes to the nNHSR of ET-1, we compared nNHSR of ET-1 after ETA- and ETB receptor blockade with that after selective ETB receptor antagonist alone. The difference was used as an index of the amount of ET-1 bound to ETA receptors in the splanchnic vasculature. We found that ETA-and-ETB receptor blockade caused a significant increase in aortic and portal venous plasma ET-1 concentrations in DOCA-salt and sham rats. The magnitude of that increase was roughly twice that in rats with ETB receptor blockade alone. This suggests that a significant amount of ET-1 is removed from the circulation by ETA receptor binding and is consistent with a previous hypothesis (13) that functional ETA receptors also act as “clearance” receptors. There was, however, no significant difference in the estimated amount of ET-1 bound to ETA receptors in the splanchnic vasculature of DOCA-salt and sham rats. This provides further evidence that the production and release of ET-1 are not increased in the splanchnic vasculature of DOCA-salt rats.

Anesthetized vs. conscious rats. Our preliminary studies were performed on anesthetized rats after acute surgery for catheter implantation. The MAP differences between DOCA-salt and sham rats were much less when they were anesthetized than when they were conscious (probably reflecting the important role of the sympathetic nervous system in regulation of blood pressure in DOCA-salt hypertension). Further studies were then performed on conscious rats to avoid the effects of surgery and anesthesia on sympathetic activity, body fluid status, and hormone synthesis or release. Rats also were in a normal crouched posture, instead of lying on their back, when blood was sampled. This is a potentially significant factor when vascular capacitance function is considered. We saw significantly lower plasma ET-1 concentration and nNHSR of ET-1 in conscious rats, presumably reflecting fewer stress-
anesthesia-induced effects on ET-1 formation, metabolism, or uptake. Furthermore, the change in aortic plasma ET-1 level and in nNHSR of ET-1 after ETB receptor blockade was greater in conscious than in anesthetized rats. This indicates that ETB receptor-mediated clearance of ET-1 was decreased in anesthetized and surgically prepared rats. The decreased uptake activity of ETB receptors in anesthetized rats may be due to desensitization of endothelial ETB receptors caused by higher circulating ET-1 level or to a lower cardiac output (and tissue blood flow) under anesthesia. Comparing data from conscious sham and DOCA-salt rats, however, we found consistent evidence that the uptake activity of endothelial ETB receptors in DOCA-salt rats is impaired in the splanchnic vasculature but remains unchanged in the whole body.

**Perspectives**

The studies reported here show that neither ET-1 content of venous capacitance vessels nor portal venous plasma ET-1 concentration is higher in DOCA-salt than in sham rats. Furthermore, estimated nNHSR of ET-1 is not higher in DOCA-salt than in sham rats, even when major processes removing ET-1 from the circulation are blocked. Collectively, the results provide strong evidence that higher endothelial cell formation of ET-1 is not likely responsible for enhanced ET-1-mediated splanchnic vеноconstriction in vivo. When combined with the fact that venous smooth muscle contractile responsiveness to ET-1 is not increased in DOCA-salt rats, the data suggest that the ability of ET receptor antagonists to reduce venomotor tone in DOCA-salt rats in vivo results from actions of the drugs on a nonvascular target. We speculate that the enhanced venoconstrictor effect of ET-1 could be mediated by an action of ET-1 on the sympathetic nervous system. A number of studies have shown prejunctional modulation of sympathetic neurotransmission by ET-1 in vitro (24); e.g., intrarenal arterial infusion of ET-1 inhibited norepinephrine release induced by renal sympathetic nerve stimulation in anesthetized dogs (23). This neuroinhibitory effect of ET-1 was mediated through activation of ETB receptor mechanisms (37). Localization of ET-1 receptors on the nerve terminals has also been reported (11). Neurotransmission in sympathetic ganglia also can be modulated by ET-1 (39). A previous study in our laboratory (16) found that pretreatment of DOCA-salt rats with an ETB receptor antagonist produced greater declines in MCFP after ganglionic blockade than after ganglionic blockade alone. These findings suggest that ET-1 acting at ETB receptors may provide an important contribution to venomotor tone through modulation of sympathetic tone to the veins. The ETB antagonist may have facilitated norepinephrine release from sympathetic nerve terminals on veins, accounting for the significantly larger response of MCFP to subsequent ganglion blockade. This action presumably was more prominent in DOCA-salt rats because of their higher pretreatment levels of endogenous vascular ET-1. The decrease in venous capacitance, previously demonstrated in essential hypertension in humans (22), might be mediated by other mechanisms, such as changes in venous wall structure or other influences on venous smooth muscle activity besides ET-1 and sympathetic input, such as reactive oxygen species.

**REFERENCES**


**GRANTS**

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