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Short communication

Ameliorating effect of an endothelin ET_A receptor antagonist on renal function of DOCA-salt hypertensive rats

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Abstract

The effects of FR139317 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]-carbonyl]amino-4-methyl-pentanoyl]amino-3-[3-(1-methyl-1*H*-indolyl)]propionyl]amino-3-2(2-pyridyl)propionic acid), an endothelin ET_A receptor antagonist, on renal hemodynamics and urine formation were examined using anesthetized deoxycorticosterone acetate (DOCA)-salt hypertensive rats, in which renal perfusion pressure was protected from FR139317-induced hypotension with an aortic clamp. An intravenous injection of FR139317 (10 mg/kg) to sham-operated normotensive control rats produced no significant changes in renal hemodynamic and excretory responses. In DOCA-salt hypertensive rats, FR139317 caused sustained renal vasodilation. Urine flow and urinary excretion of sodium were increased significantly following drug injection. We suggest that endothelin-1 and the endothelin ET_A receptor play an important role in water and sodium retention, and in renal vasoconstriction in this model of hypertension.

Keywords: Endothelin-1; Endothelin ET_A receptor; DOCA (deoxycorticosterone acetate)-salt hypertension; Renal function; Urine formation

1. Introduction

There is accumulating evidence indicating that endothelin-1 is involved in the development and/or maintenance of deoxycorticosterone acetate (DOCA)-salt-induced hypertension in rats (Schiffrin, 1995). This view is based on findings indicating that acute administration of an endothelin ET_A receptor antagonist or nonselective endothelin receptor antagonist to DOCA-salt rats produces a potent hypotensive effect and that long-term treatment with these agents efficiently suppresses the development of hypertension. In addition, we noted that the endothelin-1 content in vascular tissues was increased in DOCA-salt rats, compared with that in control rats (Fujita et al., 1995a). Lariviére et al. (1993) reported that endothelin-1 mRNA levels in aorta and mesenteric arteries were elevated in these animals. Taken together, it seems likely that stimulation of endothelin-1 production in vascular tissues is one causal factor related to DOCA-salt-induced hypertension. However, we found that an intravenous bolus injection of FR139317 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]-carbonyl]amino-4-methyl-pentanoyl]amino-3-[3-(1-methyl-1*H*-indolyl)]propionyl]amino-3-(2-pyridyl)propionic acid), an endothelin ET_A receptor antagonist (Sogabe et al., 1993), failed to decrease blood pressure in other hypertensive rats such as 2-kidney, 1-clip renal hypertensive rats and nitro-L-arginine-induced hypertensive rats (Fujita et al., 1995b), thereby suggesting that endothelin-1 is not of pathophysiologic importance in these hypertensive models.

In DOCA-salt-induced hypertension, there is a marked elevation of renal vascular resistance and a decrease in renal blood flow, and these abnormalities in renal hemodynamics are thought to play an important role in the development and maintenance of hypertension (Huang et al., 1992; Roman et al., 1988). More recently, we reported that the endothelin-1 mRNA level was significantly increased in the kidney of DOCA-salt hypertensive rats. When FR139317 was intravenously administered to DOCA-salt hypertensive rats, urine flow was not decreased, despite FR139317-induced marked hypotension. In contrast, FR139317 produced a decrease in urine flow with slight hypotension in normotensive control rats (Fujita et al., 1996), thereby suggesting that there is a functional role of endothelin-1 via the ET_A receptor in the kidney of DOCA-salt hypertensive rats but not in normotensive rats. In the present work, we investigated the effects of FR139317 on the renal hemodynamics and urine formation of DOCA-salt hypertensive rats, in comparison with those

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of normotensive control rats. To rule out the possible modification of FR139317-induced renal action by drug-induced hypotension, experiments were done using animals in which renal perfusion pressure was protected against the FR139317-induced hypotension by use of an aortic clamp.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats obtained from Japan SLC (Hamamatsu) and weighing 160–180 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the right kidney was removed via a right flank incision. After a 1-week postsurgical recovery period, the rats were treated twice weekly with DOCA suspended in corn oil, which was administered subcutaneously (15 mg/kg), and 1% NaCl was added to tap water for drinking. Sham-operated rats (normotensive control) were uninephrectomized but not given DOCA or salt. Systolic blood pressure was monitored with a tail cuff and a pneumatic pulse transducer. After 4 weeks of the above treatment, the rats with a systolic blood pressure over 180 mmHg were used for clearance studies.

2.2. Renal clearance study

The rats were anesthetized with sodium thiobutabarbital (Inactin, 100 mg/kg, i.p.) and placed on a heated surgical tray that maintained rectal temperature between 37°C and 38°C. After tracheotomy, the right femoral artery and vein were cannulated for blood sampling and for infusion of 0.9% saline containing 0.5% inulin (6 ml/h), respectively. After an abdominal midline incision was made, the left kidney was exposed, and the renal artery was carefully stripped of connective tissue, followed by the application of 5% phenol in 70% ethanol to exclude the influence of renal sympathetic nerves. An electromagnetic flow probe (1.0 mm in diameter, Nihon Kohden, Tokyo, Japan) connected to a square-wave flowmeter (MFV-2100, Nihon Kohden) was positioned on the renal artery to measure renal blood flow. To control renal perfusion pressure, a Blalock clamp was placed around the aorta just below the origin of the left renal artery. Mean arterial pressure above the clamp was recorded from a catheter inserted into the right carotid artery, and served as an index of the left renal perfusion pressure. A polyethylene cannula was inserted into the left ureter for urine collection. Mean arterial pressure and renal blood flow were continuously recorded on a polygraph (RM 6000, Nihon Kohden) throughout the experiment. A 60–90-min period was allowed for stabilization of mean arterial pressure, renal blood flow and urine flow. After the equilibration period, urine samples were collected during two 15 min control clearance periods. Results for the second control period served as the basal values for renal hemodynamics and function. Following the control periods, FR139317 (10 mg/kg) was administered intravenously by slow bolus injection (volume 1 ml/kg; duration 2 min). The dose of FR139317 used in this study has been shown to produce complete inhibition of endothelin-1-induced pressor action (Sogabe et al., 1993). During the first 5 min after injection, urine was not collected in order to take into account the dead space in the collection system. Following this, urine samples were collected during four consecutive 15-min periods (E1–E4). Blood samples (0.2 ml each) were obtained at 15 min before drug injection and at 20 min and 50 min after the injection, respectively. The blood loss was replaced by injecting an equal volume of blood from donor rats. Plasma was immediately separated by centrifugation. In the control experiment, the vehicle solution was administered.

2.3. Analytical procedure

Urine and plasma inulin levels were measured by spectrofluorometry (Hitachi 650–50), as described by Fujita et al. (1996). Glomerular filtration rate was calculated from the inulin clearance. Urine and plasma sodium concentrations were determined with a flame photometer (Hitachi 205D).

2.4. Drug

FR139317 was a kind gift from Fujisawa Pharmaceutical (Osaka, Japan). FR139317 was dissolved in 1 M NaOH and diluted with saline. Other chemicals were purchased from Nacalai Tesque (Kyoto, Japan).

2.5. Statistical analysis

All values were expressed as means ± S.E.M. For statistical analysis, we used the unpaired Student's *t*-test for two-sample comparisons and one-way analysis of variance combined with Dunnett's multiple range test for multiple comparisons. Differences were considered significant at $P < 0.05$.

3. Results

Basal mean arterial pressure of anesthetized DOCA-salt rats was significantly elevated, compared with that of normotensive control rats (153 ± 4 mmHg, $n = 20$ vs. 114 ± 3 mmHg, $n = 20$; $P < 0.001$). In DOCA-salt rats, there was a marked decrease in renal blood flow (2.41 ± 0.25 ml/g per min vs. 5.33 ± 0.38 ml/g per min of the control value, $P < 0.001$), accompanied by a significant increase in the calculated value of renal vascular resistance. Glomerular filtration rate in DOCA-salt rats tended to decrease (5–10%), compared with the rate (0.83 ± 0.05 ml/g per min) of normotensive control rats, but the ob-

served changes were not statistically significant. Basal levels of urine flow of DOCA-salt rats were significantly elevated, compared with those of normotensive control rats (10.49 ± 1.32 μl/g per min vs. 7.24 ± 1.05 μl/g per min, $P < 0.05$). Levels of urinary excretion of Na^+ and fractional excretion of Na^+ were also increased in DOCA-salt rats (about 30%).

Fig. 1 shows changes in systemic and renal hemodynamics, and urine formation after the intravenous injection of FR139317 to DOCA-salt hypertensive rats. Renal blood flow gradually increased after the drug injection, followed by a significant decrease in renal vascular resistance. No significant changes were observed in heart rate and glomerular filtration rate. There was a notable change in urine formation, i.e., urine flow, urinary excretion of Na^+ and fractional excretion of Na^+ were markedly increased after drug administration, and these effects lasted throughout the experimental period.

Fig. 2 illustrates changes in systemic and renal hemodynamics, and urine formation after the intravenous injection of FR 139317 to normotensive control rats. FR139317 did not produce significant changes in renal hemodynamics. Urine flow, urinary excretion of Na^+ and fractional excretion of Na^+ were also constant throughout the experimental period.

The injection of vehicle (0.015 M NaOH in saline) did not alter renal hemodynamics and urine formation in either normotensive control or DOCA-salt hypertensive rats (data not shown).

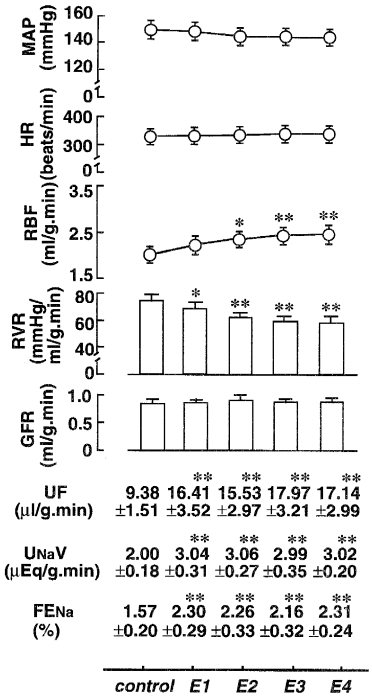

Fig. 1. Systemic hemodynamic, renal hemodynamic and excretory responses to intravenous administration of FR139317 (10 mg/kg) in DOCA-salt hypertensive rats. Each value represents the mean ± S.E.M. ($n = 6$). * $P < 0.05$; ** $P < 0.01$, compared with each control value. MAP, mean arterial pressure; HR, heart rate; RBF, renal blood flow; RVR, renal vascular resistance; GFR, glomerular filtration rate; UF, urine flow; $U_{Na}V$, urinary excretion of Na^+; FE_{Na}, fractional excretion of Na^+.

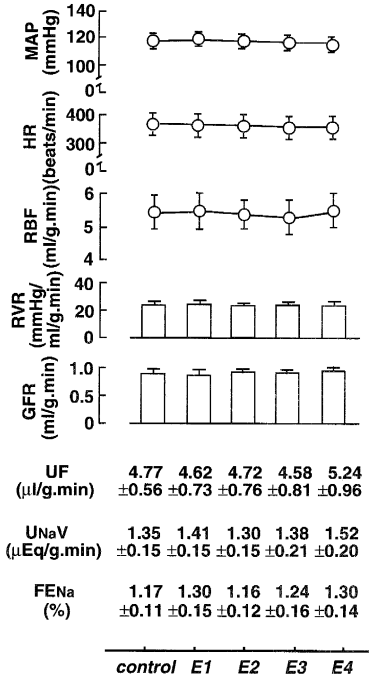

Fig. 2. Systemic hemodynamic, renal hemodynamic and excretory responses to intravenous administration of FR139317 (10 mg/kg) in normotensive control rats. Each value represents the mean ± S.E.M. ($n = 6$). MAP, mean arterial pressure; HR, heart rate; RBF, renal blood flow; RVR, renal vascular resistance; GFR, glomerular filtration rate; UF, urine flow; $U_{Na}V$, urinary excretion of Na^+; FE_{Na}, fractional excretion of Na^+.

4. Discussion

Recent studies have indicated that endothelin-1 plays an important role in the development and maintenance of DOCA-salt-induced hypertension in rats (Schiffrin, 1995). In this model, a marked elevation in renal vascular resistance and a decrease in renal blood flow often occur, and these abnormalities in renal hemodynamics are thought to be closely related to the pathogenesis of the hypertension (Huang et al., 1992; Roman et al., 1988). However, little is known about the involvement of endothelin-1 in the above renal abnormalities. In the present study, the intravenous injection of FR139317 increased renal blood flow only in DOCA-salt rats. In addition, this increase was accompanied by a marked decrease in renal vascular resistance. These results strongly suggest that endogenous endothelin-1 and ET_A receptors are involved in the renal vasoconstriction in DOCA-salt-induced hypertensive rats. It is well known that endothelin-1 is synthesized in several types of renal cells such as vascular endothelial cells, mesangial cells and tubular cells, and that this peptide functions as an autocrine and/or paracrine factor (Rubanyi and Polokoff,

1994). Recently, we observed increased endothelin-1 mRNA expression in the kidney of DOCA-salt hypertensive rats (Fujita et al., 1996). When the profile of endothelin-1 release from cultured endothelial cells obtained from DOCA-salt hypertensive rats was examined and compared with that from control rats, the level of endothelin-1 release in these hypertensive rats was higher than that of control rats (Takada et al., 1996). Using in situ hybridization histochemistry, endothelin-1 mRNA expression was seen to be increased in the endothelial cell layer of vascular tissues of DOCA-salt hypertensive rats (Day et al., 1995). However, no difference in plasma endothelin-1 concentration was observed between DOCA-salt rats and control rats (Suzuki et al., 1990). Taken together, it is reasonable to consider that endothelin-1 production is augmented in the kidney of DOCA-salt hypertensive rats and that the increased level of endothelin-1 leads to renal vasoconstriction mainly via the ET_A receptor, events which may contribute to the development and maintenance of DOCA-salt-induced hypertension.

Of particular interest in the present study is the effect of FR139317 on urine formation. In normotensive control rats, the administration of FR139317 produced no significant effect on urine flow and urinary excretion of Na^+. In contrast, in DOCA-salt hypertensive rats, notable diuretic and natriuretic effects were observed following drug injection, thereby suggesting that endothelin-1 actions mediated via the ET_A receptor are responsible for water and sodium retention in DOCA-salt hypertensive rats. Since the effect of FR139317 to increase urine formation was not synchronized with changes in renal blood flow, it is unlikely that drug-induced renal vasodilation is responsible for this effect. There is conflicting evidence with respect to the effect of endothelin-1 on urine formation in normotensive animals (natriuretic or antinatriuretic) (Rubanyi and Polokoff, 1994), perhaps related to differences in experimental systems. In addition, the effect of endothelin-1 on urine formation may be complicated by various actions of endothelin-1 on glomerular hemodynamics and endocrine systems (Rubanyi and Polokoff, 1994). Regardless of the renal effect of the peptide under normal conditions, endogenous endothelin-1, which is generated locally in the kidney of DOCA-salt hypertensive rats, appears to contribute to fluid retention in these rats.

The FR139317-induced diuretic effect observed in DOCA-salt hypertensive rats may be related to an ET_B receptor-mediated action of endothelin-1 because it has been reported that endothelin-1 inhibits arginine vasopressin-induced water reabsorption in the collecting duct of the rat. This effect is probably due to the ET_B receptor-mediated inhibitory action on arginine vasopressin-stimulated cyclic-AMP accumulation at this site (Kohan, 1996). In anesthetized dogs, the intrarenal administration of sarafotoxin S6c, a selective ET_B receptor agonist, resulted in a diuretic response with no change in sodium excretion (Clavell et al., 1995). In contrast, other investigators reported that infusion of sarafotoxin S6c into the renal artery of anesthetized dogs increased both urine flow and fractional excretion of Na^+ with little effect on renal hemodynamics, thereby suggesting that ET_B receptor stimulation inhibits sodium reabsorption at the tubular level (Brooks et al., 1994). Taken together, it seems likely that stimulation of ET_B receptor increases water excretion by inhibiting arginine vasopressin-mediated water reabsorption. However, whether the ET_B receptor modulates sodium excretion remains uncertain. There also may be functional differences between species. In the present study, when FR139317 was administered to DOCA-salt hypertensive rats, both diuresis and natriuresis occurred. It is unlikely that the diuretic and natriuretic effects following FR139317 administration are mainly due to ET_B receptor-mediated events. However, the possibility that ET_B receptor-mediated events, unmasked by FR139317 administration, might modify the renal responses to FR139317 administration will need to be ruled out.

In conclusion, our results suggest that endogenous endothelin-1 and ET_A receptors contribute to renal vasoconstriction in DOCA-salt hypertensive rats. Furthermore, endothelin-1 plays an important role in water and sodium retention in this model of hypertension. A selective ET_A receptor antagonist may be beneficial to treat subjects with mineralocorticoid-dependent hypertension.

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