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SPECIAL REPORT

Enhanced blood pressure sensitivity to DOCA-salt treatment in endothelin ET_B receptor-deficient rats

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The role of endothelin ET_B receptor-mediated action in the development and maintenance of deoxycorticosterone acetate (DOCA)-salt-induced hypertension was evaluated using the spotting-lethal (sl) rat which carries a naturally occurring deletion in the ET_B receptor gene. Homozygous (sl/sl) rats treated with DOCA-salt for 1 week exhibited an earlier onset of hypertension than heterozygous (sl/+) and wild-type (+/+) rats (systolic blood pressure, SBP; 156.7 ± 3.4 versus 128.8 ± 5.3 and 132.9 ± 3.7 mmHg, respectively). Four weeks after the start of DOCA-salt treatment, homozygous rats developed marked hypertension, with a SBP of 206.0 ± 4.5 mmHg, compared with 184.8 ± 10.7 mmHg in heterozygous and 164.3 ± 4.8 mmHg in wild-type rats. Cardiovascular hypertrophy and renal dysfunction observed after 4-weeks treatment with DOCA-salt were more severe in homozygous rats, compared to wild-type and heterozygous animals. These evidences support strongly the view that ET_B receptor-mediated actions are a protective factor in the pathogenesis of DOCA-salt-induced hypertension.
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Abbreviations: Ccr, creatinine clearance; DβH, dopamine-β-hydroxylase; DOCA, deoxycorticosterone acetate; ET, endothelin; NAG, *N*-acetyl-β-glucosaminidase; SBP, systolic blood pressure; sl, spotting-lethal; Uprotein V, urinary excretion of protein

Introduction Endothelin (ET)-1 plays an important role in the development and maintenance of deoxycorticosterone acetate (DOCA)-salt-induced hypertension in rats (Schiffrin, 1995). Lariviére *et al.* (1993) first demonstrated the increased ET-1 content in blood vessels of DOCA-salt hypertensive but not in spontaneously hypertensive rats. Long-term treatment with a nonselective ET_A/ET_B receptor antagonist bosentan was reported to attenuate the development of hypertension and vascular remodeling in DOCA-salt rats (Li *et al.*, 1994). In addition, several studies have indicated that acute administration of an ET_A receptor-selective antagonist to DOCA-salt rats produces a potent hypotensive effect and that long-term treatment with this agent efficiently suppresses the development of hypertension (Bazil *et al.*, 1992; Bird *et al.*, 1995; Fujita *et al.*, 1995; 1996; Schiffrin *et al.*, 1997). Thus, there is little doubt that ET_A receptor-mediated action is mainly responsible for the pathogenesis of this hypertension. On the other hand, the pathological role of ET_B receptor-mediated action in the hypertension including this experimental model remains controversial (Ruschitzka *et al.*, 1998). Most recently, we found that chronic treatment with an ET_B receptor-selective antagonist to DOCA-salt rats led to a deterioration in DOCA-salt-induced cardiovascular and renal injuries (Matsumura *et al.*, 1999), thereby suggesting that the blockade of this receptor subtype could be harmful in such pathological conditions. In order to confirm this view, we used spotting-lethal (sl) rat which carries a naturally occurring deletion in the ET_B receptor gene (Gariepy *et al.*, 1996), and examined the responses of blood pressure and renal function to DOCA-salt treatment.

Homozygous (sl/sl) rats exhibit abnormal development of the neural crest-derived epidermal melanocytes and the enteric nervous system, similar to that described in ET_B receptor-deficient mice and men, and do not live beyond one month due to intestinal aganglionosis and resulting intestinal obstruction. Studies using a dopamine-β-hydroxylase (DβH)/lacZ transgene indicated that enteric neuroblasts are transiently adrenergic during gut colonization and this colonization process is defective in ET_B receptor-deficient mice. Therefore, the DβH promoter was used to direct ET_B transgene expression in sl/sl rats to support normal development of the enteric nervous system (Gariepy *et al.*, 1998). DβH-ET_B sl/sl rats live into adulthood and are healthy, expressing ET_B receptor in adrenals and other adrenergic neurons. Thus, these rescued ET_B receptor-deficient rats would be useful to determine the pathophysiological roles of ET_B receptors.

Methods *Animals and experimental protocol* Male homozygous (sl/sl), heterozygous (sl/+) and wild-type (+/+) rats (weighing 160–180 g, 6 weeks of age), all of which were transgenic, were unilaterally nephrectomized. After a 1 week postsurgical recovery period, the rats were treated twice weekly with DOCA suspended in corn oil, administered subcutaneously (15 mg kg^{-1}) and 1% NaCl was added to their tap water for drinking. Sham-operated rats from all groups were unilaterally nephrectomized but were not given DOCA or salt. Systolic blood pressure (SBP) was monitored once a week by

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the tail-cuff method. After 4 weeks of treatment, urine was collected overnight, and all the rats were exsanguinated and arterial blood samples were obtained. Heart and aorta were also excised and weighed.

Analytical procedures Protein and creatinine levels in plasma or urine were determined using the Total protein-test-Wako and creatinine-test-Wako (Wako Pure Chemical Industries, Osaka, Japan), respectively. Urinary *N*-acetyl-β-glucosaminidase (NAG) activity, as an index of proximal tubule damage, was measured using the synthesized substrate sodio-m-cresolsulfonphthaleinyl N-acetyl-β-D-glucosaminide.

Statistical analysis All values are expressed as mean$\pm$s.e.mean. For statistical analysis, we used the unpaired Student's *t*-test for two-group comparison. For multi-comparisons, we used one-way ANOVA combined with Bonferroni's multiple comparison test. Differences were considered significant at $P<0.05$.

Results Basal SBPs (before the DOCA-salt treatment) of homozygous (sl/sl), heterozygous (sl/+) and wild-type (+/+) rats at 7 weeks of age were 131.8 ± 1.5 ($n=23$, $P<0.01$ vs wild), 126.4 ± 2.2 ($n=10$), and 123.0 ± 1.9 mmHg ($n=17$), respectively. As shown in Figure 1, SBP of three groups treated with DOCA and salt showed a time dependent increase for 4 weeks. However, significantly earlier and higher increases in SBP were observed in homozygous rats, than in heterozygous and in wild-type rats. Unexpectedly, three of the 12 homozygous rats died at 3 weeks (before SBP measurement). Four weeks after the start of DOCA-salt treatment, the SBP of homozygous rats was 206.0 ± 4.5 mmHg ($n=9$) ($P<0.05$ vs hetero; $P<0.01$ vs wild), whereas those of heterozygous and wild-type rats were 184.8 ± 10.7 ($n=5$) ($P<0.05$ vs wild) and 164.3 ± 4.8 mmHg ($n=9$), respectively. Sham-operated controls from three groups showed no significant changes in SBP throughout experimental periods. Four weeks after sham-operation, SBPs of homozygous, heterozygous and wild-type rats were 135.5 ± 1.9 ($n=11$), 126.9 ± 1.4 ($n=5$), and 122.9 ± 3.3 mmHg ($n=8$), respectively.

When heart weights were corrected by body weight, there were significant increases in heart weight-to-body weight ratio in DOCA-salt hypertensive groups of wild-type, heterozygous and homozygous rats, compared with each sham group. Aorta weight also showed a significant increase by the treatment with DOCA and salt. These increments induced by DOCA and salt were more marked in homozygous, than in heterozygous and wild-type rats ($P<0.05$ vs hetero; $P<0.01$ vs wild) (Table 1).

Creatinine clearance (Ccr) was significantly decreased by the treatment with DOCA and salt only in homozygous rats. DOCA-salt treatment produced significant increases in urinary excretion of protein (UproteinV), in all groups, but the extent in the homozygous group was much greater ($P<0.05$ vs hetero; $P<0.01$ vs wild). NAG activity, as an index of proximal tubule damage was also elevated by DOCA-salt treatment, and the levels in homozygous rats were significantly higher compared with wild-type and heterozygous rats ($P<0.05$ vs hetero; $P<0.01$ vs wild) (Table 1).

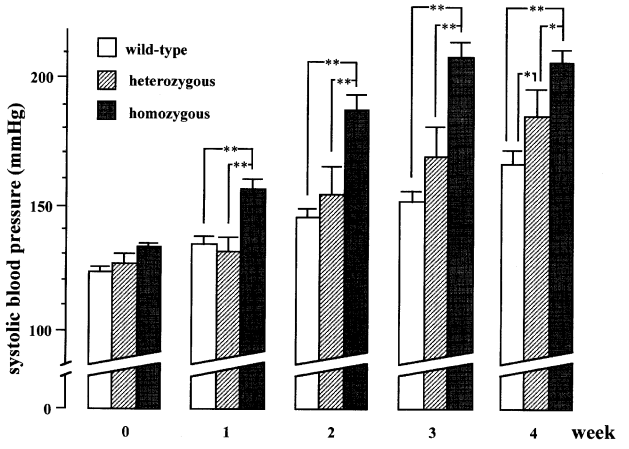

Figure 1 Time course in systolic blood pressure of wild-type (+/+) ($n=9$), heterozygous (sl/+) ($n=5$) and homozygous (sl/sl) ($n=9-12$) rats treated with deoxycorticosterone acetate and salt. Values are expressed as mean$\pm$s.e.mean $^*P<0.05$, $^{**}P<0.01$ (Bonferroni's test).

Discussion The ET_A receptor is implicated in the vasoconstrictive and mitogenic effects of ET-1, whereas the ET_B receptor mediates both vasodilation and vasoconstriction (Goto *et al*., 1996). Although a large number of pharmacological studies using ET receptor antagonists demonstrated their effectiveness in lowering blood pressure in hypertensive animal models, it still remains to be determined whether selective ET_A receptor or non-selective ET_A/ET_B receptor blockade is preferable for treatment of hypertension (Ruschitzka *et al*., 1998). On the other hand, we recently found that chronic treatment of DOCA-salt rats with A-192621, an orally active and highly potent ET_B-selective receptor antagonist, led to an exaggerated deterioration of cardiovascular and renal injuries, thereby suggesting that the blockade of this receptor subtype is harmful in such pathological conditions (Matsumura *et al*., 1999). Moreover, we and others noted that the hypertensive effects induced by an intravenous bolus injection of BQ-788 or Ro 46-8443, both of which are selective ET_B receptor antagonists, in DOCA-salt hypertensive rats were greater than those in normotensive control animals (Clozel & Breu, 1996;

Table 1 Comparative data on body, heart and aorta weights, and urinary parameters

	Wild-type		*Heterozygous*		*Homozygous*	
Parameter	*Sham* (n=8)	*DOCA-salt* (n=9)	*Sham* (n=5)	*DOCA-salt* (n=5)	*Sham* (n=11)	*DOCA-salt* (n=9)
BW (g)	303±7	281±6	356±5	319±19	304±5	216±12**
HW/BW (g 100 g^{-1})	0.291±0.006	0.362±0.009**	0.272±0.008	0.360±0.018**	0.283±0.006	0.459±0.021**
Aorta weight (mg cm^{-1} 100 g^{-1})	3.79±0.09	4.71±0.19*	3.25±0.21	4.48±0.57*	3.79±0.013	6.92±0.40**
Ccr (ml min^{-1} 100 g^{-1})	0.81±0.07	0.84±0.05	0.71±0.08	0.63±0.10	0.73±0.06	0.51±0.03**
Uprotein V (mg 24 h^{-1} 100 g^{-1})	5.56±0.41	63.20±14.39**	10.24±0.74	98.95±32.45*	11.60±0.87	210.79±19.57**
NAG (unit 24 h^{-1} 100 g^{-1})	0.065±0.010	0.281±0.023**	0.073±0.030	0.278±0.059*	0.077±0.017	0.420±0.041**

Values are mean$\pm$s.e.mean. $^*P<0.05$, $^{**}P<0.01$, compared with corresponding data in sham rats. BW, body weight; HW, heart weight; Ccr, creatinine clearance; Uprotein V, urinary excretion of protein; NAG, *N*-acetyl-β-glucosaminidase activity; DOCA, deoxycorticosterone acetate.

Hashimoto *et al*., 1998). The renal vasoconstrictor effects induced by the BQ-788 injection were also markedly enhanced in DOCA-salt hypertensive rats (Hashimoto *et al*., 1998), suggesting that ET_B-mediated systemic and renal vasodilative activities play an important role as a protective factor against DOCA-salt-induced hypertensive diseases.

In the present study, the 'rescued' ET_B receptor-deficient rats clearly exhibited an exaggerated blood pressure sensitivity to chronic DOCA-salt treatment, compared with cases in wild-type and heterozygous animals. In addition, the ET_B-deficient rats had enhanced cardiovascular hypertrophy and the worsening of renal dysfunction to the DOCA-salt treatment. Thus, ET_B receptor-mediated actions are protective in the pathogenesis of DOCA-salt-induced hypertension and related tissue injury. The use of selective ET_B receptor antagonist in mineralocorticoid-dependent hypertension should be avoided.

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