Seventh Symposium on Salt, Vol. II 309–313 (1993)
Elsevier Science Publishers B.V., Amsterdam

Role of Endogenous Endothelin in Renal Function during the Alteration of Sodium Balance: Effect of Aging

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ABSTRACT

This study was conducted to determine, firstly, the involvement of endogenous endothelin (ET), a novel potent vasoconstricting peptide, in systemic and renal hemodynamics and in the renin–angiotensin system during the alteration of sodium balance and, secondly, the effect of aging on those actions of endogenous ET. A specific ET antiserum was infused to inhibit the action of ET at a time of altered sodium balance (for 2 weeks). In conscious young rats (8 weeks, 250–280 g) fed on a low-salt diet, infusion for 80 min (60 min plus two 10 min clearance periods) at a rate of 2.5 μl/min of 1:50 diluted ET antiserum, which completely inhibited renal vasoconstriction by the exogenously administered ET (0.25–1.0 nmol/kg), caused an increase in urinary sodium excretion and fractional excretion of sodium and a decrease in plasma renin concentration without significant changes in blood pressure, heart rate, glomerular filtration rate (GFR), renal plasma flow (RPF) and urine volume, compared with the values with non-immune serum. A time control study showed no significant changes in all parameters. These results suggest that the state of low- compared with high-salt intake causes a relatively stronger activity of endogenous ET and that the endogenous ET contributes to the adaptative modulations of sodium excretion due to renal tubular action and renin release in association with the changed state of sodium balance in young rats. On the other hand, in aged rats (16 months, 680–800 g), significant decreases in relative GFR and RPF (values per 100 g body weight), and a tendency of a less marked decrease in fractional excretion of sodium were observed, as compared with those in young rats when fed on a low-salt diet, suggesting a decrease in sodium-retaining capacity of kidney due to the decrease in the function of nephron with aging. With an infusion of ET antiserum, decrease in blood pressure was observed in conscious aged rats fed only on a low salt diet. This evidence suggests that ET derived from vascular endothelial cells may in part participate in the regulation of blood pressure during the low salt intake in aged rats. Thus, ET might play an important role in regulating the renal function and systemic circulation when the state of sodium balance was altered.

INTRODUCTION

A new peptide of vascular endothelial origin, endothelin (ET) has a stronger vasoconstrictor effect on arterial strips than angiotensin II (Yanagisawa et al., 1988) and may contribute to the control of systemic blood pressure and regional blood flow. Intravenous infusion of a high dose of ET increases arterial pressure by increasing peripheral vascular resistance. Renal blood flow (RBF) and glomerular filtration rate (GFR) were markedly reduced in association with a sustained reduction in sodium excretion and an increase in plasma renin activity (Miller et al., 1989). However, vascular ET is also well known to result in a relaxation of vascular smooth muscle through the release of endothelium-derived relaxing factors (De Nucci et al., 1988; Rakugi et al., 1988). The production by the endothelium of these two substances with opposing vascular effects suggests that the endothelium possesses a complex role in the regulation of vascular smooth muscle and arterial pressure.

It has also been reported that ET inhibits renin release *in vitro* (Matsumura et al., 1989; Rakugi et al., 1988) as opposed to the stimulation observed with *in vivo* administration (Miller et al., 1989). Thus, it has not been yet determined whether and to what extent endogenous ET plays a role in modulating the activity of the renin-angiotensin system and of systemic and renal hemodynamics.

On the other hand, it is well known that *in vivo* renin release is modulated via three different pathways, i.e., baroreceptor, macula densa and the sympathetic nerve (Fray et al., 1987). A change in the state of sodium balance could be expected to cause a change in the renin–angiotensin system, renal

hemodynamics and electrolyte handling. Therefore, at first, the present study was designed to investigate the possible involvement of endogenous ET in modulating the renin–angiotensin system and systemic and renal hemodynamics by inhibiting the endogenous ET activity due to infusion of a specific ET antiserum on alteration of the state of sodium balance.

It is also well known that renal function is decreasing with aging. Depletion of extracellular fluid volume is easily introduced when sodium–water intake is inappropriate, leading to the reduction in renal blood flow. As a consequence of the decreased blood flow, imbalance of water and electrolyte, especially hyponatremia easily occurs in the elderly. Therefore, if endogenous ET actually participates in those renal functions, the second study was designed to determine the effect of aging on those actions of endogenous ET during the alteration of the state of sodium balance.

MATERIALS AND METHODS

In the first experiments, young (8 weeks) male Sprague–Dawley (SD) rats weighing 250–280 g were used. In the second experiments, aged male SD rats (16 months) weighing 680–800 g were used. Rats were divided into two groups of 20 and given either a low-salt diet (0.03 mmol of sodium/g of diet) or a high-salt diet containing 4% NaCl (normal rat chow contains 0.12 mmol of sodium/g of diet). After 2 weeks on the diets, the animals were anesthetized with sodium pentobarbitone, 50 mg/kg i.p. and catheters were inserted into the femoral artery and vein. One was for measurement of blood pressure and heart rate and the other for blood sampling and infusions of drugs. Another catheter was inserted into the bladder.

Endothelin antiserum infusion

To block the effects of endogenous ET-1, the following protocol was designed: The ET-1 antiserum used was obtained from rabbits immunized against ET-1 (porcine). This antibody completely cross-reacted with ET-1, ET-2 and ET-3 (human and rat). The IC_{50} of this serum was 1.0–1.3 pmol/ml, determined by radioimmunoassay using 1:10,000 diluted antiserum.

First, the *in vivo* neutralizing activity of this antiserum was checked. For measurement of renal blood flow, the left kidney was exposed and a calibrated electromagnetic flow probe was placed around the left renal artery. Then ET-1 antiserum or non-immune serum, diluted 1:50 in normal saline, was continuously infused at a rate of 2.5 μl/min for 60 min. ET-3 (0.25, 0.5 or 1.0 nmol/kg) was subsequently injected in a volume of 0.1 ml. When ET-3 was administered by bolus injection after an infusion of non-immune serum, there was a transient increase followed by a dose-dependent decrease in mean renal blood flow. However, when ET-1 antiserum, diluted 1:50, was preinfused, no significant change in mean renal blood flow was observed. Therefore, in the following clearance experiments, ET-1 antiserum or non-immune serum, diluted 1:50 in normal saline, was continuously infused.

Clearance study

On day 15, the rats underwent clearance determinations. Insulin (4%) and *p*-aminohippuric acid (PAH) (0.4%) in normal saline were infused at a rate of 1.2 ml/h. At first, diluted (1:50) non-immune serum was infused at a rate of 2.5 μl/min for 80 min (60 min plus two 10 min clearance periods). After a 30-min equilibration period with an infusion of inulin and PAH solution, urine was collected for two 10-min periods and 250 μl of blood was taken at the midpoint of each collection. Thereafter, ET-1 antiserum (1:50 diluted) was infused at the same rate as the infusion of non-immune serum. Then inulin and PAH solution were again infused. After an equilibration period, urine was collected again for two 10-min periods. A time control study was carried out with an infusion of non-immune serum instead of ET-1 antiserum.

Statistical analysis

All results are expressed as means ± SE. Student's paired and unpaired *t* tests were used for analysis.

RESULTS (I)

(Study I: young rats experiments)

No significant change in mean blood pressure or heart rate was observed during ET-1 antiserum or non-immune serum infusion either with low or high salt intake. In the clearance period, no significant difference in GFR or RPF was observed between the infusion of non-immune serum and ET-1 antiserum either with low salt or high salt intake (Table 1). In the time control study, there was also no change in blood pressure, heart rate, GFR, or RPF. However, urinary sodium excretion and fractional excretion of sodium (FENa) in the clearance period in rats on low salt intake were significantly increased during infusion of antiserum, compared with those with an infusion of non-immune serum or to the time control study. Such alterations were not observed in rats on high sodium intake (Table 2). The plasma renin concentration (PRC) of rats fed on a low-sodium diet

TABLE 1

Effect of ET-1 antiserum on systemic hemodynamic and whole kidney parameters in young rats fed either low- or high-salt diet

	Low salt				High salt			
	Experiment		Time control		Experiment		Time control	
	NS	Anti-ET-1	NS	NS	NS	Anti-ET-1	NS	NS
Mean BP (mm Hg)	110±3	109±2	110±3	110±3	115±2	116±4	114±3	112±4
HR (beats/min)	342±12	348±14	344±14	346±15	314±16	316±12	321±14	324±14
GFR (ml/min)	1.24±0.21	1.03±0.10	1.25±0.24	1.15±0.20	1.09±0.14	0.97±0.10	1.10±0.20	1.12±0.18
RPF (ml/min)	3.40±0.52	3.61±0.24	3.62±0.41	3.55±0.48	3.04±0.41	3.32±0.61	3.25±0.42	3.30±0.51

Values are means ± SEM: n = 10 rats in each group in experiment and 5 rats in each group in time control.
NS, non-immune serum: anti-ET-1, ET-1 antiserum.

TABLE 2

Effect of ET-1 antiserum on urinary sodium excretion and plasma renin concentration (PRC) in young rats fed either low- or high-salt diet.

	Low salt				High salt			
	Experiment		Time control		Experiment		Time control	
	NS	Anti-ET-1	NS	NS	NS	Anti-ET-1	NS	NS
$\dot{U}V$ (μl/min)	24.0±3.3	17.6±1.8	21.4±3.2	20.6±2.0	23.2±2.3	20.3±2.6	21.2±2.0	22.5±2.1
$\dot{U}Na\dot{V}$ (μEq/min)	0.30±0.08	0.59±0.16*	0.29±0.10	0.26±0.09	3.79±0.66	3.32±0.42	3.62±0.70	3.51±0.36
FENa (%)	0.17±0.02	0.42±0.12*	0.16±0.04	0.16±0.05	2.52±0.43	2.79±0.49	2.61±0.39	2.73±0.41
PRC (ng/ml/h)	10.85±1.54	7.33±1.40*	11.05±1.04	11.85±1.60	1.21±0.32	0.97±0.23	1.01±0.30	1.12±0.41

Values are means ± SEM: n = 10 rats in each group in experiment and 5 rats in each group in time control.
*$p<0.05$ vs. non-immune serum (NS) group or time-control group.

was significantly decreased with an infusion of antiserum, compared with the infusion of non-immune serum or the time-control study, whereas no significant alteration of PRC of rats fed on a high-sodium diet was observed even with the infusion of ET-1 antiserum (Table 2). In the time control study, no significant change in urine volume ($\dot{U}V$), urinary sodium excretion ($\dot{U}Na\dot{V}$), FENa, or PRC was observed.

RESULTS (II)

(Study II: aged rats experiments)

Comparison of each parameter between young and aged rats was shown in Table 3. Significant decreases in relative GFR and RPF (values per 100 g body weight, respectively) and a tendency of a less marked decrease in fractional excretion of sodium were observed, as compared with those in young rats when fed on a low-salt diet. A lower degree in the increase in PRC of aged rats during low salt intake was observed, as compared with that of young rats. With an infusion of ET-1 antiserum, decreases in blood pressure and RPF were observed in conscious aged rats fed on a low salt diet alone (Table 4). PRC decreased with an infusion of ET-1 antiserum in aged rats as well as young rats when fed on a low-salt diet alone.

CONCLUSIONS

As in a preliminary study, an infusion of antibody blocked the renal effects of exogenously administered ET-3, it could be assumed that the infusion of this antibody actually neutralized ET-1 in rat plasma. Therefore, because a decrease in renin release and an increase in sodium excretion were observed only when antibody was infused to young rats on a low-salt diet, it would appear that the plasma ET-1 level might be higher during low salt intake, compared to that with high salt intake. It has been reported that angiotensin II stimulates ET-1 release

TABLE 3

Comparison of each parameter between young and aged rats.

	BPm (mm Hg)	Hct (%)	GFR (ml/min/100 g)	RPF (ml/min/100 g)	$\dot{U}Na\dot{V}$ (μEq/min)	FENa (%)	PRC (ng/ml/h)	PAC (ng/dl)
Young rats								
Low salt	110±2	47.0±1.9	0.48±0.08	1.31±0.20	0.30±0.08	0.17±0.02	10.85±1.54	66.1±18.6
High salt	115±2	45.0±1.3	0.42±0.05	1.17±0.16	3.79±0.66	2.52±0.43	1.21±0.32	14.4±1.8
Aged rats								
Low salt	108±2	50.0±2.0	0.15±0.03*	0.58±0.07*	0.50±0.10	0.40±0.21	5.24±0.61*	87.5±17.7
High salt	110±5	47.2±2.1	0.30±0.07	1.09±0.25	1.44±0.15*	0.93±0.19*	1.18±0.22	19.3±4.0

*$p<0.05$ vs. young rats.

TABLE 4

Effect of ET-1 antiserum on renal hemodynamics in aged rats fed either low-or high-salt diet

	Low salt				High salt			
	Experiment		Time control		Experiment		Time control	
	NS	Anti-ET-1	NS	NS	NS	Anti-ET-1	NS	NS
BPm (mm Hg)	108±2	102±4*	109±2	111±3	110±5	114±8	112±4	110±3
HR (beats/min)	371±16	366±17	358±18	361±21	385±10	390±14	379±16	358±17
GFR (ml/min)	1.10±0.28	1.31±0.36	1.26±0.30	1.18±0.26	2.12±0.51	2.89±0.50	2.28±0.49	2.46±0.52
RPF (ml/min)	4.27±0.49	5.60±0.88*	4.68±0.41	4.91±0.69	6.65±0.92	7.32±0.99	6.51±1.00	6.91±1.20

*$p<0.05$ vs. non-immune serum (NS) group or time-control group.

from the endothelium (Emori et al., 1989). Thus, it could be speculated that angiotensin II may cause an increase in the endogenous ET-1 level when the salt intake is low. Alternatively, it can also be speculated that with a low-salt diet, endogenous ET-1 might have a greater action upon the regulation of sodium excretion and renin release due to the attenuation of counter-regulatory factors (such as atrial natriuretic peptide) evoked with a high-salt diet.

With a low-salt diet, we could not observe changes in blood pressure and heart rate and not even in GFR and RPF in young rats: however, an increase in sodium excretion and a decrease in renin release were evident. *In vitro* and *in vivo* analyses have demonstrated a variable ET-1 sensitivity of different vessels and a wide variety of binding sites. Therefore, it could be assumed that endogenous ET-1 affects the intrarenal hemodynamics without influencing whole renal hemodynamics and systemic blood pressure. An increase in FENa due to ET-1 antibody infusion during a low salt diet, which in this study was seen to occur without any demonstrable changes in the filtered sodium load, was evidence of a tubular effect of endogenous ET-1, whether direct or indirect.

Our present study using young rats shows that endogenous ET-1 could enhance renin release *in vivo*. In general, the *in vivo* release of renin is modulated through three major pathways: baroreceptor, macula densa and the sympathetic nervous system. Although no changes in systemic blood pressure and heart rate, nor in GFR and RPF were observed with ET-1 antibody infusion, a decrease in renin release along with increases in $\dot{U}Na\dot{V}$ and FENa were caused by the antibody, but only on a low-salt diet. Of the above-mentioned pathways, these findings suggest a possibility that the endogenous ET-1 is involved in renin release through a macula densa mechanism. However, it is alternatively suggested that with a high-sodium diet, natriuretic and vasodilatory factors, such as atrial natriuretic peptide, are activated as counter-regulation against ET-1. Thus, the change in the distribution of intrarenal hemodynamics due to ET-1 might be counter-regulated. On the contrary, with a low sodium diet, those

counter-regulatory factors are suppressed and the physiological action of ET-1 itself would be inhibited by the ET-1 antiserum, resulting in a decrease in renin release without any change in whole kidney hemodynamics in case of young rats.

In aged rats, significant decreases in relative GFR and RPF and a less marked decrease in fractional excretion of sodium (FENa) were observed, as compared with those in young rats when fed on a low-salt diet, suggesting a decrease in sodium-retaining capacity of kidney due to the decrease in the function of nephron with aging. When considering together with hematocrit showing a tendency to increase on a low-salt diet, it can be supposed that fluid volume of aged rats decreased when fed on a low salt.

With an infusion of ET-1 antiserum, decrease in blood pressure and increase in renal blood flow were observed in conscious aged rats when fed on a low-salt diet alone. This evidence suggests that ET might at least in part participate in the regulation of systemic and renal circulation during the low salt intake with in aged rats.

Thus, ET might play an important role in regulating the renal and systemic functions when the state of sodium balance was altered.

ACKNOWLEDGEMENTS

We are grateful to Mrs. J. Mabuchi and Mrs. K. Ueno for expert technical assistance. This study was partly supported by Salt Science Research Foundation (Grant 890B-5), Japan.

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