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Influence of DOCA Treatment on Administration-Time-Dependent Changes in the Effects of Furosemide in Saline-Loaded Rats

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ABSTRACT — We have previously found that the administration-time-dependent change in the effects of furosemide, a loop diuretic agent, is observed in normal rats. The present study was undertaken to examine whether an alteration in this phenomenon occurs in rats with DOCA-saline hypertension. Unilateral nephrectomized rats were divided into three groups. The first group (DOCA-saline) received a 50 mg DOCA tablet intraperitoneally and drank 1% NaCl solution. The other two groups were given sham operations. A 1% NaCl solution was given as drinking water to the second group (control-saline), while tap water was given to the third group (control-water). Furosemide (30 mg/kg) was given orally to each group at 12 a.m. or 12 p.m. Urine was collected for 8 hours after the agent, and urinary excretion of sodium and furosemide were determined. Urine volume and urinary excretion of sodium and furosemide following the agent were significantly greater at 12 a.m. than at 12 p.m. in the control-water and control-saline groups. However, the administration-time-dependent changes in these parameters disappeared in the DOCA-saline rats. These results suggest that the mode of the administration-time-dependent changes in the effects of furosemide is altered in the DOCA-saline hypertensive rats.

Keywords: Furosemide, Administration-time-dependent, DOCA hypertension, Adrenal corticoid, Diurnal variation

There is increasing evidence demonstrating administration-time-dependent changes in the effectiveness and toxicity of pharmacological agents (1). We already examined some chronopharmacological profiles of furosemide, a loop diuretic agent, in rats (2–4). These studies demonstrated that the effects of furosemide are greater when it is administered during the daytime than when it is administered during the nighttime.

Adrenal corticoids which are involved in water and electrolytes homeostasis show diurnal variations. For example, serum concentrations of corticosterone and aldosterone are remarkably higher during nighttime than during daytime in rats on a 12 : 12 hr light/dark cycle (5, 6). Because these hormones exert antinatriuretic and subsequent antidiuretic actions, it is speculated that the higher adrenal corticoids during nighttime diminish the diuresis and natriuresis following furosemide in rats. Our recent study showed that the administration-time-dependent change in the effects of furosemide disappears after adrenalectomy in rats (7). This observation supports the hypothesis that the diurnal variations in adrenal corticoids play some role in the administration-time-dependent phenomenon of furosemide.

Deoxycorticosterone acetate (DOCA)-saline hypertension is a model of mineralocorticoid-dependent hypertension. In this model, the disturbance of the diurnal variations in endogenous adrenal corticoids is speculated to occur for the following reasons: 1) The diurnal change in plasma renin activity (PRA) is involved in the mechanism responsible for the diurnal variation in adrenal corticoids (5). Because PRA is extremely suppressed in DOCA-saline hypertension, the diurnal change in PRA might be altered. 2) DOCA treatment causes hypokalemia which in turn blunts the biosynthesis of adrenal corticoids (8, 9).

Therefore, it is interesting to examine whether an alteration in the administration-time-dependent change in the effects of furosemide might occur in DOCA-saline hypertension.

MATERIALS AND METHODS

Thirty-seven male Wister rats [specific-pathogen-free (SPF) animal] (Charles River Laboratory, Kanagawa, Japan), 6-weeks-old, were maintained for more than 2 weeks under conditions of light from 7 a.m. to 7 p.m. and dark from 7 p.m. to 7 a.m. with free access to food and water. These aminals were housed and the following experiments were performed in a SPF room. These rats were subjected to unilateral left nephrectomy under pentobarbital anesthesia. Fourteen days after nephrectomy, DOCA treatment was begun. The rats were randomly divided into three groups. The first group of rats (DOCA-saline, n = 17) received a 50 mg DOCA tablet intraperitoneally and another tablet after three weeks, and drank 1% NaCl solution ad libitum. Sham operations were performed twice for the second and third groups of rats. A 1% NaCl solution was given as drinking water to the second group (control-saline, n = 10), while tap water was given to the third group (control-water, n = 10).

The study was performed at 4 weeks after the beginning of the DOCA treatment. Three percent body weight (b.w.) of 1% NaCl solution was given by gavage into the stomach at 12 a.m. (or 12 p.m.). Twenty-four hours after the vehicle alone, 30 mg/kg of furosemide in 3% b.w. of vehicle was given orally at 12 a.m. (or 12 p.m.). Urine was collected for 8 hours following vehicle alone or the drug administration at 12 a.m. (or 12 p.m.). Food and water were deprived during 8 hours after each administration. The administration of the drug was randomly assigned to 12 a.m. or 12 p.m. The washout period between the two sets of experiments was 2 days. Twenty-four hours after the end of the experiment, indirect blood pressure was measured by means of the tail cuff method (Narco Bio-Systems, Houston, TX, U.S.A.). Thereafter, blood samples were obtained under pentobarbital anesthesia.

Plasma potassium and urinary sodium concentraions were determined by flame photometry (Flame Photometer 775-A, Hitachi, Tokyo, Japan). Urinary furosemide concentraion was measured by high performance liquid chromatography (10). PRA was measured by radioimmunoassay (11).

The results are expressed as the means ± S.E. The correlation was calculated on the basis of least squares linear regression analysis. Data were analyzed by analysis of variance and the Wholly-Significant-Difference Method for paired observations (12).

RESULTS

When 3% b.w. of NaCl solution was given as a furosemide control, no significant difference was observed in urine volume or urinary sodium excretion in the collection period following the 12 a.m. administraion (day trial) compared to the collection period beginning at 12 p.m. (night trial) in any group of rats (Fig. 1). Urinary sodium, but not urine volume, was significantly ($P < 0.01$) greater in the control-saline group than in the control-water group. These parameters in the DOCA-saline group were significantly ($P < 0.01$) greater than those in the control-water and control-saline groups.

Urine volume and urinary sodium excretion following furosemide were significantly greater in the day trial than in the night trial in the control-water and control-saline groups of animals (Fig. 1). However, such a time-dependent change in the effects of furosemide was not observed in the DOCA-saline group of rats. Urinary furosemide excretion was greater at 12 a.m. than at 12 p.m. in the control-water and control-saline groups, but not in the DOCA-saline group. There were positive correlations between the urinary output of furosemide

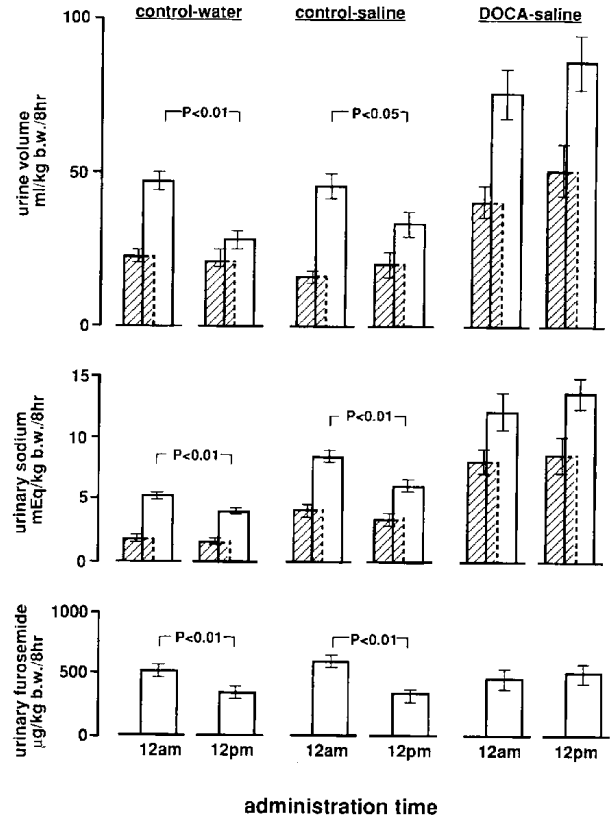

Fig. 1. Urine volume and urinary excretion of sodium and furosemide after furosemide administration in the control-water (n = 10), control-saline (n = 10) and DOCA-saline (n = 17) groups of rats. Each value is a mean ± S.E. ▨ NaCl solution alone, □ NaCl solution + furosemide.

and its effects in the control-water and control-saline groups [control-water group (n = 10): urine volume: (day trial) y = 0.062x + 15, r = 0.70 ($P < 0.05$); (night trial) y = 0.061x + 9, r = 0.68 ($P < 0.05$); urinary sodium: (day trial) y = 0.0046x + 2.9, r = 0.59 ($0.05 < P < 0.10$); (night trial) y = 0.0039x + 2.6, r = 0.65 ($P < 0.05$); control-saline group (n = 10), urine volume: (day trial) y = 0.061x + 9, r = 0.80 ($P < 0.01$); (night trial) y = 0.0064x + 14, r = 0.75 ($P < 0.05$); urinary sodium: (day trial) y = 0.0085x + 3.9, r = 0.79 ($P < 0.01$); (night trial) y = 0.0063x + 4.0, r = 0.58 ($0.05 < P < 0.10$)]. Significant correlations were also observed in the DOCA-saline group of animals (Fig. 2).

The regression lines between the urinary furosemide and its effects did not differ among the day and night trials in any group.

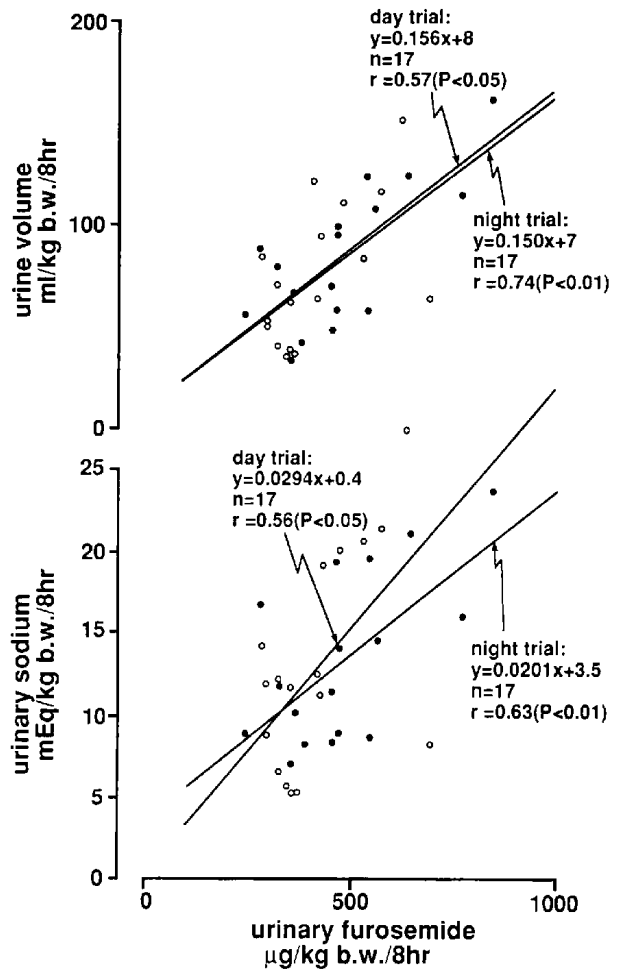

Fig. 2. Relationship between urinary furosemide and its diuretic effects in the DOCA-saline group of rats. Furosemide was given at 12 a.m. (○) or 12 p.m. (●), and urine was collected for 8 hours after the agent.

Blood pressure, PRA and plasma potassium at the end of the experiment are shown in Table 1. In the DOCA-saline group, blood pressure elevated markedly, and the values of PRA and plasma potassium decreased. The PRA in the control-saline group was slightly, but significantly lower than that in the control-water group.

DISCUSSION

The present study demonstrated that furosemide administered at 12 a.m. produces an increased diuresis compared to that administered at 12 p.m. in the control-water and control-saline groups of rats. However, such an administration-time-dependent change in the diuretic effect of furosemide disappeared in the DOCA-saline group of animals. These results suggest that the mode of the administration-time-dependent changes in the effect of furosemide is altered in this animal model.

The present as well as previous studies (2–4) showed that the administration-time-dependent chages in the diuretic effect of furosemide depend, at least in part, on the administration-time-dependent variations in the amount of urinary furosemide. The following results were obtained in the DOCA-saline rats: 1) The urinary furosemide excretion of the day and night trials did not significantly differ. 2) There were significant correlations between the urinary furosemide and its diuretic effects. 3) The regression lines did not significantly differ between the day and night trials. This means that the diuretic responsiveness to furosemide of the day and night trials does not differ in this animal model. These results suggest that the administration-time-dependent changes in the diuretic effect of furosemide disappeared as the diurnal variations in the amount of urinary furosemide disappeared in the DOCA-saline

Table 1. Blood pressure, PRA and plasma potassium at the end of the experiment

Parameter	Group		
	control-water (n = 10)	control-saline (n = 10)	DOCA-saline (n = 17)
Blood pressure mmHg	122 ± 2	128 ± 4	181 ± 6**
PRA ng/ml/hr	6.1 ± 0.7	4.0 ± 0.5*	0.8 ± 0.3**
Plasma potassium mEq/l	5.1 ± 0.3	5.0 ± 0.2	3.4 ± 0.4**

Mean ± S.E.; *$P < 0.05$, **$P < 0.01$ compared to control-water.

rats.

The mechanisms responsible for the disappearance of the administration-time-dependent changes in urinary furosemide in the DOCA-saline rats are unknown, but the following two seems possible. Our recent study suggested that normal adrenal function is involved in the administration-time-dependent phenomenon of furosemide (7). In the present study, the values of PRA and plasma potassium were low in the DOCA-saline group of rats. Because the decreased PRA and potassium suppresses the biosynthesis of adrenal corticoids (8, 9), the blood levels of endogenous adrenal corticoids might be low in these animals (13, 14). Therefore, it is speculated that normal adrenal function was disturbed and subsequently the administration-time-dependent phenomenon of furosemide was altered in the DOCA-saline group of rats. Previous studies have demonstrated that the administration-time-dependent changes in urinary furosemide disappeared following pretreatment with a β-adrenoceptor blocking agent (3) or 6-hydroxydopamine (15) or during the continuous infusion of norepinephrine (NA) (16). In addition, we recently observed that such an administration-time-dependent change is also blunted in rats with renal denervation (A. Fujimura et al., unpublished data). Based on these observations, it is concluded that the diurnal variations in the activity of sympathetic nerves including renal ones might contribute to the administration-time-dependent phenomenon of furosemide. It is well-known that plasma NA concentration elevates and renal vascular reactivity to NA increases in DOCA-saline hypertensive rats (17, 18). Therefore, it is hypothesized that the elevated plasma NA as well as the enhanced response of renal tissues to NA disturb the diurnal variations in the activity of the sympathetic nervous system, and consequently diminish the administration-time-dependent change in the urinary excretion of furosemide. However, more investigations are required because at present, we have no definite explanation for this disappearance of the administration-time-dependent phenomenon of furosemide in the DOCA-saline rats. High blood pressure per se might not reduce the diurnal variation in urinary furosemide (4). As changes in drug disposition might be caused by DOCA treatment, further studies involving determination of plasma and urinary furosemide are needed to evaluate this phenomenon.

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