Influence of Adrenalectomy on Chronopharmacological Phenomenon of Furosemide in Rats

Akio Fujimura, Kyo-ichi Ohashi and Akio Ebihara

Department of Clinical Pharmacology, Jichi Medical School, Minamikawachi-machi, Kawachi-gun, Tochigi 329-04, Japan

Received November 21, 1991 Accepted February 17, 1992

ABSTRACT—The role of adrenal corticoids in the time-dependent changes in the effects of furosemide was examined. Furosemide (30 mg/kg) was given orally to adrenalectomized or sham-operated rats at 12 a.m. or 12 p.m. Urine volume and urinary excretion of sodium and furosemide for 8 hr were significantly greater at 12 a.m. than at 12 p.m. in the sham-operated rats. However, such time-dependent changes in these parameters disappeared in the adrenalectomized animals. These findings indicate that adrenal corticoids are directly or indirectly involved in this event.

There is increasing evidence demonstrating time-dependent changes in the effectiveness and toxicity of pharmacological agents (1). We have already examined some chronopharmacological profiles of furosemide, a loop diuretic agent, in rats (2-5). These studies demonstrated that the effects of furosemide are greater when it is administered at daytime than when it is administered during nighttime. These studies also showed that the time-dependent changes in the effects of furosemide depend, at least in part, on the time-dependent variations in the urinary amount of the agent.

Adrenal corticoids, which are involved in water and electrolytes homeostasis, show diurnal variations. For example, serum concentrations of corticosterone and aldosterone are markedly higher during nighttime than during daytime in rats on a 12:12 hr light/dark cycle (6, 7). Since these exert antinatriuretic and subsequent antidiuretic actions, it is speculated that the higher adrenal corticoids during nighttime diminish a diuresis and natriuresis following furosemide in rats. The present study was the first step for addressing this issue. To examine the potential role of adrenal corticoids in the time-dependent changes in the effects of furosemide, the chronopharmacological profiles of furosemide in rats after adrenalectomy were compared to those during a control period.

Twenty-four male Wistar rats [specific pathogen free (SPF) animal] (Charles River Laboratory, Kanagawa, Japan), weighing 300 to 350 g, 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 animals were housed and the following experiments were performed in a SPF room.

In study 1, 3% 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, 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 hr following vehicle alone or the drug administration at 12 a.m. (or 12 p.m.). Food and water were deprived during 8 hr
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. Thereafter, these rats were divided into two groups. The first group of rats (n = 12) underwent bilateral adrenalectomy under ether anesthesia, and the second group of animals (n = 12) underwent a sham-operation. Seven days after operation, the identical protocol of study I was repeated (study II). Twenty-four hours after the end of study II, blood samples were obtained under pentobarbital anesthesia.

Urinary sodium concentration was determined by flame photometry (Flame Photometer 775-A, Hitachi, Tokyo, Japan). Urinary furosemide concentration was measured by high performance liquid chromatography (8). The sensitivity of this assay was 0.1 µg/ml. Plasma aldosterone concentration was measured by radioimmunoassay (9) with the sensitivity of 15 pg/ml.

The results are expressed as the means ± S.D. 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.

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. administration compared to the collection period beginning at 12 p.m. in studies I and II of any group (Figs. 1 and 2). These parameters in study II were slightly, but not significantly greater than those in study I in the adrenalectomized rats.

Urine volume and urinary sodium excretion following furosemide were significantly greater

Fig. 1. Urine volume and urinary excretion of sodium and furosemide after furosemide administration in rats during a control period (I) and after adrenalectomy (II). mean ± S.D., n = 12. ☐ NaCl solution alone, ☐ NaCl solution + furosemide.

Fig. 2. Urine volume and urinary excretion of sodium and furosemide after furosemide administration in rats during a control period (I) and after sham-operation (II). mean ± S.D., n = 12. ☐ NaCl solution alone, ☐ NaCl solution + furosemide.
at 12 a.m. (day trial) than at 12 p.m. (night trial) in study I of both groups and in study II of the sham-operated rats (Figs. 1 and 2). The values of these parameters in study II were significantly greater than those in study I in the night trial, but not in the day trial in the adrenalectomized rats.

Urine volume and urinary sodium excretion following furosemide were significantly greater at 12 a.m. (day trial) than at 12 p.m. (night trial) in study I of both groups and in study II of the sham-operated rats (Figs. 1 and 2). The values of these parameters in study II were significantly greater than those in study I in the night trial, but not in the day trial in the adrenalectomized rats. Consequently, the time-dependent changes in the effects of furosemide disappeared in the adrenalectomized animals (study II). Urinary furosemide excretion was greater at 12 a.m. than at 12 p.m. in study I of both groups and in study II of the sham-operated rats (Figs. 1 and 2). However, no significant difference was observed in urinary excretion of the agent between the day and night trials after adrenalectomy. There were significant correlations between the urinary output of furosemide and its effects (urine volume and urinary sodium) in each study: 1) between urinary furosemide and urine volume (n = 12 for each): in the adrenalectomized group, day trial: y = 0.027x + 18 (r = 0.61, P < 0.05) (study I), y = 0.012x + 29 (r = 0.58, P < 0.05); night trial: y = 0.026x + 11 (r = 0.67, P < 0.05) (study I), y = 0.023x + 20 (r = 0.69, P < 0.05) (study II); in the sham-operated group, day trial: y = 0.020x + 27 (r = 0.85, P < 0.01) (study I), y = 0.013x + 35 (r = 0.73, P < 0.05) (study II); night trial: y = 0.021x + 18 (r = 0.79, P < 0.01) (study I), y = 0.026x + 16 (r = 0.61, P < 0.05) (study II); 2) between urinary furosemide and urinary sodium (n = 12 for each): in the adrenalectomized group, day trial: y = 0.0033x + 2.9 (r = 0.58, P < 0.05) (study I), y = 0.0027x + 2.8 (r = 0.75, P < 0.01) (study II); night trial: y = 0.0046x + 2.1 (r = 0.79, P < 0.01) (study I), y = 0.0041x + 1.1 (r = 0.65, P < 0.05) (study II); in the sham-operated group, day trial: y = 0.0026x + 2.9 (r = 0.81, P < 0.01) (study I), y = 0.0019x + 4.0 (r = 0.67, P < 0.05); night trial: y = 0.0027x + 3.1 (r = 0.71, P < 0.05) (study I), y = 0.0026x + 1.3 (r = 0.70, P < 0.05) (study II). The slopes of the regression lines between the urinary furosemide and its effects did not differ among studies I and II in any parameter. Moreover, the regression lines obtained in study II were not significantly different from those in study I.

Plasma aldosterone concentration was very low in these adrenalectomized rats (adrenalectomized group: < 15 pg/ml, sham-operated group: 76 ± 12 pg/ml).

The present study demonstrated that furosemide produces an increased diuresis when administered at 12 a.m. compared to that administered at 12 p.m. in the rats with intact adrenal glands, which is similar to the previous findings (5). However, such a time-dependent change in the diuretic effect of furosemide disappeared in the rats after bilateral adrenalectomy. The observation that the existence of the adrenal gland is necessary for the chronopharmacological phenomenon of furosemide indicates that adrenal corticoids are directly or indirectly involved in this event.

Since adrenal corticoids show antinatriuretic and subsequent antidiuretic effects, these might alter the relationship between urinary furosemide and its diuretic effects. In the present study, the regression lines obtained after adrenalectomy were not significantly different from those obtained during a control period in the day or night trial. These indirect evidence suggest that the influence of adrenal corticoids on the diuretic effect of furosemide is relatively small.

The present as well as previous studies (2–5) showed that the time-dependent changes in the diuretic effect of furosemide depend, at least in part, on the time-dependent variations in the amount of urinary furosemide in control rats. The following results were obtained in the adrenalectomized animals: 1) The urinary furosemide excretion
did not significantly differ between the two administration times. 2) There was a significant correlation between the urinary furosemide and its diuretic effect. These data indicate that the time-dependent changes in the diuretic effect of furosemide disappeared as the diurnal variations in the amount of urinary furosemide disappeared in the adrenalectomized rats.

The urinary furosemide excretion after adrenalectomy was significantly greater than that during a control period in the night trial. The mechanisms responsible for this increment during the night-time in the adrenalectomized rats are unknown, but the following one is apparent. Generally, an activity of the sympathetic nervous system in rats increases during the night-time (an active period). Previous studies demonstrated that urinary furosemide excretion is smaller when it is administered during the rats' active period than when it is administered during their resting period (2–5). In addition, the diurnal changes in urinary furosemide disappeared following pretreatment with a $\beta$-adrenoceptor blocking agent (3) or 6-hydroxydopamine (10). Based on these findings, it is concluded that the diurnal variations in the activity of the sympathetic nervous system may contribute to the chronopharmacological phenomenon of furosemide and the increased activity of the system might directly or indirectly inhibit excretion of furosemide in the urine. It is well-known that the responsiveness of an organ to endogenous and exogenous norepinephrine is blunted in disease states such as adrenal insufficiency (11). Although the responsiveness to norepinephrine was not determined in the present study, it is speculated that the enhanced response of renal tissues to the sympathetic nervous system during the night-time is blunted in the adrenalectomized rats, and consequently, the urinary furosemide excretion increases. The adrenalectomy potentially removed epinephrine from the circulation and disturbed the axis of the sympathetic nervous system. This mechanism might also contribute to the chronopharmacological alteration of furosemide. Adrenal mineralocorticoid shows the diurnal variation (6, 7), and its receptors are found in the renal proximal tubule (12). Therefore, it is speculated that the diurnal variation in mineralocorticoid plays some role in the time-dependent change in the tubular secretion of furosemide. As changes in drug disposition are probably caused by adrenalectomy, further studies involving determination of plasma and urinary furosemide are needed to evaluate this phenomenon.

REFERENCES

