Influence of \(\alpha\)-Receptor Blockade on the Time-Dependent Change in the Effect of Furosemide

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ABSTRACT—Influence of \(\alpha\)-receptor blockade on the time-dependent change in the effect of furosemide (a loop diuretic agent) was examined. Furosemide (30 mg/kg) was given orally to the doxazosin (an \(\alpha_1\)-blocker) or vehicle-treated rats at 12 AM or 12 PM. Urine volume and urinary excretions of sodium and furosemide for 8 hr were significantly greater at 12 AM than at 12 PM in the vehicle-treated animals. However, such time-dependent changes in these parameters disappeared in the doxazosin-treated rats. These results suggest that the \(\alpha_1\)-receptor-mediated stimuli are involved in the mechanism of the time-dependent change in the effect of furosemide.

Keywords: Furosemide, Chronopharmacology, Doxazosin

We have previously demonstrated the time-dependent change in the diuretic effect of furosemide, a loop diuretic agent, in rats (1, 2). As such a time-dependent change disappeared following pretreatment with 6-hydroxydopamine (3), which produces a selective destruction of adrenergic nerve endings, it is suspected that adrenergic nervous system might contribute to this chronopharmacological phenomenon of furosemide. Another study using propranolol, a \(\beta\)-blocker, indicates that the \(\beta\)-receptor-mediated stimuli play some role in this event (4). On the other hand, it was not examined whether the \(\alpha\)-receptor-mediated stimuli might be also involved in the mechanism responsible for the time-dependent change in the diuretic effect of furosemide. To address this issue, the chronopharmacological profiles of furosemide following pretreatment with doxazosin, an \(\alpha_1\)-blocker, were compared to those during a control period.

Male Wistar rats (Charles River Laboratory, Kanagawa) weighing 300 to 350 g were maintained for more than 2 weeks under conditions of light from 7 AM to 7 PM and dark from 7 PM to 7 AM with free access to food and water. These animals were randomly divided into the first \((n=14)\) and second \((n=14)\) groups.

In study I, 3°10 body weight \((\text{b.w.})\) of 1% NaCl solution was given by gavage into the stomach at 12 AM (or 12 PM) in both groups of rats. Twenty-four hours after NaCl solution alone, 30 mg/kg of furosemide (Hoechst Japan Ltd., Tokyo) in 3% b.w. of NaCl solution was given orally at 12 AM (or 12 PM). Urine was collected for 8 hr following NaCl solution alone or furosemide administration at 12 AM (or 12 PM). Food and water were deprived during 8 hr after each administration. The administration of the drug was randomly assigned to 12 AM or 12 PM. The washout period between the two sets of experiments was 4 days. Thereafter, study II was done using the same groups of rats. In study II, the same protocol of study I was repeated, but 1 ml of distilled water was given orally to the first group of rats and 1 mg/kg of doxazosin (Pfizer, Tokyo) in 1 ml of water was given orally to the second group of animals at 30 min before each administration.

Urinary sodium concentration was determined by flame photometry (Flame Photometer 775-A; Hitachi, Tokyo). Urinary furosemide concentration was measured by high performance liquid chromatography (5). The sensitivity of this assay was 0.1 \(\mu\)g/ml.

The results are expressed as the means \(\pm\) S.E. The correlation was calculated on the basis of least squares linear regression analysis. Data were analyzed by analysis of variance and the paired Student’s \(t\)-test.

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 AM administration compared to the collection period beginning at 12 PM in studies I and II of any group (Figs. 1 and 2). These
parameters in study II were greater than those in study I in the doxazosin-treated rats, but the differences did not reach statistical significance (Fig. 2).

Urine volume and urinary sodium excretion following furosemide were significantly greater at 12 AM (day trial) than at 12 PM (night trial) in study I of both groups and in study II of the vehicle-treated rats (Figs. 1 and 2). The values of these parameters in study II were significantly greater than those in study I in the night, but not in the day trial in the doxazosin-treated rats (Fig. 2). Consequently, the time-dependent change in the effect of furosemide disappeared in the doxazosin-treated animals (Fig. 2). Urinary furosemide excretion was greater at 12 AM than at 12 PM in study I of both groups and in study II of the vehicle-treated rats (Figs. 1 and 2). However, no significant difference was observed in urinary excretion of furosemide between the day and night trials in the doxazosin-treated rats (Fig. 2). There were significant correlations between the urinary output of furosemide and the increment in urine volume (Δ urine volume) and urinary sodium (Δ urinary sodium) in each study: (1) between urinary furosemide and Δ urine volume (n=28 for each), vehicle-treated group: y=0.026x−3.0 (r=0.56, P<0.01) (study I), y=0.025x−2.3 (r=0.60, P<0.01) (study II); doxazosin-treated group: y=0.019x+0.8 (r=0.48, P<0.01) (study I), y=0.015x+4.2 (r=0.42, P<0.05) (study II); (2) between urinary furosemide and Δ urinary sodium (n=28 for each), vehicle-treated group: y=0.0022x+0.5 (r=0.58, P<0.01) (study I), y=0.0029x+0.2 (r=0.66, P<0.01) (study II); doxazosin-treated group: y=0.0011x+1.2 (r=0.41, P<0.05) (study I), y=0.0018x+0.4 (r=0.50, P<0.01) (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.

The present study showed that furosemide produces an increased diuresis when administered at 12 AM compared to that administered at 12 PM in the control group of rats. However, such a time-dependent change in the diuretic effect of furosemide disappeared in the doxazosin-treated animals. These results suggest that the α₁-receptor-mediated stimuli are involved in the mechanism responsible for the time-dependent phenomenon of furosemide. As the α₁-receptor blockade causes natriuresis and subsequent diuresis (6), doxazosin might alter the relationship between urinary furosemide and its diuretic
effects. In the present study, the regression lines obtained following doxazosin pretreatment were not significantly different from those obtained during the control period in the day or night trial. These indirect evidence suggest that the influence of doxazosin on the diuretic effect of furosemide is relatively small.

The present as well as previous studies (1–4) 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 the control rats. The following results were obtained in the doxazosin-treated animals: 1) The urinary furosemide excretion did not differ significantly between the day and night trials. 2) There was a significant correlation between the urinary furosemide and its diuretic effect. These data suggest that the time-dependent changes in the diuretic effect of furosemide disappeared as the time-dependent variations in the amount of urinary furosemide disappeared in the doxazosin-treated rats.

The urinary furosemide excretion following doxazosin pretreatment was significantly greater than that of the control period in the night trial. The mechanism of this increment during the night-time in the doxazosin-treated rats are unknown, but the following one is apparent: The activity of the sympathetic nervous system increases during the night-time (an active period) in rats. The present as well as previous (1–4) studies demonstrated that urinary furosemide excretion is smaller when it is administered at the rats’ active period than when it is administered during their resting period. In addition, the time-dependent changes in urinary furosemide excretion disappeared following pretreatment with 6-hydroxydopamine. Life Sci. 46, 827–831 (1990)

Furosemide is not only metabolized by the liver, but is secreted in unchanged form in the urine (7). We have no evidence indicating which pathway(s) might be influenced by pretreatment with doxazosin.

The decrease in blood pressure, which occurs during treatment with doxazosin, in itself influences the renal excretory function (8) and, therefore, might alter the chronopharmacological profile of furosemide. In addition, if doxazosin inhibits directly tubular secretion of furosemide, this might also change the time-dependent phenomenon of furosemide. Further studies are needed to evaluate the role of α₁-receptor-mediated stimuli in the time-dependent change in the diuretic effect of furosemide.

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