Circadian Rhythm of Plasma Uric Acid and Handling Stress-Induced Hyperuricemia in Conscious Cebus Monkeys

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ABSTRACT—An apparent circadian rhythm of plasma uric acid and the effect of handling stress on plasma uric acid level in conscious cebus monkeys were demonstrated. The lowest level of plasma uric acid in the circadian rhythm occurred early in the morning and the highest, before bedtime at night. With experimental handling stress, the plasma uric acid level rose to much more than the maximum level of the circadian rhythm. Stress-induced hyperuricemia could be inhibited without an increase of urinary uric acid excretion by the minor tranquilizer diazepam at doses of more than 1 mg/kg, p.o. On the other hand, benzbromarone at 20 mg/kg, p.o. significantly inhibited the hyperuricemia with a hyperuricosuric effect, while probenecid at 50 mg/kg, p.o. had no effect on either the increased plasma uric acid or urinary uric acid excretion. Accordingly, it is concluded that the plasma uric acid level in conscious cebus monkeys easily fluctuates with experimental conditions and that the animals can be utilized to evaluate the hypouricemic and hyperuricosuric property of benzbromarone-like agents.

Species differences in uric acid metabolism and excretion make it difficult to evaluate the properties of hypouricemic drugs. However, it has been generally accepted that cebus monkeys closely resemble humans as to both the high plasma uric acid level and the definite net reabsorptive flux of uric acid in the renal tubules (1). Therefore, the animals have sometimes been used to study renal handling of uric acid and to evaluate uricosuric activities. Most of such work has been done in clearance studies under anesthetic conditions (2–6). In the present study, we tried to examine the drug effects on progressive changes of plasma uric acid and urinary uric acid excretion in conscious animals. In humans, the existence of a circadian rhythm of uric acid excretion has been reported (7, 8), and stress is suggested to influence the plasma uric acid level. Thus, the circadian rhythm of plasma uric acid and the influence of experimental handling stress should be adequately investigated before trying to evaluate the drug effects using conscious cebus monkeys. In fact, the plasma uric acid level was found to easily rise due to experimental handling stress, such as body weight check, blood collection and nasotracheal tubing.

This paper discusses the circadian rhythm of plasma uric acid in conscious cebus monkeys and the influence of experimental handling stress on their plasma uric acid level. Also,
differences in the uricosuric activity of practical hypouricemic agents, benzbromarone and probenecid, under such conditions are also described.

MATERIALS AND METHODS

Experimental procedure to determine the circadian rhythm of plasma uric acid

The five cebus monkeys, three males and two females, used in the present study were Cebus apella weighing between 1.7 kg to 3.3 kg. Sessions to accustom the animals to tolerate blood collection were done for more than six months prior to the experiment. During the experiment, the animals were given apples and oranges for their breakfast at 9:00 A.M. and 35–50 g of a pellet diet consisting of 20% protein, 9% fat, 4.2% fiber and other components were given for lunch and supper at 3:00 P.M. and 7:00 P.M., respectively. Water was freely available for intake. The room light was turned on at 8:00 A.M. and off at 8:00 P.M.

To determine the circadian rhythm of plasma uric acid, 1 ml of blood was collected with a disposable syringe, moistened with heparin, from the saphenous veins of the legs or radial cutaneous veins of the arms once a day at 7:00 A.M., 11:00 A.M., 3:00 P.M., 7:00 P.M. or 11:00 P.M. Each collection was done with an interval of more than a week in order to avoid the influence of one schedule on another. Blood samples were immediately centrifuged (3000 rpm × 10) and the plasma was kept at −20°C until the measurement.

Determination of uric acid

Uric acid levels in the plasma and urine were measured by an HPLC method. A Chemcosorb-5-ODS UH column (150 × 4.6 mm i.d.) was used with an eluent of methanol/water buffer (1:7, v/v) containing PIC A reagent (Waters Association), 1 vial/1140 ml, at the flow rate of 1 ml/min with monitoring at 292 nm.

Chemicals

Drugs used in this study were diazepam, benzbromarone and probenecid. Diazepam was synthesized in our laboratories, while benzbromarone was extracted from a commercial preparation (URINORM, Torii Yakuhin). Probenecid was purchased from Sigma.

Statistics

Data are shown as the mean and standard error. The significance of the difference from the control value was evaluated by Tukey’s method (9).

RESULTS

The circadian rhythm of plasma uric acid in conscious cebus monkeys is shown in Fig. 1. The plasma uric acid level was lowest at 7:00 A.M. (1.45 ± 0.16 mg/dl) just before the animals got up in the morning. The animals actively moved around their home cages during the daytime and slept after dark. The plasma uric acid level gradually increased during the daytime reaching the highest value at 7:00 P.M. (4.10 ± 0.23 mg/dl) and then decreased during the night, with recovery of the low level by the next morning. At 11:00 P.M., when the animals were already asleep, the plasma uric acid level was significantly lower than that at 7:00 P.M.
Figure 2 shows the changes of plasma uric acid in the animals given only the vehicle. Plasma uric acid at the beginning of the experiment (11:00 A.M.) was 3.53 ± 0.15 mg/dl, which was much higher than that in the circadian rhythm at 11:00 A.M., and the higher level was maintained during the daytime. The value at 7:00 P.M. was 6.26 ± 0.38 mg/dl, corresponding to 1.5 times the value without experimental handling stress. However, the value at 11:00 A.M. the next morning was 2.42 ± 0.17 mg/dl, which was the same as that in the circadian rhythm at 11:00 A.M. These fluctuations of plasma uric acid level were repeatedly tested after handling for more than six months, and thus were confirmed to be reproducible responses.

An attempt was made to suppress the in-

Fig. 1. Circadian rhythm of plasma uric acid (PuA) levels in conscious cebus monkeys. Plots show the individual values of plasma uric acid. Blood collection was done once a day at 7:00, 11:00, 15:00, 19:00 or 23:00. The shadowed area indicates the period when the room light was off.

Fig. 2. Changes of plasma uric acid (PuA) levels in conscious cebus monkeys treated with 3% gum arabic solution. Plots show the individual values of plasma uric acid.
crease in plasma uric acid due to handling stress by the administration of three agents. The first was the minor tranquilizer diazepam. As shown in Fig. 3 and Table 1, at doses of more than 1 mg/kg, p.o., diazepam inhibited stress-induced hyperuricemia with a reduction of urinary uric acid excretion. At 5 mg/kg, p.o., it completely blocked the elevation of plasma uric acid in the experiment and sedated the animals, but it had no effect on the behavior at 1 mg/kg, p.o. Benzbromarone, a practical hypouricemic agent in humans, also showed a hypouricemic effect with apparent hyperuricosuria (Fig. 4), while probenecid (Fig. 5) had no effect on either the increased plasma uric acid level or urinary uric acid excretion.

DISCUSSION

Cebus monkeys, like chimpanzees, have been generally accepted as valuable primates for research because they have uric acid metabolism and excretion resembling those in humans, and therefore have been used to study the properties of hypouricemic agents. To evaluate drug effects on renal tubular transport of uric acid, early workers developed

![Fig. 3. Effects of diazepam on plasma uric acid (PuA) in conscious cebus monkeys. Plots show the mean values and the bars, the standard errors. ●—● diazepam, 1 mg/kg, p.o. (N = 4); ■—■ diazepam, 5 mg/kg, p.o. (N = 3); ○—○ control group (N = 6). *, **: Significantly different from the corresponding control value at P < 0.05 and P < 0.01, respectively.]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>0–4 hr (mg)</th>
<th>4–8 hr (mg)</th>
<th>8–24 hr (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treated</td>
<td>6</td>
<td>9.9 ± 2.2</td>
<td>12.4 ± 3.3</td>
<td>21.7 ± 3.7</td>
</tr>
<tr>
<td>Diazepam 1 mg/kg, p.o.</td>
<td>4</td>
<td>7.0 ± 1.2</td>
<td>5.9 ± 1.5</td>
<td>14.4 ± 3.6</td>
</tr>
<tr>
<td>Diazepam 5 mg/kg, p.o.</td>
<td>3</td>
<td>9.6 ± 2.1</td>
<td>5.1 ± 1.6</td>
<td>21.7 ± 4.1</td>
</tr>
</tbody>
</table>

Mean and standard error.
Fig. 4. Effects of benzbromarone on plasma uric acid (PUA) and urinary uric acid excretion (UUA) in conscious cebus monkeys. Plots and columns show the mean values and the bars, the standard errors. Benzbromarone, 20 mg/kg, was orally administered (N = 4) (●—●, □□). Control group (N = 4) (○—○, □□). *, **: Significantly different from the corresponding control value at P < 0.01.

Fig. 5. Effects of probenecid on plasma uric acid (PUA) and urinary uric acid excretion (UUA) in conscious cebus monkeys. Plots and columns show the mean values and the bars, the standard errors. Probenecid, 50 mg/kg, was orally administered (N = 4) (●—●, □□). Control group (N = 4) (○—○, □□).
clearance techniques in animals under anesthesia (2–6). However, these techniques put a heavy burden on the animals, because of osmotic diuresis and successive blood collection over short time periods, and therefore their use should be avoided. It would be more desirable to utilize the animals for evaluating drug effects under experimental conditions close to clinical use. As hypouricemic drugs are usually given to conscious human subjects, we tried to utilize conscious animals.

In the process of studying the properties of hypouricemic agents under conscious conditions, we found that basal plasma uric acid levels in the animals fluctuated easily during the experiment; plasma uric acid increased markedly during the daytime but returned to their original levels by the next morning. This transient hyperuricemia was partially due to circadian rhythm. However, the plasma uric acid level at the beginning of the experiment was much higher than that at 24 hours after the beginning in spite of the sample having been obtained at the same time of day (11:00 A.M.), which indicated that the hyperuricemia in the experiment was also caused by some other factors in addition to the circadian rhythm of plasma uric acid. We predicted that these factors were those of stress due to experimental handling, such as of body weight check before the experiment, blood collection and nasotracheal tubing for drug administration. Diazepam showed hypouricemic effects in the animals at doses of more than 1 mg/kg, p.o., dose-dependently without a hyperuricosuric effect.

Korotkina reported the inhibition of xanthine oxidase activity by diazepam (IC$_{50}$ = 1 × 10$^{-9}$ M) in the homogenate solution from rat brain (10), which raised the possibility of the hypouricemic effect in the present study having arisen from its inhibitory effect on xanthine oxidase. However, in our study, diazepam did not inhibit partially purified xanthine oxidase activity from rat liver at doses from 10$^{-9}$ to 10$^{-4}$ M (data not shown). On the other hand, allopurinol, a practical hypouricemic agent known to inhibit xanthine oxidase activity in both humans and experimental animals, showed a potent inhibitory effect (IC$_{50}$ = 2 × 10$^{-7}$ M). Because plasma uric acid is mainly controlled by uric acid production in the liver, the inhibitory effect of diazepam on xanthine oxidase activity, even if diazepam inhibits xanthine oxidase activity in the brain, should not reflect the reduction of plasma uric acid. Hence, the hypouricemic effect after the administration of diazepam in conscious cebus monkeys was not due to the inhibition of xanthine oxidase activity, but to the release of the animals from the experimental handling stress. These results show that plasma uric acid in conscious cebus monkeys easily increases with experimental handling stress.

Hyperuricemia in humans is thought to arise due to many environmental factors. Stress in daily life is suggested to be one factor, though this has not been confirmed experimentally. With laboratory animals, Bryan reported that the energy metabolism increased during stressful states (11). If hyperuricemia occurs due to enhancement of the energy metabolism in the body, it can be expected to be induced by experimental handling stress, as was found in the present study. As the turnover of uric acid in cebus monkey is reported to be much larger than that in humans (12), uric acid production in the animals may be more easily stimulated by experimental handling stress, with a resulting marked hyperuricemia. Although it has not been proved that stress-induced hyperuricemia can occur in humans, its occurrence in conscious cebus monkeys, which are known to resemble humans in uric acid metabolism and excretion, is very interesting. In this respect, conscious cebus monkeys may be useful for studying the causes and development of hyperuricemia in humans. Further study is needed on the mechanism that links stressful states to the development of hyperuricemia.

The present study showed quite different responses to two kinds of typical hypouricemic drugs. Benzbromarone showed hypouricemic effects with an increase of urinary uric acid excretion, as reported in humans (13, 14), while
probenecid had no effect. Evaluation of uricosuric agents using conscious cebus monkeys has been done by Dan et al. (15). They also reported that benzbromarone was more potent than probenecid in the animals, but 20 mg/kg, p.o. of probenecid showed a weak uricosuric effect without a hypouricemic effect. In a clearance technique, Fanelli reported the uricosuric activity of probenecid at more than 12.5 mg/kg, p.o. (2). These differences as to the uricosuric effect in probenecid administration might arise from differences in the net flux of uric acid in the renal tubules before dosing. Fanelli reported in the clearance study that probenecid at 12.5 mg/kg, p.o. increased the fractional excretion (FEUA) value from 0.054 to 0.174. When 100 mg/kg of probenecid was given orally, the value increased from 0.107 to 0.257. Thus, the uricosuric effect of probenecid seems to show a low ceiling in cebus monkeys, because there is no difference between the uricosuric potency at high and low doses. On the other hand, benziodarone, with a structure similar to that of benzbromarone, increased the FEUA value from 0.054 to 0.617 at 15 mg/kg, p.o., indicating a high ceiling for the uricosuric activity. These differences between the drugs seemed to have arisen from some difference in the mechanism of uricosuric action. Although the FEUA value in our conscious cebus monkeys could not be calculated, the net flux of uric acid in the renal tubules in our study might have been less reabsorptive than those under the conditions performed by Fanelli and Dan. If this is true, it can explain the uricosuric responses found with benziodarone which displays a high ceiling for uricosuric activity, while probenecid with a low ceiling had no effect. The net tubular transport of uric acid in the cebus monkey kidneys could be easily changed with the experimental conditions. The uricosuric effects of probenecid-like agents will be abolished if the FEUA value is comparatively high in the animals, which differs from the responses of benziodarone-like agents. Accordingly, serious consideration should be given in evaluating the uricosuric or hypouricemic properties of test compounds using conscious cebus monkeys.

In summary, we showed that an apparent circadian rhythm of plasma uric acid exists in conscious cebus monkeys and also that plasma uric acid in the animals can be easily increased by handling stress. In this respect, although not all hypouricemic drugs yield responses suitable for evaluation, conscious cebus monkeys may be useful for studying the causes or development of hyperuricemia in humans.

REFERENCES

11 Bryan, R.M., Jr.: Cerebral blood flow and energy


