Daily fluctuation of hepatic P450 monooxygenase activities in male rats is controlled by the suprachiasmatic nucleus but remains unaffected by adrenal hormones

Abstract Hepatic P450 monooxygenase activities, which strongly influence the efficacy and/or toxicity of drugs, are known to fluctuate daily. We also know that the P450 activities assessed by measurement of 7-alkoxycoumarin O-dealkylase (ACD) activities fluctuate daily, with apparently high values during the dark period in male rats. However, there is little knowledge about the factors that regulate daily fluctuation of P450 monooxygenase activities. In the present study using rats, we induced lesions in the suprachiasmatic nucleus (SCN) of the brain, the known site of the body’s internal clock, and examined the effects on the daily fluctuation of the ACD activities to clarify the relationship between the SCN and the daily fluctuation of P450 monooxygenase activities. In addition, adrenalectomy was performed to re-evaluate the influence of adrenal hormones on the P450 activities. Our results indicated that daily fluctuations of the hepatic ACD activities were completely eliminated in the SCN-lesioned rats. However, the ACD activities in the adrenalectomized rats showed apparent daily fluctuations with high values during the dark period and low values during the light period. Therefore, this study demonstrated that the daily fluctuation of the hepatic P450 monooxygenase activities in male rats is controlled by the SCN but remains unaffected by the adrenal hormones.

Key words Alkoxycoumarin dealkylase · Liver · Daily fluctuation · Suprachiasmatic nucleus (SCN) · Adrenal
In rodents, it was reported that fasting (Imaoka et al. 1990; Brown et al. 1995), glucocorticoid (Lloyd and Franklin 1991) and growth hormone (Kato et al. 1986; Waxman et al. 1991) all influence the P450 enzyme activities. Among these factors, fasting was reported not to eliminate the daily fluctuation of the P450 enzyme activities (Furukawa et al. 1999; Radziazlowski and Bousqet 1968). Moreover, the daily fluctuations of p-nitroanisole O-demethylase activity (Radziazlowski and Bousqet 1967) and aminopyrine N-demethylase activity (Radziazlowski and Bousqet 1968) in the rat liver were eliminated by adrenalectomy. Glucocorticoid levels also fluctuate daily within the body (Gomez-Sanchez et al. 1976; Atkinson and Waddell 1997), suggesting that this hormone may regulate the daily fluctuation of P450 enzyme activities. However, the measurements of the hepatic enzyme activities in the above studies (Radziazlowski and Bousqet 1967, 1968) were conducted within only 3 days after adrenalectomy, hence it is probable that the homeostasis of the animals was in disorder when the enzyme activities were measured. Accordingly, this point must be confirmed by an additional experiment with a sufficient recovery period.

The most important endogenous factor regulating daily variation in many kinds of physiological parameters, including glucocorticoid, is the suprachiasmatic nucleus (SCN) of the anterior hypothalamus in the brain. The SCN is known to be the site of the body's internal clock (Schwartz and Gainer 1977; Inouye and Kawamura 1979), and SCN lesion studies (Moore and Eichler 1972; Stephan and Zucker 1972; Ibuka and Kawamura 1975) have demonstrated that various physiological parameters are under the control of the SCN. Nevertheless, whether the daily fluctuation of the P450 enzyme activities is also regulated by the SCN has not been concluded. The objectives of the present study were to clarify the relationship between the SCN and daily fluctuation of P450 monoxygenase activities and to re-confirm the influence of adrenal hormones on the P450 activities in male rats. To meet these objectives, we examined the effects of SCN lesions and adrenalectomies on the daily fluctuation of P450 enzyme activities assessed by measurement of ACD activities.

Materials and methods

Experimental animals

Male F344/DuCrj rats purchased from Charles River Japan, Inc. (Yokohama) were used in this study. All animal procedures were approved by the animal care and use committee of our company. Animals were transferred and acclimatized to an animal room, maintained under an illumination intensity of ~200 lux with a light/dark cycle of 13 h:11 h, a stable temperature of 21–25 °C, and a stable humidity of 45–65%. The animals were housed in wire-meshed cages and given water and NMF laboratory rodent chow (Oriental Yeast Co., Ltd., Tokyo, Japan) ad libitum throughout the entire study except the postoperative period, when physiological saline was substituted for water in the adrenalectomized and sham-operated animals.

SCN lesion

The SCN lesion was administered with a Lesion Generator (Muramachi Kikai Co., Ltd., Tokyo, Japan) in rats between 9 and 11 weeks of age under pentobarbital anesthesia (40 mg/kg, i.p.; Dainabot Laboratories, North Chicago, Ill., USA). The anesthetized rats were mounted on a stereotaxic apparatus (Narishige Scientific Instrument Laboratories, Tokyo, Japan) and bilateral microlesions were produced with the probe of the Lesion Generator by heating (85 °C, 5 min). The stereotaxic coordinates for the SCN lesion, determined with reference to a brain atlas (Kruger et al. 1995), were set at 2.2 mm caudal from the bregma, ±0.5 mm lateral from the midline, and 8.6 mm ventral from the surface of the skull. The coordinates for the sham-operation rats were set at 6.0 mm dorsal from the SCN lesion point. After the SCN lesion was generated or the sham operation was performed, water consumption was measured continuously by the Drinkometer (O'Hara & Co., Ltd., Tokyo, Japan). This apparatus was equipped with a cartridge that made a water drop of 0.05 ml between a water tank and a drinking spout. When the rats drank, water fell into the cartridge drop by drop, and the number of drops was electrically counted (Kuribara et al. 1978). It was confirmed with the apparatus whether the drinking behavior rhythm had disappeared. The lesion in the brain was histopathologically examined at the end of the experiment.

Adrenalectomy

Adrenalectomy was conducted by bilateral dorsolateral incisions under ether anesthesia at 3 weeks of age. The sham operation was performed in the same manner as the adrenalectomy with the exception that the adrenal glands were not removed. The result of the adrenalectomy was confirmed by measurement of the serum corticosterone concentration. Blood was collected from the adrenalectomized and sham-operated rats at the removal of the liver, and corticosterone in the serum was measured by radioimmunoassay. The measurement was carried out by Biomedical Laboratories, Tokyo, Japan.

Collection of the liver

Livers were collected from the adrenalectomized and the sham-operated rats at 9 weeks of age after a 6-week recovery period, and from the SCN-lesioned and the sham-operated rats at 15 weeks of age after a 4- to 6-week recovery period. The collection was performed at 4-h intervals with the sacrifice of animals in batches of five at 0900, 1300, 1700, 2100, 0100 and 0500 hours under the light-dark condition (light: 0600–1900 hours). After the collection, a 3-g portion of each liver was frozen in liquid nitrogen and stored at about ~80 °C until measurement of the metabolizing enzymes.

Sample preparation and assays

At the time of measurement, liver samples were thawed and microsomal fractions were prepared according to the method of Omura and Sato (1964). The activities of MCD, ECD, and PCD in the microsomal fraction were determined by the method of Matsubara et al. (1983). Protein concentrations in the microsomal fraction were determined by the method of Lowry et al. (1951). 7-Methoxycoumarin, 7-ethoxycoumarin, and 7-hydroxycoumarin were purchased from Aldrich Chemical Co. (Milwaukee, Wis., USA). 7-Propoxycoumarin was synthesized according to the method of Matsubara et al. (1983).

Statistical analysis

The daily fluctuation of ACD activities was estimated by analysis of variance (ANOVA, P < 0.05). The parameters that showed significant differences in ANOVA were further analyzed by