Biochemical Pharmacology, Vol. 43, No. 9, pp. 1907–1910, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

DECREASE IN THE CONTENT OF CYTOCHROME P450IIE BY FASTING IN LIVER MICROSOMES OF HOUSE MUSK SHREW (*SUNCUS MURINUS*)

HIRONORI NAKURA, SUSUMU ITOH, HISASHI KUSANO,* HIROYUKI ISHIZONE,*
SUSUMU IMAOKA,† YOSHIHIKO FUNAE,† TSUYOSHI YOKOI and TETSUYA KAMATAKI‡

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Hokkaido 060; * Horiuchi Itaro Shouten Research Laboratories, Tokyo 189; and † Laboratory of Chemistry, Osaka City University Medical School, Osaka 545, Japan

(*Received* 19 *November* 1991; *accepted* 14 *February* 1992)

Abstract—The effects of fasting on hepatic cytochrome P450 in the mature male house musk shrew, *Suncus murinus* (suncus), were studied by Western blot analyses and enzyme assays. The content of P450IIE protein was decreased, by fasting, to 24% of the control level in contrast to the results with rats, in which P450IIE protein was increased to 172% by fasting. These changes reflected on catalytic activities, such as aniline hydroxylase and *N*-nitrosodimethylamine demethylase activities, which were decreased to about 45% and 28%, respectively, of the control levels by fasting, while in fasting rats, the catalytic activities of these enzymes were 2–3-fold higher than in controls.

Cytochromes P450 are heme-containing enzmes. This cytochrome present in liver microsomes can catalyse the oxidation and the reduction of a variety of exogenous as well as endogenous compounds such as drugs, resulting in the detoxification and intoxication of such foreign compounds. Although it has been found that there are corresponding forms of cytochrome P450 across animal species, the catalytic properties of the corresponding forms of cytochrome P450 are not necessarily identical [1].

The house musk shrew, *Suncus murinus* (suncus), is mainly distributed in southeast Asia. This animal species is classified as being in the family of *Soricidae* of the order of *Insectivora* and believed to be phylogenetically closer to primates than rodents [2]. The suncus has been established as a laboratory animal in Japan, since this animal species shows some unique characteristics. The suncus can be induced to vomit by vibration and some agents including anticancer drugs. In addition, the suncus shows a hepatic disorder with even small amounts of alcohol and ethanol at a dose of 5% in drinking water was 100% fatal [3].

The expression of cytochrome P450 is regulated by numerous endogenous and exogenous factors and varies with the class of P450. The expression of P450IIE has been recognized as one of the most interesting phenomena since this cytochrome is induced by alcohol and some other xenobiotics without a concomitant increase in the amount of mRNA coding for this cytochrome. P450IIE is also inducible by fasting associated with the increase in the amount of mRNA in the rat [4].

Despite efforts to clarify the nature of the suncus as an experimental animal, little is known about drug metabolizing enzymes in the suncus [5]. In the course of our studies on cytochrome P450 in liver microsomes of suncus, we found that fasting caused a considerable decrease in the content of cytochrome P450 in liver microsomes. We now report that fasting of suncus caused a remarkable decrease in the content of P450IIE, which was in contrast to the rat.

‡ Corresponding author: Tetsuya Kamataki, Ph.D., Division of Analytical Biochemistry, Faculty of Pharmaceutical Sciences, N12 W6 Sapporo, Hokkaido 060, Japan. Tel./FAX (81) 11-709-6390.

§ Abbreviation: NDMA, *N*-nitrosodimethylamine.

MATERIALS AND METHODS

Materials. Glucose-6-phosphate and glucose-6-phosphate dehydrogenase, $NADP^+$ were purchased from Oriental Yeast (Tokyo, Japan). *N*-Nitrosodimethylamine (NDMA§) and 3,3′-diaminobenzidine were from the Sigma Chemical Co. (St Louis, MO, U.S.A.), and 1-(hydroxymethyl)-5,5-dimethylhydantoin was from Tokyo Kasei Kogyo (Tokyo, Japan). All other chemicals were of analytical grade.

Animals. Male suncus specimens 6–7 weeks old weighing from 38 to 45 g were purchased from the Central Institute for Experimental Animals (Kawasaki, Japan). Male Sprague–Dawley rats 6–7 weeks old weighing from 158 to 176 g were purchased from Shizuoka Experimental Animals (Tokyo, Japan). They were maintained in air-conditioned quarters with 12 hr light–dark cycles. The rats and suncus were given laboratory chow (Clea Japan, Tokyo, Japan) and water *ad lib*. When necessary, suncus and rats were deprived of food for 24 hr prior to being killed but given water *ad lib*.

Preparation of hepatic microsomes. The animals were stunned by a blow on the head and decapitated. The livers were immediately removed and rinsed with 0.15 M KCl, blotted dry, and weighed. The livers were homogenized with 4 vol. of 1.15% KCl. The microsomal fraction of livers was obtained by a successive centrifugation of the homogenates at 9000 *g* for 20 min and at 105,000 *g* for 60 min at 4°.

The fraction was washed by resuspension with 1.15% KCl and recentrifugation at 105,000 *g* for 30 min at 4°. The microsomes were suspended in ice-cold distilled water at a concentration of approximately 10 mg protein/mL and stored at −80° until use.

Immunoblot analysis. Sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) was performed according to the method of Laemmli [6] and Guengerich *et al.* [7] with a 10% separating gel. Purified P450DM, corresponding to rat P450IIE1, and anti-P450DM serum were prepared by the method of Funae *et al.* [8]. The electrophoresis (gel size: 9 × 7 cm) was conducted at 30 mA for 2 hr. Western blot analyses were performed by the method of Nagayama *et al.* [9], except that anti-P450DM serum (diluted 1 : 300 with phosphate-buffered saline) was used as a first antibody. Prestained molecular size markers (Bio-Rad) were used as a standard. The amount of P450IIE was calculated from its band intensity as measured with a densitometer (Quick Scan R&D, Helena, Beaumont, TX, U.S.A.).

Enzyme assays. A typical incubation mixture for the assay of aniline hydroxylase and NDMA demethylase activities consisted of 100 mM Na–K phosphate buffer (pH 7.4), 0.05 mM EDTA, 1 mg of microsomal protein and a substrate (5 mM aniline–HCl or NDMA). The reaction was started by the addition of an NADPH-generating system (5 mM magnesium chloride, 0.5 mM $NADP^+$, 5 mM glucose-6-phosphate and 1 unit of glucose-6-phosphate dehydrogenase) in a final volume of 1 mL. After incubation for 15 min (aniline hydroxylase) or 10 min (NDMA demethylase) after 5 min preincubation at 37°, the reaction was terminated by the addition of 20% trichloroacetic acid. The activities of aniline hydroxylase and NDMA demethylase activities were estimated by determination of *p*-aminophenol [10] and formaldehyde [11], respectively. The reactions were linear with respect to protein concentration and incubation time employed in this study for both activities in the suncus and rat (data not shown).

Other methods. The content of cytochromes P450 and P420 in liver microsomes was determined according to the method of Imai and Sato [12]. Protein concentration was determined by the method of Lowry *et al.* [13] using bovine serum albumin as a standard.

RESULTS

Effects of fasting on body weight, liver weight, microsomal protein content and microsomal enzymes in the suncus and rats

The fasting of suncus and rats resulted in obvious loss of body weight and lower relative liver weight and microsomal protein content than the control suncus and rats (Table 1). Body weight and relative liver weight were decreased to 92% and 79% of control values, respectively, by fasting the suncus (Table 1). Microsomal protein content was slightly decreased (about 12%) by fasting the suncus, while it increased by about 19% in rats. The reduced CO-difference spectrum of the microsomal fraction from a fasting suncus is shown in Fig. 1. A decrease in the content of cytochrome P450 and an increase in the content of cytochrome P420 by fasing the suncus

Table 1. Effects of fasting on body weight, liver weight, microsomal protein content and activities of microsomal enzymes of suncus and rats

	Treatment* (N)	Body weight (g)	Relative liver weight (g/100 g body weight)	Microsomal protein content (mg/g liver)	Cytochrome P450 (nmol/mg protein)	Cytochrome P420 (nmol/mg protein)	Aniline hydroxylase (nmol/min/mg protein)	NDMA demethylase (nmol/min/mg protein)
Suncus	Control (5)	59.5 ± 0.9	5.0 ± 0.1	41.7 ± 0.8	0.31 ± 0.02	0.15 ± 0.01	0.43 ± 0.04	0.119 ± 0.07
	Fasting (5)	54.5 ± 0.6†	4.0 ± 0.1§	36.6 ± 0.4‡	0.18 ± 0.01§	0.31 ± 0.02§	0.24 ± 0.03§	0.033 ± 0.02§
Rat	Control (4)	235 ± 5.4	4.9 ± 0.0	19.1 ± 0.5	1.02 ± 0.06	0.19 ± 0.01	0.76 ± 0.06	0.32 ± 0.05
	Fasting (4)	214 ± 2.3	3.7 ± 0.1§	22.7 ± 0.7‡	1.02 ± 0.06	0.22 ± 0.02	1.38 ± 0.05‡	1.10 ± 0.06§

Suncus and rats were treated as described in Materials and Methods.
* The control animals were given food and water *ad lib.* while the fasting animals were given only water *ad lib.* for 24 hr prior to being killed.
N, number of animals used.
All values represent the mean ± SE.
Significantly different from the control group: † $P < 0.05$, ‡ $P < 0.01$, § $P < 0.001$.

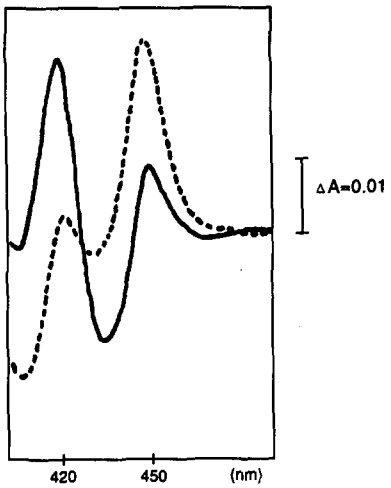

Fig. 1. Effect of fasting on reduced CO-difference spectra of liver microsomal fraction of a suncus (solid line, fasting group; dotted line, control group). Spectra were recorded in the presence of 0.2% Emulgen 911 and 20% glycerol. The protein concentrations were 0.6 mg/mL.

Table 2. Effect of fasting on the amount of P450IIE protein detected by Western blot analysis

	Control*	Starvation†
Suncus	100.0 ± 8.6	24.1 ± 2.7§
Rat	100.0 ± 9.6	172.3 ± 9.6‡

The intensity of bands was densitometrically determined.

* Relative intensity of P450IIE protein bands on Western blot analysis.

† The starved animals were given water *ad lib.* for 24 hr prior to being killed.

All values represent mean ± SE.

The numbers of suncus and rats are five and four, respectively.

Significantly different from the control; ‡ $P < 0.01$, § $P < 0.001$.

was observed; this did not occur in rats. Aniline hydroxylase and NDMA demethylase activities were decreased by fasting the suncus to about 45% and 28%, respectively, in contrast to rats, in which significant increases (up to 3.4-fold) were seen (Table 1).

Effects of fasting on expression of cytochrome P450IIE protein in hepatic microsomes of suncus and rats

Expression levels of P450IIE protein in hepatic microsomes were examined with immunoblot analysis. When anti-rat P450DM (P450IIE1) serum was applied to Western blot analysis, only one major protein was recognized to give a visible band on an immunoblot filter at a molecular mass of 52 kDa in the suncus (Fig. 2), probably indicating that a form of cytochrome P450 corresponding to rat P450IIE1 was present in suncus livers. The presence of P450IIE protein in suncus livers has been supported by our recent preliminary results: we have obtained a partial cDNA clone which showed a sequence highly similar to rat P450IIE1 (Nakura H, unpublished result). The P450IIE protein from microsomes of the suncus showed a slightly lower molecular mass than that of rats. P450IIE protein was decreased to 24% of the control level by fasting the suncus (Table 2). On the contrary, P450IIE1 protein was increased to 172% by fasting the rats (Table 2), in accordance with the reported results [14].

DISCUSSION

Cytochrome P450 proteins are considered to be induced under various conditions by multiple mechanisms. Among these, the effects of fasting have been studied by several investigators. It is also reported that fasting of rats results in an increase in P450IIE1 and P450PCN-E (P450IIIA) proteins while P450UT-A (P450IIC11) was reported to be reduced and P450UT-F and P450PB-C were unchanged [14, 15]. Aniline hydroxylase and NDMA demethylase activities, most of which are attributable to P450IIE isoform, were increased by fasting of rats (Table 1), in agreement with results obtained by Ma *et al.* [14]. In the present experiments, we found that both of these catalytic activities were decreased by fasting of suncus. The decrease in the activities of aniline hydroxylase and NDMA demethylase were associated with a decrease in the content of P450IIE protein.

Recently, Johansson *et al.* [16] reported that the P450IIE1 gene is mainly regulated at a post-transcriptional level in rats. Further, the rate of transcription of the *CYP*IIE1 gene in rats appears to be activated by factors present under specific physiological conditions such as fasting [15]. The results, shown in Table 2, strongly suggest that different mechanisms exist in fasting suncus and fasting rats for the regulation of the expression of P450IIE protein. Another explanation for the apparent discrepancy between rats and suncus is that the starvation of suncus specifically results in liver damage which may be responsible for the decreased content of all forms of cytochrome P450. However, this may not be the case, since the content of P450IIE protein decreased to an extent larger than total content of cytochrome P450.

Ketones, such as acetone and isopropanol, are potent inducers of hemoprotein, mainly of P450IIE1 (P450j) [17]. Ketone bodies are known to be generated by fasting without medication. Since ketosis has also been noted in fasting suncus (Saito, personal communication), the ketosis alone may be insufficient to account for the induction of P450IIE1 protein by fasting, as previously demonstrated [18]. The apparent discrepancy between the suncus and the rat in the effects of fasting on the content of P450IIE1 cannot be explained at present.

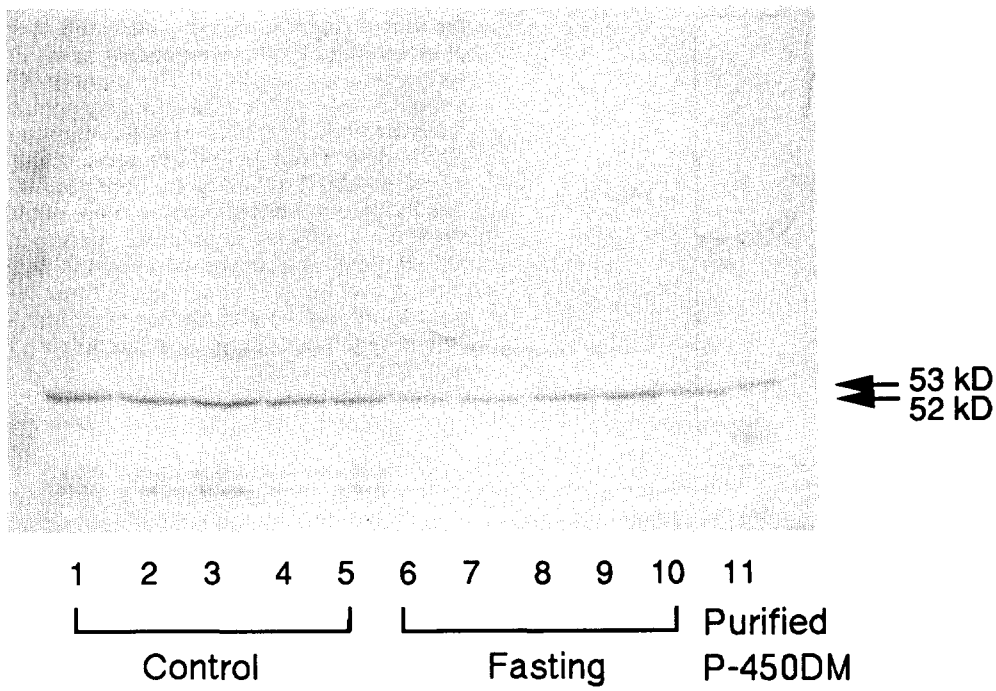

Fig. 2. Immunoblot analysis for the cytochrome P450IIE protein in hepatic microsomes from control (lanes 1–5) and fasting suncus (lanes 6–10). The amount of microsomal protein applied to the filter was 25 μg. Purified P450DM was loaded as a positive control (lane 11).

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