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The influence of sex steroid status on the depression of hepatic drug-metabolizing enzyme activity cytochrome P-450 content by carrageenan-induced inflammation in rats

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Abstract

The present study investigated the influence of sex steroid status on the carrageenan-induced depression of hepatic drug-metabolizing enzyme activity and cytochrome P-450 content. The presence of sex steroids seems to be a main factor in the sex-related depression by carrageenan-induced inflammation in rats.

Introduction

Pathological and abnormal physiological states in animals and man have been shown to alter hepatic drug-metabolizing activity[1]. Recently, we reported that the sex of the animal influenced the pattern of depression seen with carrageenan-induced inflammation[2~4]. Decreased hepatic drug-metabolizing enzyme activity (hexobarbital hydroxylase, aminopyrine N-demethylase, ethylmorphine N-demethylase and benzphetamine N-demethylase) and cytochrome P-450 content were observed in male Wistar rats after carrageenan administration, but hepatic drug-metabolizing enzyme activity and cytochrome P-450 content was not depressed in female Wistar rats after treatment with carrageenan. In a preliminary study, carrageenan-induced inflammation caused a sex-dependent depression of hexobarbital metabolism in rats[2]. The present study investigates the influence of sex steroid status on the carrageenan-depression of hepatic drug-metabolizing enzyme activity and cytochrome P-450 content.

Materials and Methods

Male and Female Wistar rats were divided into castrated and sham-operated groups. Surgery was performed under pentobarbital anesthesia. Animals were allowed 25 days to recover prior to the start of the treatment schedule. Sham-operated male or castrated female rats were given corn oil (Nakarai Chemical Co., Tokyo, Japan) alone (1 ml/kg, *s.c.*) or β-estradiol (Nakarai Chemical Co., Tokyo, Japan, 0.5 mg/kg, *s.c.*) in corn oil every other

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day for 28 days. Sham-operated female or castrated male rats were given corn oil (1 ml/kg, *s.c.*) alone or testosterone propionate (Sigma Chemical Co., Tokyo, 20 mg/kg, *s.c.*) in corn oil every other day for 24 days. Twenty four hr after the last administration of the corn oil or steroids, the sex steroid- and corn oil-treated groups were further divided with half of the animals receiving carrageenan and half saline. The hind paw edema was induced by carrageenan (PICNIN-A, Lot No. P-9) as previously described[2]. Hexobarbital hypnosis and drug metabolism were measured 24 hr after carrageenan injection. Each experimental group consisted of 6-8 animals.

The duration of hexobarbital (100 mg/kg, *i.p.*) hypnosis was measured as the time elapsing from the loss of the righting reflex until the animal could successfully right itself from the supine position twice within 30 sec.

Enzyme source and incubation conditions for assay of hexobarbital (10 mM hexobarbital), aniline (10 mM aniline) and aminopyrine (10 mM aminopyrine) have been described in previous reports[2,4]. Hexobarbital activity was determined by measuring the hexobarbital using the method of Brodie et al.[5]. Aniline hydroxylase or aminopyrine N-demethylase activity was determined by measuring formaldehyde formation by method of Nash[6], or by measuring p-aminophenol formation by the Imai and Aoto[7], respectively. The cytochrome P-450 content was assayed by the method of Omura and Sato[8]. Protein content was determined by the method of Bradford[9]. Statistical comparisons were made between carrageenan-treated animals and their corresponding saline controls utilizing Student's t-test with significance at $P<0.05$.

Results and Discussion

The data in figure 1 depict the effect of carrageenan on the hypnotic response induced by hexobarbital and the hepatic biotransformation of hexobarbital in the native and castrated male rats. Native or castrated male rats received carrageenan 25 days after the surgery and the next day duration of hexobarbital-induced hypnosis and *in vitro* hepatic metabolism of hexobarbital and aniline were measured. Hexobarbital, aniline and aminopyrine were selected as substrate since hexobarbital and aminopyrine are classified as type I substrate and this biotransformation has been reported to be sex-dependent in rats, while the metabolism of aniline, a type II substrate, has been reported to be sex-independent[10]. The hexobarbital hypnosis is one of the simplest and most widely used *in vivo* tests of cytochrome P-450-mediated drug metabolism. Duration of hexobarbital hypnosis was significantly potentiated by carrageenan-induced inflammation in both native and castrated male rats as compared to their respective control. However, the duration of hexobarbital hypnosis in native rats strongly increased than that in castrataed male rats. Namely, the carrageenan-treated, native male rats slept 480% longer, whereas the carrageenan-treated, castrated male rats slept only 60% than longer that of the appropriate control animals (Fig. 1).

Similarly, hepatic hexobarbital oxidation was significantly reduced by 45% in native male animals following carrageenan, compared to a reduction of only 30% in castrated male rats. These results suggest that hepatic drug-biotransformation is less susceptible to carrageenan-induced inhibition in castrated male rats and that part of the inhibitory effects of carrageenan is mediated through those components of the hepatic monooxygenase system

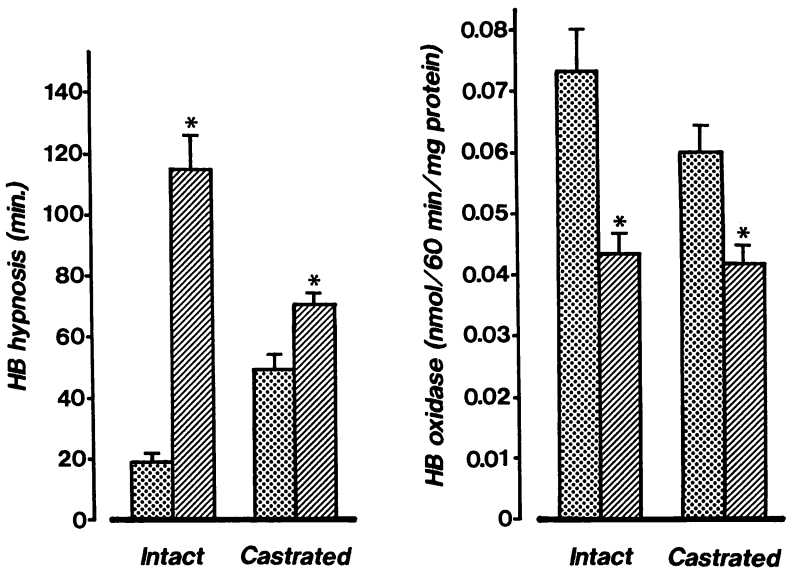

Fig. 1 Effect of carrageenan-induced inflammation on hexobarbital hypnosis and hexobarbital oxidase in castrated male rats

Intact or castrated male rats received either saline (dotted column) or carrageenan (shaded column) following surgery. Hexobarbital hypnosis (left panel) and hexobarbital oxidase (right panel) were measured 24 hr later. Each histogram represents the mean±S.E. for 6-8 animals. Data were analyzed by Student's t-test : *, $P<0.05$ with respect to respective control value. Abbreviation : HB, hexobarbital.

regulated by androgen.

In native and castrated male animals, carrgeenan-induced inflammation did not produce a statistically significant inhibition in aniline biotransformation (data not shown).

To examine further the role of testosterone in carrageenan-induced inhibition of drug metabolism, an experiment was performed in which testosterone was administered exogenously. Following the administration of carrageenan to sham-operated male rats, aminopyrine metabolism and cytochrome P-450 content were significantly reduced in corn oil-treated male rats, but not in estradiol-treated male rats (Fig. 2). In castrated male rats receiving corn-oil, carrageenan treatment did not reduce aminopyrine metabolism and cytochrome P-450 content. However, these parameters were significantly inhibited by carrageenan treatment in the castrated, testosterone-treated male rats.

Since carrageenan-iduced inflammation has been shown to decrease hepatic metabolism of type I substrates in male, but not female rats[2~4], it was of interest to determine the effect of estrogen treatment in male rats on the inhibitory effect of carrageenan on hepatic drug metabolism. Carrageenan treatment did not produce a significant decrease in the aminopyrine metabolism and cytochrome P-450 content in sham-operated or castrated female rats. In sham-operated female rats treated with testosterone, carrageenan administration significaltly reduce the ethylmorphine metabolism and cytochrome P-450 content. In estrogen-treated, castrated female rats, carrageenan was without effect on aminopyrine

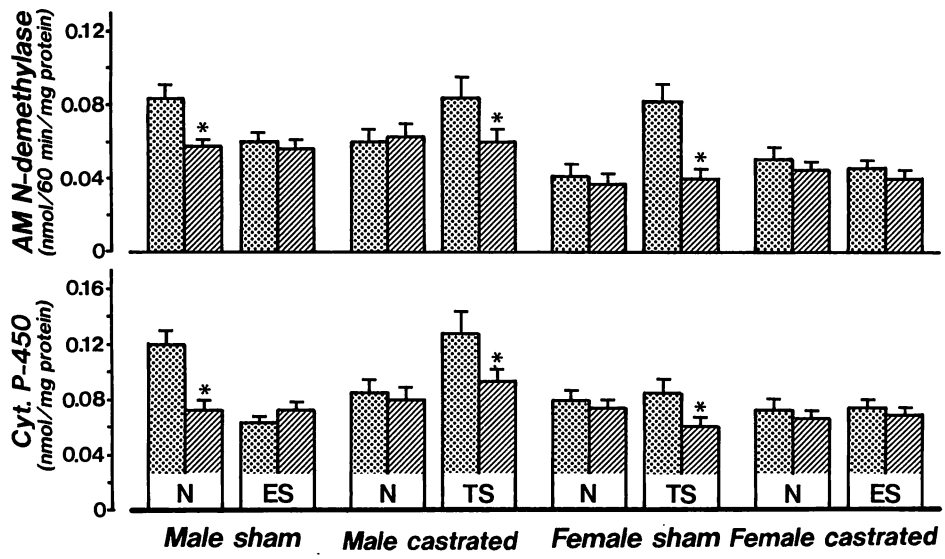

Fig. 2 Effect of steroids on the modulation of hepatic aminopyrine N-demethylase and cytochrome P-450 by carrageenan-induced inflammation in rats

Sham-operated or castrated rats pretreated with corn oil (N), 17β-estradiol (ES), or testosterone propionate (TS), then received either saline (dotted column) or carrageenan (shaded column). They were killed and the aminopyrine N-demethylase activity and cytochrome P-450 content were measured, as described in Materials and Methods. Each histogram represents the mean±S.E. for 6 animals. Asterisks indicate significance at $P<0.05$ with respect to respective control value. Abbreviations : AM N-demethylase ; aminopyrine N-demethylase, Cyt. P-450 ; Cytochrome P-450.

metabolism and cytochrome P-450 content. Thus, estrogen treatment may provide some protection against the carrageenan-induced inhibition of hepatic drug metabolism in sham-operated male rats. Therefore, these data suggest that the presence of testosterone may be a factor in determining the extent of carrageenan-induced inhibition of drug metabolism in the rats.

Administration of androgenic steroids to female rats has been shown to enhance the ability of liver to metabolize many substrates[11]. Therefore, female rats were treated with testosterone. Testosterone treatment elevated aminopyrine N-demethylase activity. In testosterone-treated female rats, carrageenan-induced inflammation depressed the testosterone-elevated rate of aminopyrine metabolism and cytochrome P-450 content, thus suggesting that part of the carrageenan-inhibitory effect is, indeed, manifested through the androgen-dependent components of the hepatic microsomal drug-metabolizing system.

The mechanism of the sex-dependent effect of carrageenan-induced inflammation on depression of hepatic drug-metabolism is unclear. One possibility may involve sex steroidal control over different isozymes of cytochrome P-450 as described by Kato and Kamataki[12]. They isolated P-450 (M), found exclusively in the male and which metabolizes ethylmorphine, and P-450 (F), found exclusively in the female but poorly metabolizes aminopyrine. Estradiol decreased the content of P-450 (M) in male rats. That carrageenan-induced

inflammation may decrease a similar isozyme which is induced by testosterone could explain our results on aminopyrine N-demethylase results. In this case, estradiol would have be sufficient to overcome the effects of endogenous testosterone in male sham-operate rats and depress the formation of P-450 (M). That estradiol enabled carrageenan-induced inflammation to depress aminopyrine N-demethylase activity in these rats supports this contention. Kato and Kamataki[12] also demonstrated that P-450 (M) and P-450 (F) metabolize hexobarbital to a similar extent. These authors have suggested that other isozymes of P-450 may be decreased by estradiol which metabolize hexobarbital. It could be speculated thet carrageenan-induced inflammation and estradiol decrease similar isozymes of cytochrome P-450 resulting in decreased ethylmorphine activity. Thus, carrageenan-induced inflammation and estradiol may depress a similar factor necessary to initiate the depression of these isozymes. Their actions would be additive unless either had produced maximal depression. This could explain why no further depression was seen after carrageenan administration when estradiol was present such as in female sham-operated animals treated with corn oil and female castrated rats given estradiol as discussed previously. The more consistent decreases in cytochrome P-450 levels in males following carrageenan administration parallels their greater metabolic capability and may reflect differential depression of various isozymes of cytochrome P-450.

Since it is known that rat liver contains a variety of cytochrome P-450 forms[13], it is interesting to speculate that carrageenan-induced inflammation may exert a selective activity toward certain specific forms of these hemoproteins ; e.g., whose activities are androgen-dependent. Such speculation requires further studies to determine the effect of carrageenan-induced inflammmation on each of the forms of cytochrome P-450.

In summary, the presence of sex steroides seems to be a main factor in the differential depression by carrageenan-induced inflammation of the content of cytochrome P-450 and the activities of ethylmorphine N-demethylase and hexobarbital hydroxylase in the rats. The testosterone in some way direct the development of the sex difference since, in its presence, carrageenan-induced inflammation is able to depress the content of cytochrome P-450 and activities of hexobarbital oxidase and aminopyrine N-demethylase, while in its absence, the sex difference dose not manifest itself.

Acknowledgement

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