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Dual Regulation of 21-Hydroxylase Activity by Sex Steroid Hormones in Rat Hepatocytes

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In the rat liver, cytochrome *P*450 catalyzes the hydroxylation of steroid hormones. The expression and activity of some *P*450 isozymes are regulated by sex steroid hormones. Steroid 21-hydroxylase activity in rat liver is provided mainly by CYP2C6. We studied the regulation of 21-hydroxylase activity by sex steroid hormones in rat primary hepatocyte culture. We added estrogens (estrone, estradiol, estriol) and androgens (testosterone, dihydrotestosterone), ranging from 10^{-9} to 10^{-5} M) to the culture. The 21-hydroxlyase activity was stimulated by estrogens and was suppressed slightly by androgen in a dose-related manner. The results of our studies demonstrated that sex steroid hormones act differently on 21-hydroxylase activity in rat hepatocytes and, thus, support the hypothesis that the extra-adrenal production of deoxycorticosterone from circulating progesterone is increased during pregnancy by the massive presence of estrogens.

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INTRODUCTION

Steroid hormones have been shown to be substrates for the hepatic *P*450 isozymes. Progesterone is catalyzed by several rat hepatic cytochrome *P*450 isozymes to 2α, 6β, 15α, 16α, 21-hydroxylated forms [1]. In the rat, liver microsomal 21-hydroxylation is catalayzed largely by a cytochrome *P*450 designated CYP2C6 and as a result, deoxycorticosterone (DOC) is produced in the liver. Steroid hormones also play an important role in regulating the expression of several constitutive hepatic *P*450s, by mechanisms that are only partially understood at the present time. In clinical observations, estrogen treatment resulted in increased plasma levels of DOC in pregnant women [2]. Our previous study demonstrated that 17β-estradiol stimulated rat liver 21-hydroxylase activity [3], however, there are few reports on how androgens affect these enzyme activities. The constitutive CYP2C6 is expressed at comparable levels in both sexes and is not significantly affected by hormonal factors [4]. Hornsby demonstrated that in bovine adrenocortical cells, C19 steroids decreased 21-hydroxylase activity by inactivation of the enzyme through oxygen-derived radicals that are generated as C19 steroids bound to the enzyme [5], but that report did not address the question of whether androgen acts directly on the liver.

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Primary cultures of rat hepatocytes are well established as simple *in vitro* systems in which the cells maintain their functions without being influenced by other endo-organs. The aim of this study was to investigate the *in vitro* effects of androgens and estrogens on 21-hydroxylation activity in cultured rat hepatocytes.

MATERIALS AND METHODS

Sprague–Dawley male rats (6–8 weeks old) were obtained from Nippon SLC (Shizuoka, Japan). Cell culture supplies were obtained from Sumitomo Bakelite (Tokyo, Japan), fetal calf serum, penicillin G and streptomycin were purchased from GIBCO Laboratories (Grand Island, NY, U.S.A.), Hanks Balanced Salt Solution (HBSS) from Nissui Pharmaceutical Co. (Tokyo, Japan), William's medium E from Flow Laboratories (Irvine, Scotland), and Collagenase S-1 from Nitta Gelatin (Osaka, Japan). Steroids were removed from fetal calf serum by treatment with Dextran coated charcoal. Estrone (E_1), 17β-estradiol (E_2), estriol (E_3), dexamethasone, testosterone and dihydrotestosterone were obtained from Sigma Chemical Co. (St Louis, MO).

Culture experiments

Hepatocytes were isolated by collagenase perfusion of livers from 6–8-week-old male Sprague–Dawley rats, as described previously [3]. The cells were

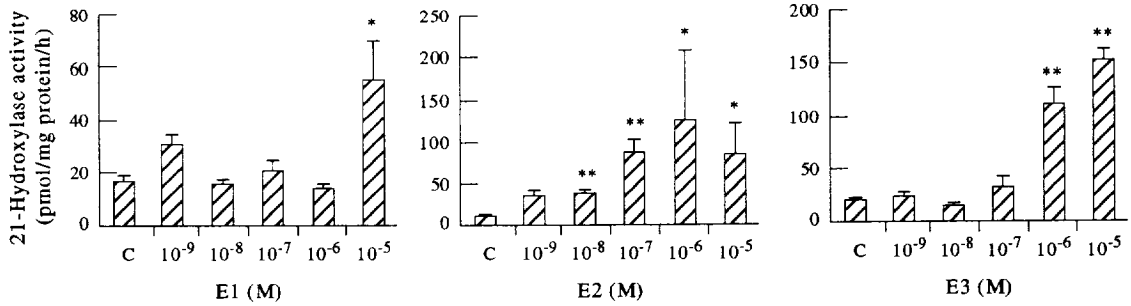

Fig. 1. Dose-dependency of the responses to estrogen of 21-hydroxylase activity in primary culture of rat hepatocyte. Four wells of cultured cells were analyzed at each estrogen concentration. Vertical lines indicate ±SEM. *$P < 0.05$; **$P < 0.01$ vs control.

suspended in William's E medium with 10% (v/v) fetal calf serum, penicillin G (100 IU/ml), streptomycin (100 μg/ml), amphotericin B (0.25 μg/ml) and dexamethasone (1×10^{-9} M) and insulin (1×10^{-9} M), and cultured at a cellular density of 2×10^5 cells/0.5 ml in 24-multiwell culture plates. All cultures were incubated at 37°C with 95% air–5% CO_2. 4 h after plating, the medium was removed, washed with HBSS, replaced with serum free medium containing the test steroids and then changed every 24 h thereafter. After 48 h incubation, the medium containing the test steroids was removed, and the cells washed with HBSS for assay of the steroid 21-hydroxylase activity.

Cellular 21-hydroxylase activity was determined in quadruplicate by supplementing the culture medium with 50 μM progesterone. After 2 h, DOC levels were assayed in the medium by EIA with HPLC separation as described previously [3].

Data analysis

The statistical significance of the difference between sample means was calculated according to the unpaired Student's t-test.

RESULTS

To examine the estrogen effect on 21-hydroxylase activity, rat hepatocytes were maintained in the presence of E_1, E_2 and E_3 at various concentrations (10^{-9}–10^{-5} M) for 48 h. As shown in Fig. 1, at each estrogen concentration a significant increase in the activity of 21-hydroxylase occurred, thus showing that the activity is stimulated in a dose-dependent fashion.

To examine the androgen effects on 21-hydroxylase activity in rat hepatocytes, the animals were treated with testosterone or dihydrotestosterone (DHT) at various concentrations (10^{-9}–10^{-5} M) for 48 h. In contrast to the effects of estrogen, the addition of testosterone or DHT caused a significant decrease in 21-hydroxylase activity (Fig. 2).

DISCUSSION

Few studies have addressed the effects of sex steroid hormones on 21-hydroxylase activity of rat liver microsomes. Waxman demonstrated that the content of CYP2C6, which is responsible for much of the 21-hydroxylation in microsomes from rat liver, is not affected by sex or age [6]. The results presented in this study show that 21-hydroxylase activity in rat hepatocytes is stimulated by estrogen and inhibited by androgen.

Estradiol is known to have a dose-dependent effect on cell growth. The present experiments provide evidence that the action of estrogen on 21-hydroxylase activity is not the consequence of increased cell growth since cell number and non-collagen proteins from

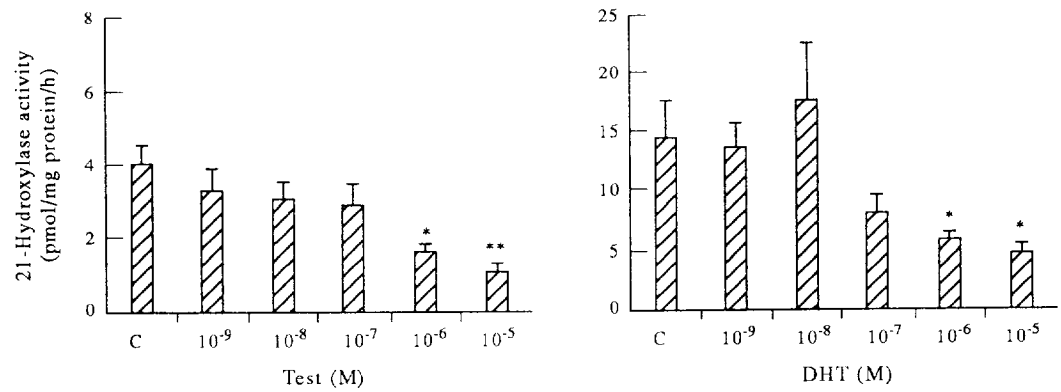

Fig. 2. Dose-dependency of the responses to androgen of 21-hydroxylase activity in primary culture of rat hepatocytes. Four wells of cultured cells were analyzed at each androgen concentration. Vertical lines indicate ±SEM. *$P < 0.05$; **$P < 0.01$ vs control.

hepatocyte were barely affected by this treatment (data not shown). Several pathways have been demonstrated to participate in estrogen effects, including the direct regulation of gene expression through the estrogen–estrogen receptor complex [7]. Another pathway is an indirect mechanism, whereby estrogen affects the receptor of cell growth factors, e.g. growth hormone, which in turn potentiates the regulatory effect on hepatic *P*450s [8]. However, this mechanism is not important in our experiment, as the hepatocytes were maintained under a serum-free condition.

It has been shown that C19 steroids, i.e. androgens, decrease steroidogenic enzyme activities in cultured cells [9]. These findings are consistent with the hypothesis that C19 steroids bind to the steroidogenic enzyme, and that this complex enhances the generation of oxygen-derived radicals that inactivate the enzyme. To support this concept, several investigators demonstrated that the mechanism of action of C19 steroids did not involve gene expression through the androgen–androgen receptor complex, but involved the destruction of the enzyme protein by C19 steroids [5, 10]. This was observed not only with 21-hydroxylase in bovine adrenal cells but also with 11β-hydroxylase in bovine adrenal cells and 17α-hydroxylase in Leydig cells [10]. Although this suppression mechanism is not dependent on androgenic activity, it is known which combinations of side-chain moiety (functional group) and steroid nucleus it requires. Further experiments with hepatocytes maintained under different oxygen concentrations, are required to clarify this mechanism. It is also possible that the enzyme–inhibitor complex converts to products that inactivate the enzyme. In microsomal protein preparations from rat and rabbit liver, 17β-substituted steroid analogs reduced the binding of C21-steroids to the enzyme at the active site [11].

Liver cytochrome *P*450s have been demonstrated to be classified into three categories based on the sex specificity and responsiveness to androgens: neonatally imprinted by exposure to androgen, reversibly inducible by androgen, and regulated primarily by other factors [12]. From the results of the present study, 21-hydroxylase in rat liver is a member of the third group. Interestingly, estrogen stimulation of 21-hydroxylase activity in the liver leads to a reduction of circulating progesterone during pregnancy.

In conclusion we have demonstrated that estrogens increase and androgens decrease 21-hydroxylase activity in rat hepatocyte primary cultures. This model should help to elucidate the precise mechanism of hormonal regulation of 21-hydroxylase activity in the liver.

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