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Role of Prolactin in the Regulation of Cytosolic NADP Isocitrate Dehydrogenase in the Liver of the Male Rat

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ABSTRACT

The activity of cytosolic NADP-linked isocitrate dehydrogenase (ICDH) in rat liver was determined. The administration of 2-bromo-α-ergocryptine (CB-154) to male rats produced a significant increase of the enzyme activity and a decrease of serum prolactin (PRL) levels in relation to control animals. Male rats 21 days after castration had lower levels of serum prolactin and higher activity of the enzyme than controls. Injection of PRL to castrated male rats lowered the enzymatic activity to control values. In intact rats injected with prolactin, the activity of the enzyme also decreased. Female rats were separated into the following groups: (a) virgins; (b) rats on day 15 of lactation; (c) ovariectomized rats. The enzymatic activity was similar in the different groups, but significantly higher than in male rats. However, serum PRL was significantly increased in 15 days lactating rats and decreased in ovariectomized ones in relation to virgins. We conclude that PRL regulates hepatic ICDH activity in male, but not in female rats. Incubation of isolated hepatocytes from intact or castrated male rats maintained the difference in ICDH activity observed in vivo, while there were no differences in ICDH

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activity in non-parenchymal cells. Addition of PRL, CB-154, androgens or antiandrogens to isolated hepatocytes from intact and castrated rat, had no effect on the ICDH activity, suggesting that the effect of PRL is exerted at the transcriptional level.

Key Words: Prolactin; Isocitrate dehydrogenase; Liver; Male rat.

INTRODUCTION

The particular role in lipid metabolism of the hepatic cytosolic NADP-isocitrate dehydrogenase (ICDH, EC 1.1.1.4.2), which is the predominant isoform of this enzyme, remains obscure, but it has been shown that the activity and expression of this enzyme is subjected to hormonal regulation. In a teleost fish, Anabas testudineus, it has been reported that ovine prolactin (PRL) significantly inhibited liver malic enzyme, glucose 6-phosphate dehydrogenase and ICDH activities in vivo and in vitro, and reversed the action of bromocryptine on these enzymatic activities. Hence, PRL seems to have an inhibitory effect on lipid metabolism in this teleost (1). It has also been shown that estradiol (2) and thyroid hormone (3–4) regulate the activity of this enzyme, most probably mediated through changes in the cellular requirements of NADPH. Previously we have reported that surgical and pharmacological castration in rats produced an increase in the hepatic cytosolic NADP-ICDH activity, which is reverted to values observed in non castrated rats after injection of testosterone (5–6). It is known that in castrated rats serum testosterone and PRL concentrations decrease (7–9), and testosterone replacement normalizes PRL levels. Thus, the effects of castration on ICDH activity may be indirect, through changes in PRL secretion. Other results obtained in our laboratory suggest that maternal exposure to agrochemicals, such as glyphosate, during pregnancy induces a variety of functional abnormalities in the specific activity of the dehydrogenases in liver, heart, and brain of the pregnant rats and their fetuses (10). The aim of this report was to determine if the high activity of ICDH in the liver of castrated rats is a consequence of the low concentration of PRL in serum and whether PRL regulates ICDH activity in vivo and in vitro, and if the ICDH activity is regulated in the same way in animals of both sexes. For this purpose, we measured the effects on ICDH activity of PRL or bromocryptine, an inhibitor of PRL secretion (11, 12), given to intact or castrated male rats or to primary cultures of isolated hepatocytes. We also measured ICDH activity in virgin, lactating or castrated female rats, which have widely different levels of circulating PRL (13).

MATERIALS AND METHODS

Adult male rats (270–290 g body weight) maintained in standard conditions were used. Eight groups of eight rats each were used.

Group I—Control: Sham-operated rats.

Group II—Control + PRL: seven days before sacrifice, control rats (sham-operated) were injected subcutaneously with PRL 100 μg/kg body weight, in saline solution, twice a day (08:00 am and 08:00 pm).

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Group III—Control + Bromocryptine: 3 mg/kg body weight in 0.1 mL of 30% ethanol in saline solution, subcutaneously, during 7 days.

Group IV—Pharmacological castration: this was done by subcutaneous injection of flutamide 20 mg/kg body weight, dissolved in 5% ethanol using gelatin as vehicle.

Group V—Pharmacological castration + PRL: intact rats were injected with flutamide and prolactin using doses and time of administration as mentioned above.

Group VI—Pharmacological castration + bromocryptine: intact rats injected with flutamide and bromocryptine, using doses and time of administration as mentioned before.

Group VII—Castrated rats: the castration was performed by orchidectomy through a small scrotal incision, under light ether anesthesia; the animals were sacrificed 21 days later.

Group VIII—Castrated + PRL: castrated rats were injected with PRL the last seven days before sacrifice by the same method as for Group II.

Adult female rats were maintained in the same conditions as male rats. Four groups of eight rats each were used. Group I—Control virgin rats. Group II—Ovariectomized virgin rats. Rats were ovariectomized through two bilateral incisions under ether anesthesia; they were used at least 2 weeks after ovariectomy. Group III—Lactating rats: rats in the 15th day post partum, nursing a litter of eight pups. Group IV—Weaned rats: lactating rats were separated from their litters on the 7th day postpartum and sacrificed on the 15th day.

The rats were sacrificed by decapitation under ether anesthesia. The liver was excised, weighed and homogenized with 0.5 M Tris–HCl buffer, pH 7.4 containing 1 mM dithiothreitol. The liver homogenate was centrifuged at 100,000 × *g* for 1 h in a Beckman LS-65B ultracentrifuge, the supernatant was used for enzyme and protein determinations. Isocitrate dehydrogenase activity was determined by following the rate of NADPH formation at 340 nm in a Shimadzu UV 190 spectrophotometer. The determinations were made by duplicate in a final volume of 1.35 mL containing 0.7 mL 0.1 M Tris–HCl buffer pH 7.4, 0.2 mL 10 mM $MgSO_4$, 0.2 mL 0.2 mM NADP, 0.05 mL enzyme preparation and 0.2 mL 1 mM DL-isocitrate (14). The results were expressed as μmol NADP/min/mg of protein. Proteins were determined by the Biuret reaction (15).

Primary culture of hepatocytes: all reagents used were of analytical grade.

Intact or castrated Wistar male rats weighting 180–250 g were used. The animals were kept on a 12 h light: 12 h dark, in an air-conditioned room (22° to 23°) and received laboratory diet and water *ad libitum*. Isolation of hepatocytes was performed according to Wang et al. (16). Briefly, the perfusion medium consisted of a Hanks solution with 2 mM EDTA, equilibrated with $O_2 : CO_2$, 95 : 5, v/v, pH 7.4, as disaggregation agent. Livers were perfused through cannulation of the portal vein. Non-parenchymal cells were obtained from the supernatant by passing them through a gradient of Percoll. The final pellet, consisting mainly of hepatocytes, was suspended and used for the experiments. Microscopic examination of the incubated suspension showed cell debris and isolated cells, of which 90–95% did not stain with trypan blue. After cell dissociation, hepatocytes were washed in a modified Hanks solution, salt-free with 0.4 mM $MgSO_4 \cdot 7H_2O$, 1.85 mM $CaCl_2$ (anh, pa), 0.5 mM $MgCl_2 \cdot 6H_2O$ and albumin 1%, but no EDTA. The isolated hepatocytes and non-parenchymal cells were counted and plated

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to a density of a million cells per mL and incubated for 3 hours, in Minimum Essential Medium (Gibco) supplemented with 5% (v/v) fetal bovine serum, 2 mM glutamine, 100 μg/mL streptomycin, 100 U/mL penicillin G, and 50 μg/mL gentamycin. Cells from control rats were incubated with 10^{-7} M of flutamide, ciproterone acetate and bromocryptine. The cells of castrated rats were incubated with 10^{-7} M of testosterone, dihydrotestosterone, PRL, and cycloheximide. For ICDH determination, cells (500,000) were homogenized in 0.5 M Tris–HCl buffer, pH 7.4 containing 1 mM dithiothreitol, and processed as described above for whole livers. Serum PRL was measured by double antibody radioimmunoassay using materials generously provided by the NHPP (National Hormone and Pituitary Program, NIADDK, USA). The hormone was radio-iodinated using the Chloramine T method and purified by passage through Sephadex G75. The results were expressed in terms of the rat prolactin RP-3 standard preparation. Assay sensitivity was 0.5 μg L^{-1} serum and the inter- and intra-assay coefficients of variation was less than 10%. Testosterone in serum was measured by RIA using a commercial kit (DPC Coat-a-count testosterone, coated tube RIA from Diagnostic Products Corporation, Los Angeles, CA).

Statistical Analysis

Significant differences among means were considered at a level of $p < 0.05$ and identified by one-way ANOVA, Kolmogorov-Smirnov and Newman-Keul procedures. In all the cases, the variances were homogeneous.

RESULTS

In Vivo Experiments

Table 1 shows that surgical as well as chemical (flutamide) castration induced increases in ICDH activities accompanied with decreases in circulating testosterone and

Table 1. Effect of different levels of PRL on ICDH activity in intact and castrated male rat liver.

Group	Treatment	Enzyme activity (μmol NADP/min/ mg protein)	Serum PRL (ng/mL)	Serum testosterone (ng/mL)
Intact	Vehicle	27.24 ± 1.1	11.3 ± 3.7	5.98 ± 1.20
Intact	PRL	19.39 ± 1.4*	—	—
Intact	CB-154	54.35 ± 2.8**	1.5 ± 0.1*	3.28 ± 0.73
Intact	Flutamide	40.33 ± 1.5**	3.3 ± 0.1*	2.85 ± 0.48
Intact	Flutamide + PRL	25.73 ± 3.4	—	—
Intact	Flutamide + CB-154	41.13 ± 2.1**	—	—
Castrated	Vehicle	52.66 ± 5.8**	4.3 ± 1.7	2.25 ± 0.91
Castrated	PRL	32.15 ± 3.6[a]	—	—

Note: Values represent the mean ± SD of groups of eight rats. *$p < 0.05$; **$p < 0.01$ compared with intact controls treated with vehicle.
[a]$p < 0.05$ compared with castrated treated with vehicle.

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PRL. Treatment with bromocryptine (CB-154), which also reduced circulating PRL, increased ICDH activity, while PRL treatment reduced ICDH activity in intact and in castrated male rats. These results indicate that PRL seems to regulate ICDH negatively in male rat liver.

Female rats had higher liver ICDH activity compared with male rats, and the values did not vary regardless of the physiological state of the rat, or the circulating PRL levels (Table 2).

In Vitro Experiments

As in the in vivo experiments, ICDH activity in hepatocytes isolated from castrated rats was significantly higher than that of hepatocytes isolated from intact rats (Table 3), while there were no differences in the enzymatic activities measured in the non-parenchymal cell fraction (Table 3).

In contrast with the effects observed in vivo, short-term incubations of hepatocytes from intact rats with bromocryptine or flutamide, did not modify the activity of ICDH (Fig. 1). Incubation of hepatocytes from castrated rats with androgens such as testosterone, DHT or with prolactin also had no effect on the elevated levels of ICDH activity (Fig. 2).

DISCUSSION

The present results demonstrate sexual dimorphism in the endocrine regulation of hepatic ICDH activity. They confirm that ICDH activity in the liver of male rats is regulated by the endocrine status of the animal, and that PRL, rather than or along with gonadal steroids, mediates the effect of castration on enzyme activity. Thus, the effect of gonadectomy on ICDH levels may be a consequence of the reduction of circulating levels of PRL observed in the castrated rats. Thus, treatment with bromocryptine, which blocks pituitary PRL release, mimicked the effect of gonadectomy. Furthermore, exogenous PRL decreased enzyme activity, regardless of whether the rats were intact or surgically or

Table 2. Effect of different levels of PRL on ICDH activity in female rat liver.

Group	Enzyme activity (μmol NADP/min/ mg protein)	Serum PRL (ng/mL)	Serum testosterone (ng/mL)
Intact virgins	75.29 ± 2.6	5.1 ± 1.5	0.24 ± 0.20
Ovariectomized	75.75 ± 4.4	1.3 ± 0.1	0.02 ± 0.02
Day 7 of lactation	67.29 ± 4.9	93.3 ± 24.4*	—
7 Days lactation + 8 days weaned	72.61 ± 6.8	9.8 ± 3.3	—

Note: Values represent the mean ± SD of groups of eight rats. *$p < 0.05$ compared with intact virgin rat.

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Table 3. Determination of ICDH activity in isolated hepatocytes and non-parenchymal cells from intact or castrated rats.

	Enzymatic activity (μmol NADP/10^6 cells)	
	Intact	Castrated
Hepatocytes	418.3 ± 198.7	1307.2 ± 256.3*
Non-parenchymal cells	90.26 ± 44.9	191.5 ± 95.7

Note: Values represent the mean ± SD of three incubations performed in triplicate. *$p < 0.001$ compared with incubations from intact rats.

chemically castrated. It has been shown (17), that bromocryptine does not affect circulating testosterone levels. Our results (Table 1) confirm this, since there was no significant reduction in serum testosterone in the bromocryptine-treated rats. Although hypoprolactinemia is associated with a marked reduction in testicular LH receptors, it does not affect testosterone production. On the other hand, hyperprolactinemia causes a short-term increase in plasma testosterone, which decreases with time (18). Prolonged hyperprolactinemia produces involution of the gonads accompanied with decreased response to LH and diminished testosterone secretion that leads to infertility (19). Conversely, PRL secretion patterns in male rats seem to be strongly dependent upon circulating testosterone levels during development and in adults (20,21).

Thus, although the effects of PRL administration could be mediated through changes in circulating testosterone, the effect of bromocryptine was not mediated by changes in androgen levels. This strengthens the idea that PRL has a direct effect on ICDH expression or activity, and, furthermore, that the effects of castration or testosterone replacement may be enacted through the changes they provoke in circulating PRL.

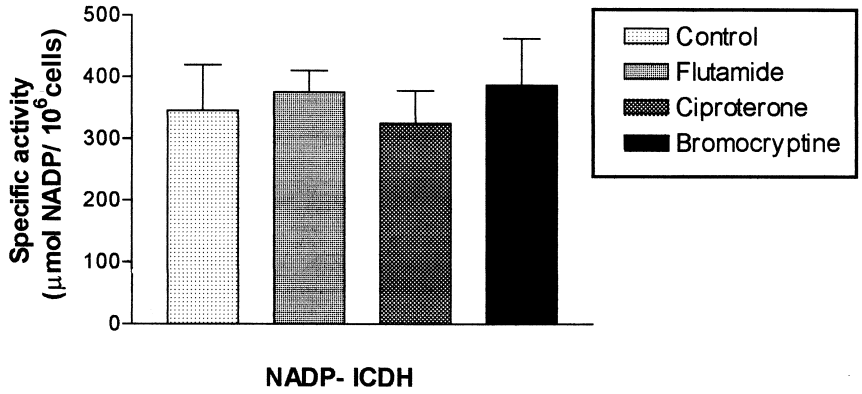

Figure 1. Variation of ICDH activity in cultured hepatocytes of control rats. Effect of flutamide, cyproterone and bromocryptine. Isolated hepatocytes (500,000 cells per well) were incubated for 3 h in modified Hanks medium with no additions or in the presence of 10^{-7} M flutamide, cyproterone or bromocryptine. The cells were homogenized and ICDH activity measured. Results are the mean ± SD of triplicate incubations. For details see Materials and Methods section.

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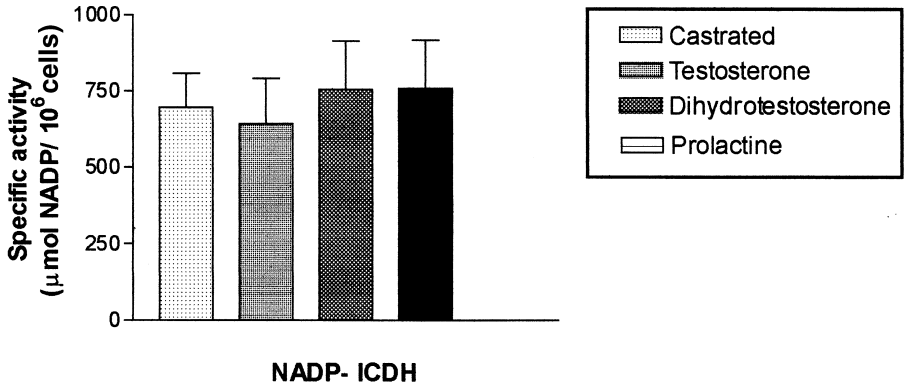

Figure 2. Variation of ICDH activity in cultured hepatocytes of castrated rats. Effect of testosterone, dihydrotestosterone or prolactin. Isolated hepatocytes (500,000 cells per well) were incubated for 3 h in modified Hanks medium with no additions or in the presence of 10^{-7} M testosterone, dihydrotestosterone or prolactin. The cells were homogenized and ICDH activity measured. Results are the mean ± SD of triplicate incubations. For details see Materials and Methods section.

There are also described instances where PRL synergizes with testosterone in the regulation of enzymatic activities, such as acid phosphatases in prostate and seminal vesicles (22).

In contrast, PRL does not seem to have a regulatory role on hepatic ICDH in female rats. Females showed elevated activity compared to male rats, and these values were not modified in the different reproductive states that are associated with different serum PRL concentrations. Thus, the variations in circulating PRL caused by lactation, weaning or ovariectomy did not influence ICDH activity.

There are several reported instances of hepatic sexual dimorphism, such as in PRL and GH receptor levels and estrogens along with GH are mainly responsible for these differences (23–27). In the case of liver PRL receptors, the pattern of GH secretion, which is regulated by estrogens, is a determinant factor in the expression of PRL receptors (27). Thus, estrogen administration "feminizes" male liver in terms of PRL and GH receptor expression, while ovariectomy in females takes the receptor levels and the pattern of GH secretion to values typical of male rats (25,28,29). Thus, actual or previous estrogen impregnation may have permanently altered ICDH expression in female rats, and may have produced a desensitization of the liver to PRL in this particular response in spite of the elevated hepatic PRL receptors.

Prolactin is also able to stimulate several hepatic enzymes, such as ornithine decarboxylase (ODC), thymidine kinase and protein kinase C (PKC), acting directly on the liver cells (30). The effect on ODC seems to be due to a direct effect of PRL on the activity of enzyme rather than from its induction by hormone (31). Furthermore, the subsequent rapid induction of the growth-related gene expression suggests a role for PRL as a hepatic mitogen (12). Nevertheless, PRL seems to be generally stimulatory in these instances, and is effective in livers from both sexes, indicating a different response in this instance.

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Isolation and short-term incubation of hepatocytes obtained from intact or castrated male rats did not alter the differences in ICDH activity observed in vivo in these two situations, thus, castration increased the enzymatic activity. On the other hand, the addition of bromocryptine, PRL or androgenic or antiandrogenic agents to these incubations had no effect on enzymatic activities, suggesting that the effects observed in vivo may be due to changes in the expression of the enzyme, rather than on activation or inactivation of preexisting enzyme protein. Furthermore, the effect of castration seems to be exclusive of hepatocytes, since there was no effect of gonadectomy on the enzyme activity of isolated non-parenchymal cells. In conclusion, our results show a sexually dimorphic regulation by prolactin of hepatic ICDH activity that seems to be exerted at the expression level and is observed in male rats.

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