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EFFECTS OF GROWTH HORMONE ON CYTOCHROME P-450$_j$

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Summary: Hypophysectomy of male and female rats resulted in a 5 and 10-fold increase respectively, in cytochrome P-450$_j$ as determined by immunoblotting. Treatment of hypophysectomized rats with growth hormone resulted in a decrease in P-450$_j$. Microsomal N-nitrosodimethylamine demethylase activity was increased approximately 23 and 75 per cent in male and female rats respectively, over the activity in microsomes of intact animals. These studies suggest that hypophysectomy results in an increase in cytochrome P-450$_j$ and that growth hormone acts as a repressive factor for constitutive P-450$_j$.

Cytochrome P-450 refers to a family of hemoproteins which function as the terminal oxidase in the metabolism of various drugs, steroids and xenobiotics(1-3). These cytochromes are selectively inducible by many different inducing agents. More than 10 isozymes of cytochrome P-450 have been isolated from rat livers and can be grouped into several families based on their spectral and catalytic properties, and sequence and immunochemical relatedness (4-7). Previous studies have clearly shown that a low K_m form of N-nitrosodimethylamine demethylase (NDMAd) in rat liver microsomes involves cytochrome P-450$_j$. This isozyme is induced by fasting, acetone treatment, diabetes and ethanol (9-11). P-450$_j$ is probably identical to P-450$_{ac}$ (12,13).

Recent studies by Yamazoe et. al. (14) demonstrated the effects of a pituitary factor on the constitutive and inducible levels of hepatic cytochromes P-450$_b$ and P-450$_e$. Results of these studies suggested the growth hormone (GH) might be the pituitary factor involved in modulating the activity of P-450$_b$ and P-450$_e$. Studies presented in this communication were done to evaluate the possible involvement of the pituitary gland and GH

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Abbreviations used: P-450, cytochrome(s) P-450; NDMA, N-nitrosodimethylamine; NDMAd, N-nitrosodimethylamine demethylase; GH, growth hormone; HYPOX, hypophysectomized.

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in modulating constitutive levels of cytochrome P-450$_j$ in hepatic microsomes.

MATERIALS AND METHODS

Animals

Spague-Dawley rats weighing 120-140 grams were obtained from the Holtzman Co. (St. Louis, Mo.) Hypophysectomized animals were obtained from Harlan (Indianapolis, ID.) Hypophysectomized animals were allowed 7 days to stabilize upon arrival. All animals were fed a commercial laboratory chow-diet and water ad libitum. The animals were given a subcutaneous injection of GH (0.2IU/100g of body weight) twice a day for 6 days according to the procedures of Yamazoe et. al. (14). Control animals received buffer saline.

Preparation of Microsomes and Enzyme Assays

Rat liver microsomes were prepared according to the procedure of Williams and Pendleton (15). Microsomes were either used immediately or stored in pellet form at -70C. Microsomal NDMAd activity was assayed according to the procedure of Yoo et. al. (16). Microsomal NADPH cytochrome P-450 reductase activity was determined by using cytochrome c as an artifical electron acceptor according to the procedure of Yang et. al. (17). Total cytochrome P-450 was determined spectrophotometrically according to the procedure of Omura and Sato (18). All protein determination were performed using the method of Lowry et. al. (19). Student's t-test was used for statistical analyes. Values greater than 0.05 were not considered statistically significant.

Immunoblot Analysis

Cytochrome P-450$_j$ content in hepatic microsomes was determined by immunoblot analyses (12). Anti P-450$_{3a}$ IgG was used in these assays. This antibody was raised against the ethanol inducible cytochrome P-450$_{3a}$ in rabbits. However, this antibody has been shown to specifically recognize cytochrome P-450$_j$ in immunoblots of rat liver microsomes (12). Previous analysis of rat liver microsomes using this IgG showed only one major band with a Mr of 52,000 in the immunoblot analysis. No cross-reactivity with other P-450 isozymes was observed (12).

RESULTS

Effects of hypophysectomy on microsomal P-450$_j$ content

The immunoblot shown in Fig. 1 demonstrates the effects of hypophysectomy and GH treatment on hepatic microsomal cytochrome P-450$_j$. As can be seen in Fig. 1 hypophysectomy results in a marked increase in immunodetectable cytochrome P-450$_j$ protein in both male and female rats compared to intact animals. When animals were administered GH, the amount of P-450$_j$ was decreased. This decrease was more pronounced in microsomes from female rats compared to that from male rats.

Cytochrome P-450$_j$ has been shown to be associated with the metabolism of N-nitrosodimethylamine (20). Data in Table 1 show the effects of hypophysectomy and GH on NDMAd activity. NDMAd

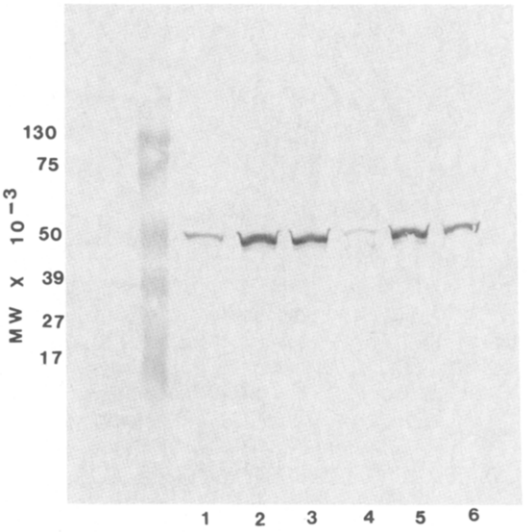

Fig. 1. **Immunoblot of rat liver microsomal proteins.** Hepatic microsomes from intact (well 1), hypox (well 2), and hypox and GH-treated (well 3) male rats, and intact (well 4), hypox (well 5) and hypox + GH (well 6) female rats were added at a level of 10ug of protein. Electrophoresis was performed and the proteins were transferred to immobilon and immunologically stained with anti-P-450$_{3a}$ IgG.

activity is increased in both hypox male and female rats and GH administration slightly increases NDAMd activity in female rats. Although hypophysectomy results in a 5 and 10-fold increase in immunodetectable P-450$_j$ in male and female rats respectively, NDMAd activity is increased only by 23 and 75 percent in male and female rat liver microsomes (Table 1).

Data in Table 2 show effects of hypophysectomy and GH treatment on NADPH cytochrome P-450 reductase activity. Hypophysectomy results in a marked decrease in reductase activity in microsomes from both male and female rats. GH administration results in a significant increase in reductase activity compared to microsomes from hypox animals.

DISCUSSION

Results presented in this communication show that hypophysectomy in both male and female rats resulted in a significant increase in immunodetectable P-450$_j$ protein. This increase is greater in microsomes from female rats compared to male rats. Administration of GH results in a decrease in immunodetectable P-450$_j$ (Fig 1). While hypophysectomy results in a 5 to 10-fold increase in P-450$_j$, a corresponding increase in NDMAd activity is not observed in these microsomes. NDMAd activity is only moderately increased. This lack of correlation between NDMAd activity and increase in P-450$_j$ protein might be

Table 1. Effects of Hypophysectomy and GH on NDMAd Activity in Rat Liver Microsomes

Treatment	NDMAd Activity	$P\text{-}450_j$ Content
Male		
Intact	1.08 ± 0.18	2.37
Hypox	1.33 ± 0.15	12.0
Hypox + GH	1.42 ± 0.04*	9.0
Female		
Intact	0.83 ± 0.17	1.0
Hypox	1.46 ± 0.13*	10.0
Hypox + GH	1.84 ± 0.30*	4.2

NDMAd activity is shown as nmol formaldehyde formed/min/mg microsomal protein. $P\text{-}450_j$ content is presented in arbitrary unit (peak area) from the scanning densitometry on the immunoblot analysis shown in Fig. 1. Values represents mean ± SD from 4 rats.

* Values significantly different from the corresponding controls ($P<0.01$).

explained by the fact that hypophysectomy also results in a significant decrease in NADPH cytochrome P-450 reductase activity. As shown in Table 2, reductase activity is reduced 65 percent and 55 percent in male and female rats respectively. Since this enzyme catalyzes the rate-limiting step in xenobiotic

Table 2. Effects of Hypophysectomy and GH on NADPH Cytochrome P-450 Reductase Activity and Total P-450 in Rat Liver Microsomes

Treatment	NADPH Cytochrome P-450 Reductase (nmol/min/mg protein)	Total Cytochrome P-450 (nmole/mg protein)
Male		
Intact	91.5 ± 14.0	0.65 ± 0.13
Hypox	32.7 ± 4.8*	0.73 ± 0.22
Hypox + GH	47.4 ± 6.8*	0.76 ± 0.16
Female		
Intact	78.4 ± 8.8	0.50 ± 0.17
Hypox	35.0 ± 5.2*	0.82 ± 0.29*
Hypox + GH	50.6 ± 3.2*	0.75 ± 0.19*

*Values significantly different from corresponding controls ($P<0.01$).

metabolism (21,22), decrease in the activity of this enzyme could alter the overall rate of demethylation of NDMA.

The regulation and induction of several isozymes of cytochrome P-450 have been investigated (23). A report by Song et. al. (24) suggested that the induction of $P\text{-}450_j$ may be regulated differently under different conditions. A recent report by Hong et. al. (20) suggested that the induction of $P\text{-}450_j$ by acetone may be due to post-transcriptional events such as an increase in rate of translation or protein stabilization. Results of our studies suggest a possible role for GH in regulation of basal levels of $P\text{-}450_j$ protein. As was seen with $P\text{-}450_b$, cytochrome $P\text{-}450_j$ levels may be repressed by GH or other pituitary factors. The mechanism of the GH-repression of $P\text{-}450_j$ remains to be investigated.

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