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DOSE-DEPENDENT EFFECTS OF MEDROXYPROGESTERONE ACETATE ON THE HEPATIC DRUG-METABOLIZING ENZYME SYSTEM IN RATS*

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Abstract—The activity of the hepatic microsomal drug metabolism was examined *in vitro* in rats pretreated with 10–600 mg/kg medroxyprogesterone acetate intraperitoneally daily for seven days. In both sexes there was a significant increase in the liver weight, amount of cytochrome P-450, activity of NADPH-cytochrome *c* reductase, benzo[*a*]pyrene hydroxylase and 2,5-diphenyloxazole hydroxylase. The increase in 7-ethoxycoumarin-*O*-deethylase activity was also significant in female rats, but not in male rats. In the female rats pretreated with medroxyprogesterone acetate, the ability of α-naphthoflavone and SKF 525A to inhibit benzo[*a*]pyrene hydroxylase was decreased and slightly increased, respectively. The results show that medroxyprogesterone acetate has a dose-dependent inducing effect on the hepatic drug metabolism in rats. Female rats seem to be more sensitive to the inducing effect of medroxyprogesterone acetate than the males. The characteristics of medroxyprogesterone acetate induction resemble mostly those caused by phenobarbital and pregnenolone-16α-carbonitrile.

Medroxyprogesterone acetate (17α-acetoxy-6α-methylpregn-4-ene-3.20-dione)(MPA) is a synthetic progesterone. The drug has been used as a long-acting injectable contraceptive [1], in slowing sexual development in children with precocious puberty [2], and in the treatment of cancer of endometrium [3], breast [4], kidney [5] and prostate [6]. In clinical therapy MPA is often given in large doses [4].

There are only a few experiments concerning the effect of MPA on hepatic drug metabolism. In female rats treated with MPA, the activity of liver enzymes metabolizing *p*-nitroanisole, aniline and aminopyrine were significantly higher [7]. In male rats, MPA caused an increase in the activity of hepatic testosterone A-ring reductase [8]. The same enzyme is also induced in man by MPA [9]. In patients with liver diseases treated with MPA, the plasma antipyrine half-life decreased and the content of liver cytochrome P-450 increased [10].

In previous studies of the effect of MPA on hepatic drug metabolism, only few doses of MPA have been used [7, 8]. Although for therapeutic purposes a wide dosage range of MPA is used, there is little information about the dose-dependent effect of MPA on the drug metabolism. The present study was undertaken to clarify the effect of various doses of MPA on hepatic drug metabolism and also to characterize the type of induction.

METHODS

Chemicals. Medroxyprogesterone acetate (Lutopolar) and Lutopolar vehicle (macrogol. 4000 28.8 mg, sorbimacrogol. oleas. 300 1.92 mg, NaCl 8.65 mg, methyl.*p*-oxybenz. 1.73 mg, propyl.*p*-oxybenz. 0.19 mg, aq. steril ad. 1 ml) were kindly supplied by Farmos Oy, Finland. Bovine albumin, ethyl isocyanide, 7-ethoxycoumarin and horse heart cytochrome *c*, type III, were obtained from the Sigma Chemical Co., St. Louis, MO, 2,5-diphenyloxazole from NEN, Chemicals GmbH, Frankfurt am Main, α-naphthoflavone and benzo[*a*]pyrene from Fluka AG, Buchs, Switzerland, and SKF 525A (β-diethylaminoethyl-2.2-diphenylvalerate) was purchased from Smith, Kline & French Laboratories, Philadelphia, PA). All other chemicals were of the highest grade commercially obtainable.

Treatment of animals. Two months old female and male (170–250 g) Sprague–Dawley rats were used. Medroxyprogesterone acetate in Lutopolar vehicle was injected intraperitoneally 10, 50, 100, 200 and 600 mg/kg (0.4 ml/100 g body wt) daily for seven days. Control rats were given an equal amount of Lutopolar vehicle. The last dose was given 24 hr before the rats were killed. Food was removed 12 hr before the rats were decapitated.

Preparation of microsomes. Livers were removed and washed quickly with 0.25 M sucrose, homogenized in four volumes of 0.25 M sucrose with a glass Potter–Elvehjem homogenizer. The homogenates were centrifuged at 10,000 *g* for 20 min. Part of the 10,000 *g* supernatant fraction was stored at −70° for enzyme determinations. The remaining part of the supernatant fraction was centrifuged at 105,000 *g* for 60 min and the pellet obtained was resuspended

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in 0.15 M KCl and recentrifuged as above. The washed pellets were stored at −70°. Before use, the pellets were resuspended in 0.1 M Tris–HCl buffer, pH 7.4, so that 1 ml suspension contained microsomes from 1 g of liver [11].

Cytochrome P-450 and b_5 content. The amount of cytochrome P-450 and b_5 in the microsomal fraction was determined by the method of Omura and Sato [12].

NADPH-cytochrome c reductase. The activity of NADPH-cytochrome *c* reductase in the microsomal fraction was determined as described by Masters *et al.* [13].

Monooxygenase enzyme assays. The activities of benzo[*a*]pyrene (BP) hydroxylase, 2,5-diphenyloxazole (PPO) hydroxylase and 7-ethoxycoumarin-*O*-deethylase were determined in the 10,000 *g* supernatant fraction by the methods described by Nebert and Gelboin [14], Cantrell *et al.* [15] and Jacobson *et al.* [16], respectively.

Inhibition of BP-hydroxylase and PPO-hydroxylase. For studying the inhibition of BP-hydroxylase and PPO-hydroxylase, the incubate was made 0.1 mM with respect to α-naphthoflavone (α-NF) or 0.2 mM with respect to SKF 525A [11].

Ethyl isocyanide difference spectrum ratio. The difference spectrum ratio $(\Delta A_{455-490})/(\Delta A_{430-490})$ was determined by addition of ethyl isocyanide to sodium dithionite reduced microsomal fraction [11].

Protein determination. Protein content was determined by the method of Lowry *et al.* [17] with bovine albumin as a standard.

Statistical analysis. Statistical treatment of the data was performed by using the Student *t*-test.

RESULTS

The effect of MPA on some parameters of the hepatic drug-metabolizing enzyme system is shown in Table 1 and Fig. 1.

During the seven day treatment the absolute and relative liver weight increased in a dose-dependent manner in the female and the male rats. The relative amount of microsomal protein decreased significantly in the male rats given a large dose of MPA. There was also a similar, but not significant, change in the females.

The content of cytochrome P-450 and the activity of NADPH-cytochrome *c* reductase increased in a dose-dependent manner in both sexes. The amount of cytochrome b_5 increased only slightly.

The activity of BP-hydroxylase, PPO-hydroxylase and 7-ethoxycoumarin-*O*-deethylase increased as a function of MPA dose. In the male rats, however, the changes were smaller than in the females. With respect to the activity of BP-hydroxylase, the female rats were about ten times more sensitive to the inducing effect of MPA than the male rats.

The ratio $(\Delta A_{455-490})/(\Delta A_{430-490})$ of the ethyl isocyanide induced difference spectrum slightly increased in the male rats, but in the female rats there was no significant change as a result of MPA pretreatment.

Figure 2 shows the effect of MPA on the ability of SKF 525A and α-NF to inhibit BP- and PPO-hydroxylase. In the case of the female rats, α-NF lost its ability to inhibit BP-hydroxylase, although in the control rats there was about 50 per cent inhibition. In contrast, in the male rats the degree of inhibition by α-NF changed only in the groups treated with 100 and 200 mg/kg MPA. In these groups the inhibition of BP-hydroxylase by α-NF was less than in the control groups. There was a slight increase in the ability of SKF 525A to inhibit BP-hydroxylase. This change was greater in the female rats than in the males. MPA pretreatment caused only slight changes in the degree of inhibition

Table 1. The effect of MPA on liver weight (g), liver weight/body weight × 100, the amount of microsomal protein (mg/g liver), cytochrome P-450 and cytochrome b_5 (nmoles/mg microsomal protein), the activity of NADPH-cytochrome *c* reductase (nmoles cytochrome *c* reduced/min/mg microsomal protein), benzo[*a*]pyrene hydroxylase, 2,5-diphenyloxazole hydroxylase and 7-ethoxycoumarin-*O*-deethylase (fluorescence units/min/mg 10,000 *g* supernatant protein) and the ratio $(\Delta A_{455-490})/(\Delta A_{430-490})$ of the ethyl isocyanide induced difference spectrum with the reduced cytochrome P-450 in rats pretreated with 600 mg/kg MPA daily for seven days

	Female			Male		
		Pretreated			Pretreated	
Parameter	Control $X \pm$ S.D.	$X \pm$ S.D.	% of control	Control $X \pm$ S.D.	$X \pm$ S.D.	% of control
Liver weight	5.98 ± 0.62	11.0 ± 0.84	184 ± 14‡	8.04 ± 1.22	12.6 ± 1.24	157 ± 15‡
Liver weight/body weight	3.01 ± 0.24	6.16 ± 0.45	205 ± 15‡	3.23 ± 0.07	5.76 ± 0.25	178 ± 8‡
Microsomal protein	19.8 ± 0.4	17.8 ± 3.7	90 ± 19	19.5 ± 2.5	13.6 ± 1.6	70 ± 8†
Cytochrome P-450	0.53 ± 0.05	1.03 ± 0.14	194 ± 26‡	0.68 ± 0.09	1.07 ± 0.12	157 ± 18†
Cytochrome b_5	0.59 ± 0.05	0.74 ± 0.07	125 ± 12	0.72 ± 0.06	0.87 ± 0.04	121 ± 6†
NADPH-cytochrome *c* reductase	23.6 ± 2.9	54.3 ± 10.6	230 ± 45†	23.2 ± 4.8	64.7 ± 11.3	279 ± 49‡
Benzo[*a*]pyrene hydroxylase	39 ± 10	117 ± 14	303 ± 37‡	111 ± 18	200 ± 16	180 ± 14‡
2,5-Diphenyloxazole hydroxylase	211 ± 48	491 ± 47	232 ± 22‡	247 ± 40	336 ± 50	136 ± 20*
7-Ethoxycoumarin-*O*-deethylase	354 ± 69	801 ± 64	226 ± 18*	519 ± 36	629 ± 118	121 ± 23
Ratio $(\Delta A_{455-490})/(\Delta A_{430-490})$ of the ethyl isocyanide induced difference spectrum	0.69 ± 0.02	0.68 ± 0.04	99 ± 6	0.41 ± 0.02	0.54 ± 0.02	132 ± 5‡

* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$. Each value represents the mean ± S.D. from four separate experiments.

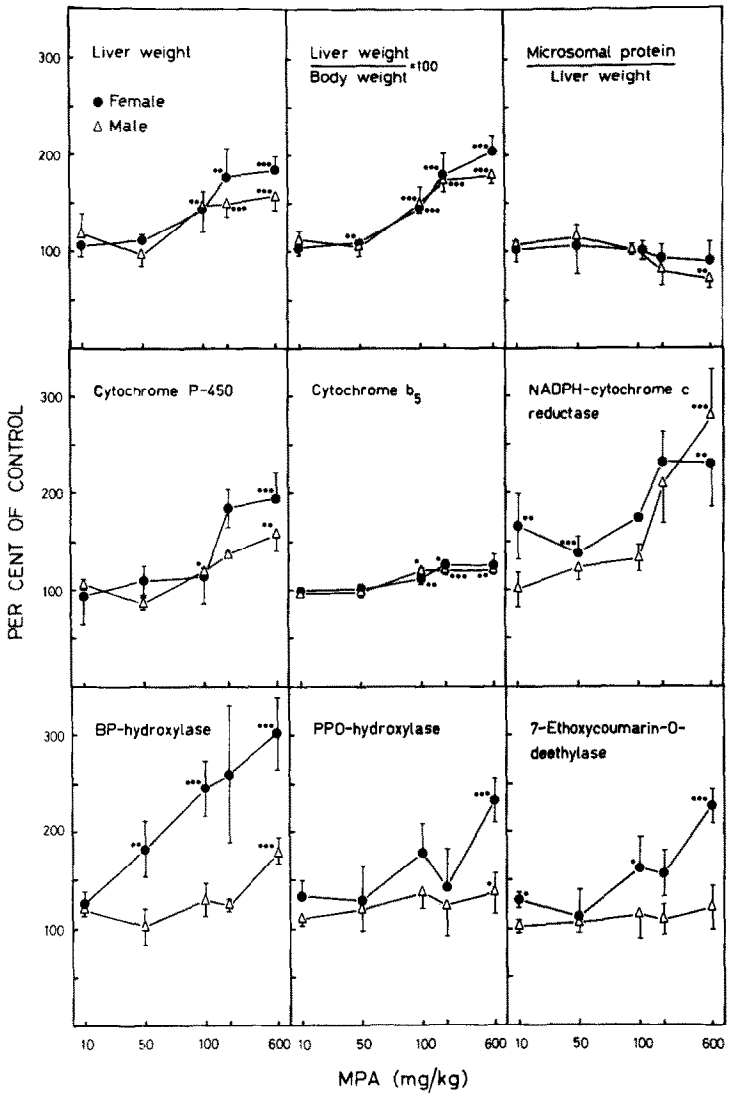

Fig. 1. The effect of MPA on liver weight (g), liver weight/body weight × 100, amount of microsomal protein (mg/g liver), cytochrome P-450 and cytochrome b_5 (nmoles/mg microsomal protein), activity of NADPH-cytochrome *c* reductase (nmoles cytochrome *c* reduced/min/mg microsomal protein), benzo[*a*]pyrene (BP) hydroxylase, 2,5-diphenyloxazole (PPO) hydroxylase and 7-ethoxycoumarin-*O*-deethylase (fluorescence units/min/mg 10,000 *g* supernatant protein) as a function of dose of MPA. The rats were treated i.p. with daily doses of MPA for seven days. The values of treated rats are presented as per cent of the values of control rats. Each value represents the mean ± S.D. from four separate experiments. Full circles represents female and open triangles male rats. Differences between treated and control groups are significant at level (+) $P < 0.05$, (++) $P < 0.01$ and (+++) $P < 0.001$.

of PPO-hydroxylase by SKF 525A and α-NF. These changes were similar to those seen on BP-hydroxylase.

DISCUSSION

The present results show that medroxyprogesterone acetate has a dose-dependent inducing effect on the hepatic drug-metabolizing enzyme system in rats. Female rats seem to be more sensitive to the effect of MPA than the males. This phenomenon was clearest in the case on the induction of BP-hydroxylase activity.

As a result of pretreatment with MPA, the character of drug metabolism in the female rats changed towards that in the males. The absolute activities of the different enzymes in the female rats increased to the level of the male rats. α-NF and SKF 525A inhibited BP-hydroxylase activity to the same extent in both sexes. The sex-dependent effect of MPA could also be seen in that the male rats lost more body weight during the treatment (data not shown) that the female rats. Also the amount of relative microsomal protein decreased more in the male rats than in the females.

Long-term treatment with MPA (10 mg/kg p.o.)

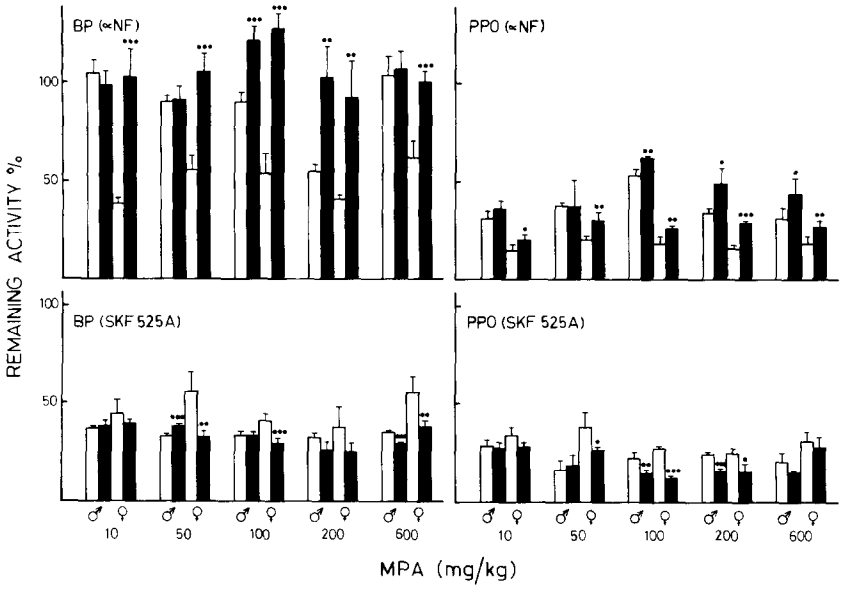

Fig. 2. The effect of MPA on the ability of α-naphthoflavone (α-NF) and SKF 525A to inhibit benzo[a]pyrene hydroxylase (BP) and 2,5-diphenyloxazole hydroxylase (PPO) in rats treated i.p. with daily doses of MPA for seven days. The activities (fluorescence units/min/mg 10,000 *g* supernatant protein) in pretreated (black columns) and control (open columns) groups are given as per cent. Each value represents the mean ± S.D. from four separate experiments. The symbols are the same as in Fig. 1.

has been shown to induce metabolism of *p*-nitroanisole, aniline and aminopyrine in female rats [7]. Our data show a clear change in hepatic drug-metabolizing enzymes even after a few days therapy. Furthermore, the inducing effect was dose-dependent up to 600 mg/kg. NADPH-cytochrome *c* reductase and BP-hydroxylase activities seem to be more sensitive to the inducing effect of MPA than the other enzymes.

Dahm *et al.* [18] demonstrated that MPA has an inducing effect on the hepatic protein and lipid synthesis. In our study, MPA significantly increased the absolute and relative liver weight in both sexes. This was associated with a similar increase in the synthesis of overall protein in liver, because the amount of protein per liver weight did not change in microsomal and 10,000 *g* supernatant fractions (data not shown). Moreover, MPA also increased the ratio of cytochrome P-450 per microsomal protein. This suggests that MPA, as well as inducing overall protein synthesis associated with enhanced hepatic weight, also has a specific effect on the synthesis of enzymes of microsomal fraction; the amount of cytochrome P-450 increased in relation to the microsomal protein.

Phenobarbital (PB) and pregnenolone-16α-carbonitrile (PCN) increase the protein content in liver microsomes in female rats [19]. Our results showed that MPA differs from the above-mentioned inducers in that it has only slight effect on the amount of microsomal protein per gram liver. However, it may be inferred that MPA induced mostly cytochrome(s) P-450 related to PB inducible cytochromes [11], as the degree of inhibition of BP-hydroxylase by SKF 525A increased. Moreover, α-NF, which is a very effective inhibitor of 3-methylcholanthrene pretreated rat liver microsomal BP-hydroxylase [11], inhibited less BP-hydroxylase in the MPA pretreated groups than in the control groups.

In our study MPA behaved in many respects like PCN, which effectively increases liver weight, NADPH-cytochrome *c* reductase and BP-hydroxylase activity and has only a slight effect on the ratio $(\Delta A_{455-490})/(\Delta A_{430-490})$ of the ethyl isocyanide induced difference spectrum [19].

It has been shown that testosterone enhances the activity of liver microsomal enzymes [20]. MPA has an androgenic effect which relates to its specific binding *in vivo* to the androgen receptor in prostate and seminal vesicle in rats [21]. Therefore it may be that the sex-dependent inducing effect of MPA is due to its androgenic action. However, in the females the ratio $(\Delta A_{455-490})/(\Delta A_{430-490})$ of the ethyl isocyanide induced difference spectrum did not change towards that in the males. Moreover, testosterone does not decrease the growth rate of rats and also it does not significantly alter the liver to body weight ratio [22] like MPA in our study.

MPA has certain glucocorticoid-like effects on the liver [23] and it could be possible that part of its inductive effect is due to this glucocorticoid character. There seem to be many similarities between MPA and glucocorticoid-like substances. Dexamethasone has been shown to induce hepatic drug metabolism and impair body growth rate like MPA, but it does not, however, affect liver weight [24].

Progesterone given to female rats causes an increased hepatic demethylation of *p*-chloro-*N*-methylaniline, liver weight, microsomal protein and body growth rate. In contrast, in males progesterone decreases demethylation, microsomal protein and body growth rate and has no significant effect on liver weight [25].

From the long-acting contraceptives, norethindrone is known to increase relative liver weight [26]. In chronic administration, norethynodrel also induces hepatic drug metabolism in male rats [27], but acute treatment does not induce drug metabolism in female rats [28].

This experiment shows that MPA has a specific dose-dependent inducing effect on the hepatic drug-metabolizing enzyme system. The induction seems to be sex-dependent so that female rats are more sensitive to the inducing effect of MPA. The induction of MPA resembles in many respects that of phenobarbital and pregnenolone-16α-carbonitrile. When rats were given 600 mg/kg MPA, the only examined parameters which were impaired were body growth rate and the amount of relative hepatic microsomal protein in the male rats. By smaller doses MPA increased hepatic protein synthesis associated with increased liver weight. This might be one of the effects by which MPA treatment gave good results in patients with liver diseases [10], although direct extrapolations from animal studies with large doses to the human situation where much smaller doses are used are uncertain.

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