Further Studies on the Persistence of Neonatal Androgen Imprinting on Sex-Specific Cytochrome P-450, Testosterone and Drug Oxidations

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Abstract—Neonatal castration completely suppressed the expression of P-450-male and expressed P-450-female; and testosterone treatment in a neonatal period partially reversed the effect of castration, i.e., neonatal imprinting (Kamataki et al., 1984; Waxman et al., 1985). In the present communication, we investigate the reversibility and persistency of neonatal imprinting on the expression of P-450-male and P-450-female. To our surprise, testosterone treatment at adulthood (8 weeks old) caused full expression of P-450-male and restored the activities of 2α- and 16α-testosterone hydroxylases in neonatally castrated rats. The levels of ethylmorphine N-demethylation, propoxycoumarin O-depropylation and benzo(a)pyrene hydroxylation were increased to the levels of adult male rats by adult testosterone treatment. Moreover, treatment with testosterone of neonatally castrated rats at the age of 19 weeks did not cause a complete recovery of P-450-male content and drug-metabolizing activities. Testosterone administration into neonatal female rats did not significantly alter the contents of sex-dependent cytochrome P-450 and drug and steroid metabolizing activities in adulthood. Additional testosterone treatment in adulthood only slightly affected these parameters. All these results indicate that neonatal androgen imprinting on sex-dependent cytochrome P-450 and drug and steroid metabolizing activities in rat liver microsomes is not a permanent programming process and is modified by the presence and absence of sex steroid hormones.

Mammalian liver cytochrome P-450 is comprised of a family of heme protein enzymes that oxidatively metabolize structurally diverse lipophilic chemicals including drugs, environmental agents and other xenobiotics as well as steroid hormones and fatty acids. Sex-related differences in cytochrome P-450-mediated metabolisms of exogenous and endogenous compounds have been recognized in rat liver microsomes (1-5). The 20-30% lower levels of electron transport activity in hepatic microsomes and total cytochrome P-450 in female as compared to male rats are consistent with the slower metabolisms of some drugs in female rats, but they are not able to explain the larger sex-related differences, up to 20-fold in propoxycoumarin O-depropylation (6), observed for the rates of metabolisms of a variety of drugs (1, 7). Therefore, these large sex-related differences probably reflect the contributions of androgen-dependent cytochrome P-450 in male rat liver microsomes to the overall androgen-dependent cytochrome P-450-mediated drug metabolisms. To support this hypothesis, we made an effort to demonstrate sex-specific forms of cytochrome P-450 by means of a purification procedure (2, 8, 9).

We finally purified male-specific and
female specific cytochrome P-450 from male and female rat livers, respectively, and designated them P-450-male and P-450-female (10). We proved immunochemically that P-450-male and P-450-female exist specifically in liver microsomes from adult male and female rats, respectively, and that their contents are about one-fourth of the total cytochrome P-450 (10). P-450-male and P-450-female are not expressed in rats of less than 20 days old (11).

Sex-related metabolic differences in rats are believed to be determined primarily by events occurring during the neonatal period, when secretion of testicular androgens imprints a latent masculine potential, neonatal imprinting, onto an otherwise feminine pattern (12). Neonatal castration of male rats results in the expression of a feminine pattern of several sexually differentiated characteristics in adult life, including gonadotropin secretion and sexual behavior (12). The effects of neonatal castration are reversed by the administration of testosterone. Similar neonatal imprintings have been demonstrated on the androgen-dependent drug and steroid metabolizing enzymes (13-15).

We (16) and Waxman et al. (17) also demonstrated that neonatal castration of male rats results in complete loss of the expression of male-specific cytochrome P-450 and partial expression of female-specific cytochrome P-450. Moreover, neonatal administration of testosterone reversed the effect of neonatal castration (16, 17).

Studies on the oxidative metabolism of drugs and steroid hormone carried out by several groups indicated that the administration of testosterone at adult age into neonatally castrated rats produced a limited effect to manifest a male-type metabolic pattern (4, 5, 13-15).

However, to our surprise, an almost complete reversal of the effect of neonatal castration on P-450-male was observed with testosterone treatment at adult age (Y. Yamazoe et al., unpublished observation). Sex-specific forms of cytochrome P-450, such as P-450-male, have been considered to play the major role of sex-related differences in the oxidations of a variety of drugs and steroid hormones (10, 11, 18-23). Even though the quantities are low, other forms of sex-specific cytochrome P-450 have also been reported to contribute some of the oxidative activity of microsomes in the hormonally modified rats (17, 24-28). Further studies, therefore, have been required to determine the relationship between the regulation of sex-specific forms of cytochrome P-450 and oxidative activities of drugs and steroid hormones.

In this report, we have studied the persistence of neonatal androgen imprinting on sex-specific cytochrome P-450, testosterone hydroxylation and drug oxidations through neonatal castration and androgen supplementation during the neonatal or adult periods and neonatal testosterone treatment of female rats. In contrast to previous reports, we have observed that neonatal androgen imprinting is an important factor for manifesting some male specific metabolisms in the absence of androgen, but the lack of neonatal androgen imprinting was almost completely reversed by the administration of androgen in some period of adulthood.

Materials and Methods

Treatment of animals and preparation of liver microsomes: Male and female Sprague-Dawley rats were purchased from Clea Japan, Tokyo. Testosterone propionate was given as follows: neonatal testosterone treatment (10 mg/kg, s.c.), at 1, 3 and 5 days after castration or at 1 day after birth; adult testosterone treatment (20 mg/kg s.c.), at 8 or 19 weeks of age 4-5 times for alternative days. Neonatal castration was done within 24 hr after birth. Some male and female rats were gonadectomized at 20-21 days of age. All the animals were killed at 9 weeks or 20 weeks of age. Livers were homogenized with 1.15% potassium chloride, and the microsomes were prepared by sequential centrifugations at 9,000×g and 105,000×g, respectively.

Chemicals: Goat anti-rabbit immunoglobulin G (IgG) was purchased from Cappel Lab. (Cochranville, PA, U.S.A.), and horseradish peroxidase - rabbit anti-peroxidase complex from Miles Yeda (Rohovot, Israel). All other chemicals used were obtained as described
previously (19).

Immunocchemical quantification of P-450-male and P-450-female: A peroxidase staining technique coupled with SDS-polyacrylamide gel electrophoresis (Western blot) was used to quantify the content of P-450-male and P-450-female in hepatic microsomes (29, 30). The characteristics of P-450-male, P-450-female and monospecific antibodies to these cytochromes were described previously (10, 30).

Assays of the microsomal steroid and drug-metabolizing activities: A typical incubation mixture (1 ml) consisted of NADP (0.8 mM), glucose 6-phosphate (8.0 mM), glucose 6-phosphate dehydrogenase (1 IU), magnesium chloride (6 mM), potassium, sodium phosphate buffer (pH 7.4, 100 mM), ethylenediaminetetraacetic acid (0.1 mM), liver microsomes (0.25 mg for the metabolisms of testosterone, benzo(a)pyrene and O-ethylresorufin, and 1.0 mg for 7-n-propoxycoumarin, ethylmorphine and aniline), and a substrate (2 mM testosterone, 0.1 mM benzo(a)pyrene, 2 μM O-ethylresorufin, 0.5 mM 7-n-propoxycoumarin, 5 mM ethylmorphine and aniline). The incubation was started by addition of the NADPH generating system (NADP, glucose 6-phosphate, glucose 6-phosphate dehydrogenase and magnesium chloride), which was preincubated at 37°C for 3 min, and was reacted at 37°C for 15 min, except for O-ethylresorufin dealkylation which was reacted for 5 min. Testosterone hydroxylations were measured as previously described with a JASCO model TRIROTAR VI chromatograph equipped with an AS-L350 sample processor (20). For the metabolisms of 7-propoxycoumarin, benzo(a)pyrene and O-ethylresorufin, fluorescent phenolic metabolites were determined by the methods of Aitio (31), Nebert and Gelboin (32), Burke and Mayer (33), respectively. 3-Hydroxybenzo(a)pyrene was used as the standard for the assay of benzo(a)pyrene hydroxylation. For aniline hydroxylation, p-aminophenol was measured colorimetrically (34). Formaldehyde, detected by the method of Nash (35), was used for the ethylmorphine N-demethylation assay. Testosterone 5α-reductase activity was determined by the method of Lax et al. (36). Microsomal protein was determined by the method of Lowry et al. (37) with bovine serum albumin as the standard. Cytochrome P-450 content was measured from the CO-reduced difference spectrum (38) except that 20% glycerol and 0.2% Emulgen 913 (Kao-Atalas, Ltd., Tokyo) were included.

Results

Effect of neonatal castration and testosterone treatment on P-450-male and P-450-female contents of rat liver: Castration of male rats at birth caused complete inhibition of the expression of P-450-male in the liver of adult male rats, whereas it caused the full expression of P-450-female (Fig. 1). However, treatment with testosterone in the neonatal period partially restored the expression of P-450-male. Surprisingly, testosterone treatment in the adult period almost completely restored the content of P-450-male. Treatment with testosterone in the neonatal or adult period inhibited the expression of P-450-female in male rats that were castrated when they were 1 day old.
These results indicate that the persistence of neonatal imprinting is not an absolutely programmed phenomenon.

Effect of neonatal castration and testosterone treatment on testosterone metabolisms in male rat liver: Castration at 1 day old markedly depressed testosterone 2α- and 16α-hydroxylations in the liver of adult male rats in accordance with previous studies (5, 7) (Fig. 2). However, treatment with testosterone in the neonatal period partially restored the hydroxylase activities.

In contrast to previous reports (13, 39, 40), treatment with testosterone in the adult period almost completely restored testosterone 2α- and 16α-hydroxylase activities in neonatally castrated rats. The change in testosterone 6β-hydroxylation is similar to those in testosterone 2α- and 16α-hydroxylations, but the effects of neonatal and adult testosterone treatment seem to be less. The combined testosterone treatment in both the neonatal and adult periods restored the activity. These results indicate that a cytochrome P-450 less sensitive toward neonatal androgen imprinting is involved in testosterone 6β-hydroxylation.

In contrast to testosterone 2α- and 16α-hydroxylations, testosterone 7α-hydroxylation is higher in female rats than in males in accordance with previous reports (5, 17) (Fig. 3). Castration at 1 day old markedly increased testosterone 7α-hydroxylation, and neonatal testosterone treatment did not reverse the effect of the castration. However, adult testosterone treatment and the combined testosterone treatment in both the neonatal and adult periods completely reversed the effect of neonatal castration. Similar results were observed with microsomal testosterone 5α-reduction in male rat livers, except for the effect of neonatal testosterone treatment.

Effect of neonatal castration and testosterone treatment on the activity of microsomal drug-metabolizing enzymes in male rat liver: Castration at 1 day old markedly depressed ethylmorphine N-demethylation, propoxycoumarin O-depropylation and benzo(a)pyrene hydroxylation in the liver of adult male rats (Fig. 4). The treatment with
testosterone in the neonatal period partially restored these drug-metabolizing activities and treatment with testosterone in adult period, in contrast to previous reports (41, 42), almost completely restored these activities. The changes of drug-metabolizing activities are roughly in accord with the changes of P-450-male content and testosterone 2α- and 16α-hydroxylations.

Aniline hydroxylation and O-ethylresorufin O-deethylation are known as drug metabolisms which do not show clear sex-related differences (19, 43, 44). Castration at birth did not cause any significant changes in aniline hydroxylation and O-ethylresorufin O-deethylation levels in the adult period (Fig. 5). Moreover, treatment of neonatally castrated rats with testosterone in the neonatal period or in adulthood did not change either of the activities. These results are in accordance with the previous report which showed the indiffrence of aniline hydroxylation and O-ethylresorufin O-deethylation to P-450-male content in varying hormonal conditions (19).

Prolonged effect of neonatal or prepubertal castration on P-450-male content and drug oxidation activity in male rat liver:
Since the results obtained in the present studies indicate a lack of persistence of absolute programming, we examined the prolonged effect of castration by maintaining the neonatally castrated rats for 20 weeks without a testosterone supply.

Castration at 1 day old inhibited the expression of P-450-male and fully stimulated the expression of P-450-female in the liver of 20 week-old rats (Table 1). P-450-male content was decreased to 31% of the intact male level, in prepubertally (20 days old) castrated rats lacking testosterone for 16 weeks, which is markedly low in comparison with rats killed at 8 weeks of age. In addition, the levels of benzo(a)pyrene hydroxylation and propoxyecoumarin O-depropylation in neonatally or prepubertally castrated rats seemed to decrease with prolonged absence of androgen. Moreover, testosterone treatment at 19 weeks of age of neonatally castrated rats only partially restored P-450-male content. Surprisingly, P-450-male was markedly decreased and P-450-female was expressed in 20 week-old rats which were castrated at 20 days old, although P-450-female was not expressed in 8 weeks old rats castrated at 7, 14 or 22 days old (10, 16).
P-450-female was detectable only in 5 in 7 rats which had been neonatally castrated, but there was a reverse correlation between P-450-female and P-450-male amounts (Fig. 6). Moreover, the administration of testosterone to 19 weeks old rats castrated at birth or 20 days of age completely suppressed expression of P-450-female.

**Table 1. Prolonged effect of neonatal or prepubertal castration on P-450-male and P-450-female contents and drug oxidation activity in male rat liver**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cast</th>
<th>Cast+Ta</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-450-male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(nmole/mg prot)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cast 1 day</td>
<td>0.27±0.02</td>
<td>N.D.(a) (0%)</td>
<td>0.16±0.02 (59%)</td>
</tr>
<tr>
<td>Cast 20 day</td>
<td>0.22±0.06</td>
<td>0.07±0.03 (31%)</td>
<td>0.15±0.04 (68%)</td>
</tr>
<tr>
<td>Benzo(a)pyrene hydroxylation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pmole/mg prot/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cast 1 day</td>
<td>246±22</td>
<td>57±9 (23%)</td>
<td>113±18 (46%)</td>
</tr>
<tr>
<td>Cast 20 day</td>
<td>224±28</td>
<td>78±25 (35%)</td>
<td>163±31 (73%)</td>
</tr>
<tr>
<td>Propoxycoumarin O-depropylation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(nmole/mg prot/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cast 1 day</td>
<td>1.58±0.25</td>
<td>0.16±0.03 (10%)</td>
<td>0.57±0.23 (36%)</td>
</tr>
<tr>
<td>Cast 20 day</td>
<td>1.47±0.23</td>
<td>0.25±0.06 (17%)</td>
<td>0.87±0.21 (56%)</td>
</tr>
</tbody>
</table>

\(a\): P-450-female was expressed in all rats, 0.30±0.04 (n=10). \(b\): P-450-female was expressed in five rats, 0.18±0.05 (n=5/7). Cast: castration, Ta: adulthood testosterone treatment. The rats were castrated at 1 day or at 20 days of age and killed at 20 weeks of age. The number of rats in the neonatally castrated group was 10; and in the prepubertally castrated group, it was 7. The results are given as means±S.D. from 10 (Cast 1 day) and 7 (Cast 20 day) rats.

**Fig. 6. Effect of testosterone treatment at 19 weeks of age on the expression of P-450-female in relation to P-450-male content in male rats castrated at birth or 20 days of age. **

P-450-female was detectable only in 5 in 7 rats which had been neonatally castrated, but there was a reverse correlation between P-450-female and P-450-male amounts (Fig. 6). Moreover, the administration of testosterone to 19 weeks old rats castrated at birth or 20 days of age completely suppressed expression of P-450-female.

**Effect of testosterone treatments on P-450-male and P-450-female content in normal adult, prepubertally ovariectomized or neonatally testosterone-imprinted female rats:** Testosterone treatment of adult female rats did not cause any significant effect on P-450-female and did not stimulate the expression of P-450-male. On the other hand, in adult female rats neonatally treated with testosterone, additional testosterone-treatment in adulthood significantly decreased P-450-female content (Table 2). Ovariectomy at 21 days of age did not cause any significant change in P-450-female, but testosterone treatment of these ovariectomized rats caused a complete loss of P-450-female concomitantly with the expression of P-450-male.

**Effect of testosterone treatment on testosterone hydroxylations in intact adult, prepubertally ovariectomized or neonatally testosterone-imprinted female rats:** Testosterone treatment in adulthood did not produce significant changes in 2α-, 16α- and 6β-hydroxylations of testosterone in intact female rats (Fig. 7). However, the same treatment given to prepubertally ovariectomized female rats markedly increased 2α-
and 16α-hydroxylations of testosterone to the levels of intact male rats. Testosterone 6β-hydroxylation also increased, but to a lesser extent. Neonatal administration of testosterone did not cause any significant effect on testosterone hydroxylations in adulthood.

However, the neonatal androgen imprinting caused a small facilitation effect on stimulation of the testosterone hydroxylations by postpubertal testosterone treatment.

**Effect of testosterone treatments on the activity of microsomal drug-metabolizing enzymes in intact adult, prepubertally ovariectomized or neonatally testosterone-imprinted female rats:** Testosterone treatment in adulthood did not produce any significant change of ethylmorphine N-demethylation, propoxycoumarin O-depropylation and benzo(a)pyrene hydroxylation in intact female rats. However, the same treatment of prepubertally ovariectomized rats markedly increased ethylmorphine N-demethylation and benzo(a)pyrene hydroxylation to the levels of intact male rats (Fig. 8). Propoxycoumarin O-depropylation also clearly increased, but to a lesser extent. Neonatal testosterone-treatment did not cause significant change or showed a tendency to decrease in these three activities. However, the additional treatment of these adult rats with testosterone slightly increased the activities.

On the other hand, the levels of aniline hydroxylation and O-ethylresorufin O-deethylation were not changed in prepubertally ovariectomized female rats by adult treatment with testosterone (Fig. 9). The neonatal testosterone treatment decreased the levels of aniline hydroxylation and O-ethylresorufin O-deethylation, and the testosterone treatment of these adult rats did not cause any further change in either of the enzyme activities. The level of testosterone 7α-hydroxylation was slightly decreased in

### Table 2. Effect of testosterone treatments on P-450-male and P-450-female in normal adult, prepubertally ovariectomized or neonatally testosterone-imprinted female rats

<table>
<thead>
<tr>
<th>Testosterone treatment</th>
<th>P-450-male</th>
<th>P-450-female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Neonatal T</td>
<td>&lt;0.01</td>
<td>0.20±0.03</td>
</tr>
<tr>
<td>Neonatal T</td>
<td>&lt;0.01</td>
<td>n.d.</td>
</tr>
<tr>
<td>Prepubertal ovarex</td>
<td>&lt;0.01</td>
<td>0.12±0.05</td>
</tr>
<tr>
<td>Prepubertal ovarex</td>
<td>0.15±0.03</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Fig. 7.** Effect of testosterone treatments on testosterone 2α-, 16α- and 6β-hydroxylations in intact adult, prepubertally ovariectomized or neonatally testosterone-imprinted female rats. O: ovariectomy at 21 days of age. Ta: adulthood testosterone treatment; Tn: neonatal testosterone treatment; Tn, Ta: neonatal and adulthood testosterone treatments. The results are expressed as means±S.D. from 3–5 rats.
the prepubertal ovariectomy group, and the adulthood testosterone treatment caused a further marked decrease in enzyme activity. Moreover, neonatal testosterone treatment markedly decreased testosterone 7α-hydroxylation to a level similar to that of adult male rats. The adulthood testosterone treatment of neonatally testosterone-treated rats seemed to cause a further decrease in testosterone 7α-hydroxylation.

These results indicate that the persistence and intensity of neonatal androgen imprinting is different depending upon the metabolic pathway.

**Discussion**

Mammalian liver cytochrome P-450's, are products of several gene families (45). Except for a few endogenous substrates, the oxidations of drugs and steroid hormones are catalyzed by several species of cytochrome P-450, although the extent of the contribution made by each cytochrome P-450 depends on the substrate used (19, 46). Sex-related differences of drug and steroid metabolism, gonadotropin secretion and sexual behavior in rats are believed to be determined primarily by events occurring in the neonatal period, when secretion of testicular androgens imprints a latent musculine potential, "neonatal imprinting", onto an otherwise feminine pattern. (12).

It is now clear that sex-related differences of drug and steroid oxidation are due to the presence of sex-specific cytochrome P-450, such as P-450-male and P-450-female and the very similar cytochrome P-450 (10, 21–23, 25, 47). In addition, other minor forms of sex-specific cytochrome P-450 have been reported to be involved in the sex-related differences (25–28, 48).

Neonatal imprinting on the oxidation of drugs and steroid hormones in rat liver has been widely studied (4, 5, 39–42). Previously, we reported neonatal imprinting of
P-450-male and P-450-female (16); and later, Waxman et al. (17) reported neonatal imprinting of P-450 2c/UT-A and P-450 2d/UT-I, corresponding to P-450-male and P-450-female, respectively, and of P-450 PB 2a/PCN-E. Castration at birth completely expunged the capability to express P-450-male and stimulated the expression of P-450-female in adulthood, but clear expression (44% of intact male level) of P-450-male was observed, if the rats were castrated at the age of 7 days (16). The administration of testosterone to neonatally castrated rats at 1, 3, 5 days after castration clearly restored the expression of P-450-male and completely suppressed the expression of P-450-female (16). Similar results have been reported on the expression of P-450 2c/UT-A, P-450 2d/UT-I and P-450 PB 2a/PCN-E (17). These results indicate that neonatal androgen imprinting on the expression of P-450-male is almost completed within 7 days after birth.

In the present studies, in contrast with any expectation based on the established concept of neonatal imprinting on the oxidation of drugs and steroid hormones (4, 5, 15, 39–43), we have observed an almost complete restoration of P-450-male in neonatally castrated rats by treatment with testosterone in adulthood (Fig. 1). Similarly, the levels of 2α- and 16α-hydroxylations of testosterone were completely restored in neonatally castrated rats by treatment with testosterone in adulthood (Fig. 2). Moreover, the levels of ethylmorphine N-demethylation, propoxy-coumarin O-depropylation and benzo(a)-pyrene hydroxylation were restored by treatment with testosterone in adulthood (Fig. 3). Thus these results clearly reflected the change of P-450-male content.

Although those results are not in accordance with most previous reports, very recent results reported by Dannan et al. (49) on sex-specific cytochrome P-450 and testosterone hydroxylations support our results. At the present time, we do not know the reason for the discrepancy found between our/Dannan’s results (49) and the previous reports (13–15, 39–43).

Prolonged lack of androgen in neonatally or prepubertally castrated rats caused a decreased responsiveness to testosterone treatment (Table 1). Moreover, the expression of P-450-female in 20 week-old male rats, which were castrated at birth or 20 days of age, was observed. This is in contrast with our previous results in which the expression of P-450-female in 8 week-old male rats castrated prepubertally was not detected.

These results indicate that neonatally castrated rats did not show a reduced responsiveness to testosterone during a certain period after castration, but a prolonged lack in androgen caused a reduced responsiveness to testosterone in neonatally or prepubertally castrated rats. The expression of P-450-male and P-450-female is clearly dependent on the secretion pattern of growth hormones (19, 20, 23, 47). Therefore, further studies will be needed to determine whether or not the altered expression of cytochrome P-450 is due to an altered state in growth hormone secretion (50–52).

Moreover, although testosterone treatment in adult female rats did not cause any significant change in the expression of P-450-male and P-450-female, the same testosterone treatment caused a slight decrease in the level of P-450-female in neonatally testosterone-imprinted female rats, and it caused a complete loss in P-450-female and the expression of P-450-male in prepubertally ovariectomized rats (Table 2).

In accordance with these results, 2α- and 16α-hydroxylation of testosterone and ethylmorphine N-demethylation, propoxy-coumarin O-depropylation and benzo(a)-pyrene hydroxylation were markedly stimulated by testosterone treatment in prepubertally ovariectomized rats (Figs. 7 and 8). Although neonatally testosterone-imprinted female rats did not show increased activities in testosterone hydroxylations and drug oxidations, testosterone imprinting slightly increased their responsiveness to the adulthood testosterone treatment.

Testosterone treatment also caused a marked decrease in testosterone 7α-hydroxylation in prepubertally ovariectomized rats and neonatally imprinted female rats (Fig. 9). To our surprise, the activity was also decreased in neonatally imprinted female rats without adult testosterone treatment. These results indicate that the presence and
intensity of neonatal androgen imprinting is different depending upon the metabolic pathway.

Recently, Dannan et al. (49) have observed that neonatally administered testosterone in neonatally ovariectomized rats caused a complete inhibition of the expression of P-450 2d/UT-I and a full expression of P-450 2c/UT-A and testosterone 16α-hydroxylase activity. These and our results indicate that the presence of the ovary may reverse the effect of neonatal imprinting on P-450-male, P-450-female, testosterone hydroxylation and drug oxidations except for testosterone 7α-hydroxylation, which may be highly susceptible to neonatal androgen imprinting.

All these results indicate that neonatal androgen imprinting is an event which is not permanently programmed. The effects may be modified by testosterone treatment in adulthood, by prolonged lack of androgen or by the presence of estrogen.

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