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A POSSIBLE PRIMARY ROLE FOR THE PITUITARY IN THE CONTROL OF SEX DEPENDENT DIFFERENCES IN HEPATIC FOREIGN COMPOUND METABOLISM IN THE RAT

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Abstract—The role of the pituitary in the control of the neonatally imprinted sex difference in the metabolism of a dimethylated chlorocyclodiene epoxide (DME)* was investigated. Hypophysectomy of adult female rats resulted in an effective masculinization of the hepatic metabolism of DME. Adrenalectomy, thyroidectomy, castration or hypophysectomy of adult male rats did not prevent liver enzymes from displaying a basically masculine pattern of DME metabolism. However, castration of male rats at one day old resulted in a basically feminine pattern of metabolism being displayed by liver enzymes in adult life. The effects of adult castration on the metabolism of DME and ethylmorphine by rat liver enzymes were completely reversed by testosterone propionate. The effects of combined hypophysectomy and castration on the hepatic metabolism of these substrates, however, were not reversed by testosterone treatment. These results suggest that sex dependent differences in the metabolism of foreign compounds by adult rat liver enzymes are mediated directly by the pituitary gland.

A role for testicular androgens in the control of sex differences in hepatic drug metabolism is well established [1–7]. Castration of adult male rats reduces the rates of metabolism of substrates that exhibit a sex difference [3, 4] and, conversely, treatment of adult female rats and of castrated adult male rats with testosterone increases the rates of metabolism of substrates whose metabolism is sex dependent [5, 6]. Theories put forward to explain observed sex differences in hepatic drug metabolism, involve a primary anabolic role for testicular androgens [1, 7], acting directly upon the liver so as to induce the higher levels of drug metabolism often seen in the adult male rat.

Recent work indicates, however, that the testes alone are not solely responsible for observed sex differences in drug metabolism. Castration of adult male rats has been shown to be ineffective in abolishing neonatally imprinted sex differences in the metabolism of DME [8], in the apparent K_m of ethylmorphine *N*-demethylase [9–11], and in the half life of cytochrome P-450 [12]. Furthermore, it has been shown that hepatic drug metabolizing enzymes of the rat respond to different extents to the stimulatory effects of testosterone [2, 9, 10]. For example, injections of testosterone propionate (3.8 mg/rat), on alternate days for four weeks, enhances ethylmorphine *N*-demethylase activity of adult male castrates five-fold, whereas the same treatment only doubles this enzyme activity in adult females [2]. This 'responsiveness' [9] of ethylmorphine *N*-demethylase has been shown to be dependent on the actions of neonatal testosterone [9, 10]. In addition, Kramer *et al.* [13] have reported that the stimulatory effects of testosterone on drug metabolizing enzymes are not seen in hypophysectomized rats. Further evidence implicating the pituitary in the control of sex differences in drug metabolism has been reported by Chung [10], who has postulated the existence of two novel (masculinizing and feminizing) hypophyseal factors, responsible for the maintenance and the regulation of neonatally imprinted sex differences in drug metabolism. Additional support for the existence of a pituitary 'feminizing factor' in the control of hepatic drug metabolism has been reported by Burke *et al.* [14], who have postulated that the control of sex differences in hepatic drug metabolism is similar, in certain respects, to the control of sex differences in hepatic steroid metabolism [15–17].

The work presented here describes the role of the pituitary in the control of the neonatally imprinted sex difference in the metabolism of DME [8]. Efforts are made to correlate previous data on neonatal imprinting [9, 10] with a possible primary role for the pituitary gland in the control of sex differences in drug metabolism.

MATERIALS AND METHODS

Animals and surgical procedures. Adult male and female Sprague–Dawley rats, CD strain were used. Adrenalectomized, thyroidectomized, gonadectomized or hypophysectomized rats were purchased from Charles River Animal Suppliers, Margate, Kent, U.K. All surgical procedures were performed under diethyl ether anaesthesia. Control animals received equivalent amounts of diethyl ether.

The effectiveness of hypophysectomy was monitored by direct visual examination of the pituitary

* Abbreviations used: DME, 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,7,8,8a - octahydro - 6,7-dimethyl-6,7-epoxy-1,4-methanonaphthalene; M1, M2, two metabolites of DME.

stalk under the median eminence at death. A significant decrease in both body weight and adrenal weight following surgery was noted in hypophysectomized rats. The effectiveness of adrenalectomy and thyroidectomy was monitored by direct visual post mortem examination. Animals were kept on a pellet and water diet *ad lib.* except for those hypophysectomized or adrenalectomized who received 5% (w/v) glucose or 0.9 (w/v) saline, respectively, in place of drinking water. Unless otherwise specified the time between surgical treatment and actual analysis of hepatic enzymes was four weeks.

Incubation procedures. Animals were decapitated and crude liver microsomal fractions consisting of 9000 $g \times 20$ min supernatants of the liver homogenate were prepared as previously described [8]. All drug metabolism studies were carried out using this 9000 $g \times 20$ min supernatant fraction. Incubation conditions for the determination of ethylmorphine *N*-demethylase and metabolism studies using DME were performed as previously described [8].

RESULTS

Table 1 shows the effects of adrenalectomy, thyroidectomy, ovariectomy or hypophysectomy on the metabolism of DME by adult female rat liver preparations. Hepatic microsomal enzymes from adrenalectomized, thyroidectomized or ovariectomized adult female rats produced small but detectable amounts of metabolite M2 (Fig. 1) not seen in the control female, but similar to those seen in the prepubertal female rat [8].Hypophysectomy of adult female rats, however, had the most pronounced effect on the metabolism of DME, in that, after removal of the pituitary gland, liver enzymes from the female rat produced metabolite M2 in quantities comparable to those found in the hypophysectomized male (Table 2). Typical chromatograms are shown in Figs. 1 and 2.

Adrenalectomy, adult castration, thyroidectomy or hypophysectomy of adult male rats (Table 2; Fig. 2) did not prevent hepatic enzymes from the male

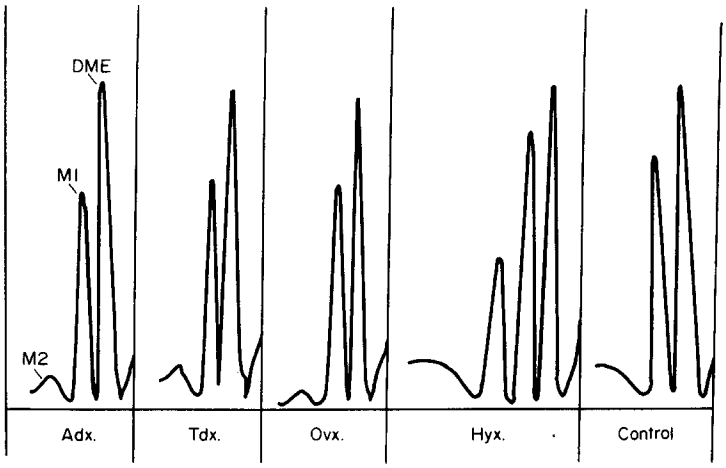

Fig. 1. Gas chromatograms typical of those obtained from four or more independent experiments showing the effect of: ovariectomy, Ovx; thyroidectomy, Tdx; adrenalectomy, Adx; or hypophysectomy, Hyx, on the metabolism of DME by adult female rat liver enzymes.

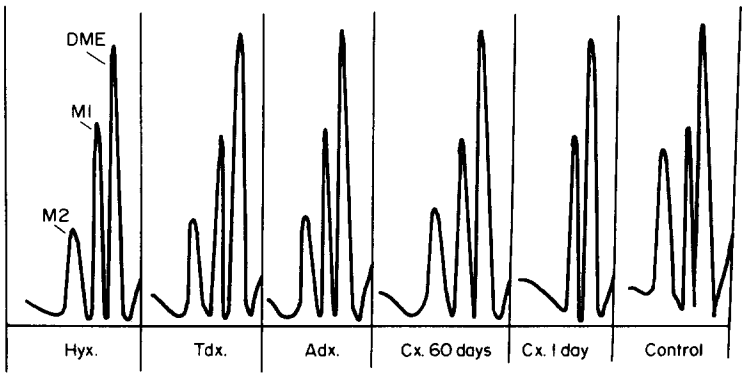

Fig. 2. Gas chromatograms showing the effect of: hypophysectomy, Hyx; thyroidectomy, Tdx; adrenalectomy, Adx; and castration, Cx, of 60 day old and 1 day old male rats, on the metabolism of DME by adult male rat liver enzymes. Results are typical of those obtained from four or more independent experiments.

Table 1. Effects of surgical treatments on the metabolism of DME by adult female rat liver 9000 $g \times 20$ min supernatant fraction*

	DME Metabolite peak area ratio M2/M1
Adrenalectomy	0.03 ± 0.01
Thyroidectomy	0.03 ± 0.01
Ovariectomy	0.04 ± 0.01
Hypophysectomy†	0.93 ± 0.06†
Control female	0‡

* Values represent mean ± S.E. for four independent experiments except for † which represents eight independent experiments and ‡ which represents more than twenty independent experiments. A metabolite peak area ratio of 0 denotes no detectable metabolite M2 formed. Typical chromatograms are shown in Fig. 1.

Table 2. Effects of surgical treatment on the metabolism of DME by adult male rat liver 9000 $g \times 20$ min supernatant fraction*

	DME Metabolite peak area ratio M2/M1
Adrenalectomy	1.11 ± 0.05
Thyroidectomy	1.25 ± 0.05
Castration (60 days)	1.04 ± 0.03
Hypophysectomy	0.95 ± 0.06
Castration (1 day)	0
Control male	1.48 ± 0.07

* Values represent mean ± S.E. for four independent experiments. A metabolite peak area ratio of 0 denotes no detectable metabolite M2 formed. Typical chromatograms are shown in Fig. 2.

producing metabolite M2, although a decrease in metabolite peak area ratio M2/M1 was noted. Castration of male rats at one day old, however, resulted in hepatic enzymes from the adult male rat exhibiting a basically feminine pattern of metabolism of DME (Fig. 2) in that only metabolite M1 was produced in detectable quantities.

Table 3 shows the effects of combined castration and hypophysectomy on the metabolism of DME and ethylmorphine. Castration of adult male rats reduced rates of metabolism of ethylmorphine and decreased the metabolite peak ratio for DME. The effects of castration alone on both these parameters were fully reversed by treatment with testosterone propionate. Combined castration and hypophysectomy af adult males reduced the activity of hepatic ethylmorphine *N*-demethylase even more than did castration alone, and also further reduced the metabolite peak area ratio for DME. However, the effects of combined castration and hypophysectomy, unlike those of castration alone, were not reversed by testosterone treatment.

DISCUSSION

The data presented here support previous suggestions [10, 13, 14, 16] that the pituitary plays a major role in the control of sex differences in drug metabolism by liver enzymes of the rat. Hypophysectomy of adult female rats results in an effective masculinization of hepatic metabolism of DME (Table 1), in that both metabolites M1 and M2 are produced after removal of the pituitary. It can be postulated that the 'masculinization' of DME metabolism following hypophysectomy is due to the removal of a pituitary factor(s), presumed to be present in the normal female, and which, when present, prevents the formation of metabolite M2. Adrenalectomy, thyroidectomy or ovariectomy of female rats had a similar, but far less pronounced, effect on the metabolism of DME, but are known to have opposite effects to hypophysectomy on plasma concentrations of ACTH, TSH, FSH and LH [18]. This would appear to militate against mediator roles for ACTH, TSH, FSH and LH.

The concept of the existence of a pituitary factor acting to feminize hepatic microsomal metabolism in the female rat is not new. The work of Gustaffson and co-workers [15, 16, 19–21] has indicated the presence of a novel pituitary factor 'feminotropin' that acts to 'feminize' the hepatic microsomal metabolism of steroids in the rat. 'Feminotropin' has been shown to be distinctly different from GH, LH, FSH, TSH and from prolactin [16], and has been impli-

Table 3. Effects of combined hypophysectomy and castration on the metabolism of DME and ethylmorphine by adult male rat liver 9000 $g \times 20$ min supernatant fraction*

	DME Metabolite peak area ratio M2/M1	Ethylmorphine (nmoles HCHO/min/g liver
Control male	1.48 ± 0.06	186 ± 6
Castrated male	1.04 ± 0.03	81 ± 1
Castrated male + TP†	1.52 ± 0.04	190 ± 3
Castrated + hypophysectomized male	0.90 ± 0.05‡	56 ± 2‡
Castrated + hypophysectomized male + TP†	0.88 ± 0.05	55 ± 1

* Values represent mean ± S.E. of four independent experiments.

† Rats were injected with testosterone propionate (TP) 2 mg/day on alternate days for two weeks.

‡ Statistically significant difference from castrated male at $P < 0.05$.

cated in the control of drug metabolizing enzymes [14]. Evidence presented in Table 1 suggests that the apparent qualitative sex difference in the metabolism of DME is under the control of a similar if not identical pituitary factor to that which controls the metabolism of steroids by the female rat liver microsomes.

Table 2 shows the effects of surgical treatments on the metabolism of DME by adult male rats. Hypophysectomy, adrenalectomy, thyroidectomy or adult castration was unable to prevent the formation of metabolite M2 by liver enzymes from the adult male rat, although a decrease in the metabolite peak area ratio M2/M1 was observed in each case. In agreement with previous observations [8], castration at one day old totally prevented the formation of metabolite M2 in adult life. This observation further supports previous suggestions [17, 20] that the presence of a 'feminizing factor' in adult life is determined by the absence of androgens during the neonatal period. The presence of testicular androgens during this period can be said to masculinize a basically feminine pattern [17]; the absence of testicular androgens during the neonatal period resulting in the basically feminine pattern being retained in adult life. This scheme (a basically feminine pattern becoming masculine by the action of neonatal androgens) is in agreement with current theories on sexual differentiation [22–26] whereby bipotential organs deviate from a basically feminine pattern first by a regression of female characteristics and then by a development of masculine potentialities. These developmental changes can be divided into two aspects. Firstly, the appearance of new functions, and secondly, a process of facilitation whereby certain parts of the body become (more) sensitive to testosterone in adult life [26].

The results shown in Table 3 agree with the findings of Kramer *et al.* [13]. Testosterone was found to have no effect on the hepatic metabolism of xenobiotics in hypophysectomized animals. One possible explanation for this observation is that androgenic effects on drug metabolism are mediated directly via the pituitary gland. Evidence has been presented for the existence of a masculinizing factor, secreted by the male pituitary, that controls the metabolism of steroids [16] and of foreign compounds [10]. In the present context a masculinizing factor could account for:

(1) the absolute requirement of the pituitary gland for the expression of stimulatory androgenic effects on foreign compound metabolism; and

(2) differing levels of responsiveness of drug metabolizing enzymes to the actions of androgens in adult life [2, 9, 10], differences that arise as a result of neonatal exposure to testosterone [9, 10].

It can be postulated that in addition to preventing the secretion of a 'feminizing factor' in adult life, neonatal androgens also facilitate the ability to secrete a counterpart masculinizing factor. A masculinizing pituitary factor can be envisaged as mediating androgenic effects on hepatic drug metabolism by a positive feedback to the pituitary. The responsiveness of liver enzymes to androgens in adult life would therefore be dependent on the ability to secrete a masculinizing factor, which is in turn determined by neonatal androgens. This need not preclude a quantitative rather than a qualitative relationship between male and female rats, since the effects of neonatal androgens need not be all-or-none, but could include the possibility of a gradation of responsiveness, according to the level of exposure to androgens.

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