Biochemical Pharmacology, Vol. 29, pp. 2759–2762.

0006-2952/80/1015-2759 $02.00/0

THE EFFECTS OF GONADECTOMY AND HYPOPHYSECTOMY ON THE METABOLISM OF IMIPRAMINE AND LIDOCAINE BY THE LIVER OF MALE AND FEMALE RATS

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(*Received* 7 *March* 1980; *accepted* 31 *May* 1980)

Abstract—The sex differences in the hepatic metabolism of imipramine and lidocaine were studied in relation to the effect of gonadectomy and hypophysectomy on these differences. It was found that gonadectomy in male animals led to a more feminine pattern of metabolism. Hypophysectomy mimicked this effect except in the case of lidocaine *N*-deethylation which was unaffected by hypophysectomy. Castration/hypophysectomy gave a similar result to hypophysectomy alone. These data indicate a gonadal control of metabolism in the male, except for lidocaine *N*-deethylase, which is under more complex control via the pituitary gland. In the female, ovariectomy was without effect while hypophysectomy caused a masculinization of hepatic metabolism, indicating a dominant role of the pituitary in the control of drug metabolism in the female. This agrees well with reports of hepatic steroid metabolism and lends further support to the hypothesis of a pituitary 'feminizing factor' secreted by the female. The nature of the 'feminizing factor' is, as yet, unknown.

Sex differences exist in the metabolism of many substrates by the hepatic microsomal monooxygenase system in the rat [1–6]. The differences have been attributed entirely to the presence of androgen in the male [3–7]. Studies on the metabolism of steroids in rat liver, both from this laboratory and others [8–14], have shown a control of liver microsomal metabolism by the testes in the male and the pituitary gland in the female. Evidence has also been presented that indicates a mediating role of the pituitary gland in the gonadal control in the male [13]. The existence of a novel pituitary hormone, 'feminizing factor', has been postulated to account for the effects of hypophysectomy on steroid metabolism in the female rat [13, 15, 16].

Pituitary hormones are known to affect drug metabolism. Wilson [17–19] showed a change in the developmental pattern of drug metabolism following transplantation of a pituitary tumour and subsequently showed that the effect was due to growth hormone, and Burke *et al.* [20] indicated that hypophysectomy could modify the induction of drug metabolism by 3-methylcholanthrene.

The drugs chosen for this study, lidocaine (a local anaesthetic and antiarrhythmic) and imipramine (an antidepressant) have a wide range of metabolites which are easily separated, are cheap and are readily available in radio-labelled form. They thus make good substrates in an investigation of drug metabolism. The clinical relevance of drug metabolism is also more apparent when using commonly used drugs.

This project was designed to investigate the applicability of the steroid metabolism model discussed above to drug metabolism and, in particular, to answer the following questions:

(1) Does the pituitary control drug metabolism?
(2) Is the known effect of androgens on drug metabolism mediated via the pituitary gland?

METHODS

Animals of the Sprague–Dawley strain, seven weeks of age at the start of the treatment, were used throughout the study. The following groups of animals were used: in experiment 1, control males, castrated males, hypophysectomized males and castrated/hypophysectomized males and in experiment 2, control females, ovarectomized females and hypophysectomized females. The operations were carried out under ether anaesthesia and control animals were sham-operated under the same conditions. The animals were maintained, five to a cage, in a light- and temperature-controlled room (lights on 6:00 a.m.–8:00 p.m.; 23 ± 1°) and allowed free access to standard laboratory diet and water or saline/glucose (0.9% sodium chloride, 10% glucose) solution, in the case of the hypophysectomized animals. The animals were kept for 14 days following the operation before incubation. This time period has previously been found to be adequate for the complete effect of the operations to be seen [21, 22].

On the morning of the incubation, the animals were taken from the animal house and immediately killed by a blow on the head followed by exsanguination. The liver was quickly excised and rinsed in 20 ml ice-cold Bucher medium [23]. The animals were examined visually for success of the operation and suspect animals rejected. The liver was finely chopped and homogenized in 4 vol. Bucher medium using a Potter–Elvehjem homogenizer fitted with a teflon pestle. The homogenate was separated into

its component fractions by differential centrifugation as previously described [24, 25]. The microsomal pellet was resuspended in Bucher medium and kept on ice until needed for the incubation.

One millilitre of microsomal suspension (containing 5–8 mg of protein) was incubated with [^{14}C]imipramine hydrochloride (Radiochemical Centre, Amersham, U.K.) (2×10^5 d.p.m. containing 2 μmoles of imipramine) or [carbonyl-^{14}C]lidocaine hydrochloride (New England Nuclear, Winchester, U.K.) (2×10^5 d.p.m. containing 0.45 μmoles of lidocaine) in the presence of an NADPH-regenerating system [24] in a final volume of 3 ml. The incubation was allowed to proceed for 60 min at 37° and the reaction was terminated by the addition of 200 μl of 10 N sodium hydroxide solution followed by 5 ml of extraction solvent (dichloroethane for imipramine and chloroform for lidocaine). The mixture was agitated for 10 min on a rotary shaker and the two phases separated by centrifugation for 10 min at 1500 r.p.m. in an MSE Mistral 4L refrigerated centrifuge. The lower, organic layer was removed and the procedure repeated three times. The solvent was removed by rotary evaporation under reduced pressure using a Büchi ROTAVAPOR-R and the residue redissolved in a small quantity (< 200 μl) of chloroform. The resultant solution was applied to a Merck thin-layer chromatography (t.l.c.) plate (silica gel F-254) as a 1.5–2 cm band. The plates were developed once in solvents comprising chloroform–1-propranol–ammonia (50:50:1) for imipramine and chloroform–methanol (9:1) for lidocaine. Radioactive bands were localized and quantitated using thin-layer scanning on a Berthold Model II scanner (Berthold, Winblad, F.R.G.) or autoradiography followed by scintillation counting [24]. Metabolites were identified by their t.l.c., gas chromatographic and mass spectrometric properties as compared to authentic standards obtained from Astra Läkemedel AB, (lidocaine, monoethylglycylxylidide and 3-hydroxylidocaine) and Ciba-Geigy, Basle, Switzerland (imipramine, desmethylimipramine, didesmethylimipramine, 2-hydroxyimipramine and imipramine *N*-oxide).

Protein concentrations were determined using the method of Lowry *et al.* [26] with bovine serum albumin as standard.

Statistical analysis was by the Duncan Multiple Range Test and the level of significance set at $P < 0.05$.

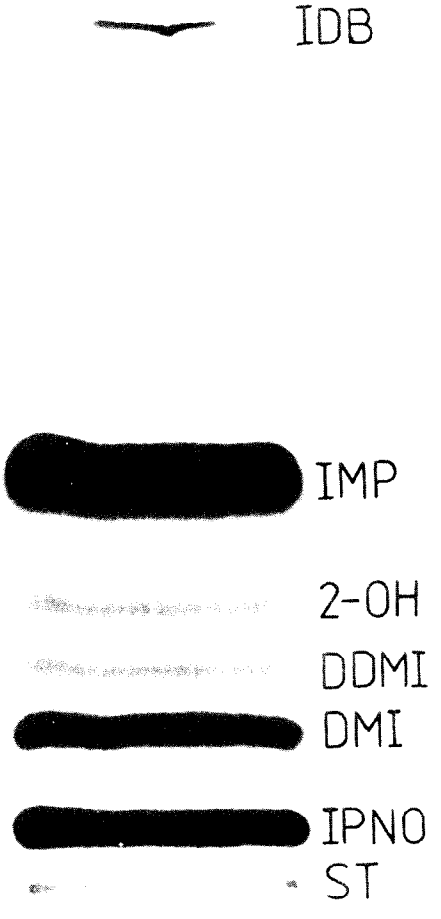

Fig. 1. Autoradiograph of the metabolites of [^{14}C] imipramine following separation on a t.l.c. plate as described in Methods. IDB = iminodibenzyl, IMP = imipramine, 2-OH = 2-hydroxyimipramine, DDMI = didesmethylimipramine, DMI = desmethylimipramine, IPNO = imipramine *N*-oxide, ST = start band.

RESULTS AND DISCUSSION

Incubation of imipramine and lidocaine with the microsomal fraction of rat liver resulted in the formation of the following metabolites at a linear rate for at least 1 hr: for imipramine (IMP) (see Fig. 1), iminodibenzyl (IDB)—a non-enzymic breakdown product, 2-hydroxyimipramine (2OH) didesmethylimipramine (DDMI), desmethylimipramine (DMI) and imipramine-*N*-oxide (IPNO); for lidocaine (LID) (see Fig. 2), 3-hydroxylidocaine (3OH-LID) and monoethylglycylxylidide (MEGX)—the *N*-deethylated product.

In experiment 1 (Table 1), castration of male animals caused a significant decrease in imipramine *N*-oxidase and *N*-demethylase activity and lidocaine *N*-deethylase activity while having no effect on 2-hydroxylation of imipramine or 3-hydroxylation of lidocaine. If the values for the control male in experiment 1 and the control female in experiment

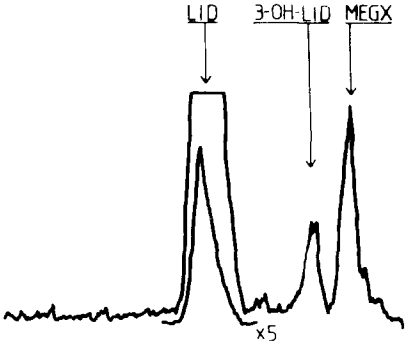

Fig. 2. Thin-layer scanning of the metabolites of [^{14}C] lidocaine following separation as described in Methods. LID = lidocaine, 3OH-LID = 3-hydroxylidocaine, MEGX = monoethylglycylxylidide.

Table 1. The effect of castration and/or hypophysectomy on the metabolism of lidocaine and imipramine by male rat liver

	Imipramine			Lidocaine	
	N-Oxidase	*N*-Demethylase	2-Hydroxylase	*N*-Deethylase	3-Hydroxylase
Control males (A)	1.06 ± 0.03 (4)*	1.65 ± 0.11	0.43 ± 0.02	1.78 ± 0.33	0.21 ± 0.03
Castrated males (B)	0.78 ± 0.18 (5)	0.83 ± 0.16	0.48 ± 0.04	0.88 ± 0.20	0.25 ± 0.02
Hypophysectomized males (C)	0.86 ± 0.11 (4)	1.13 ± 0.12	0.48 ± 0.07	1.50 ± 0.04	0.29 ± 0.06
Castrated and hypophysectomized males (D)	0.70 ± 0.20 (5)	0.87 ± 0.17	0.48 ± 0.06	1.48 ± 0.09	0.48 ± 0.16
	D B C A †	D B C A	A B C D	B D C A	A B C D

* Figures in brackets are the numbers of animals in group. Results expressed as nmoles product/min/mg protein—mean ± 1 S.D.

† Group means arranged in rank order. Those groups underlined are not significantly different from one another ($P > 0.05$).

2 (Table 2) are compared, it can be seen that castration of the male animals led to a more feminine pattern of metabolism but that the change was not complete. The sex differences seen in this study were similar to those reported by Bickel *et al.* [27, 28] and von Bahr *et al.* [29, 30] for imipramine and lidocaine, respectively. Hypophysectomy of male animals caused a similar decrease to castration in imipramine *N*-oxidase and *N*-demethylase activities (although not as marked as castration for the *N*-demethylase) but had no effect on lidocaine *N*-deethylation. Combined castration/hypophysectomy was also without effect on lidocaine *N*-deethylation while affecting imipramine *N*-oxidase and *N*-demethylase activities in the same manner as castration alone. These results indicate that, in the case of *N*-oxidation and *N*-demethylation of imipramine, hypophysectomy was mimicking the effect of castration. Hypophysectomy leads to a lowering of blood lutropin and follitropin levels and subsequent hypertrophy of the testes and decrease in blood androgen levels. Thus, in this case hypophysectomy can be seen as an indirect castration. The primary control, therefore, was via the testes and agrees with the accepted gonadal control of drug metabolism [3–7]. This was not the case for the *N*-deethylation of lidocaine, which was decreased by castration but not by hypophysectomy or hypophysectomy/castration. These data might suggest a vital role for the pituitary in the effect of castration on lidocaine *N*-deethylase. A similar phenomenon has been reported for the effect of castration and hypophysectomy on 4-androstene-3,17-dione metabolism by rat liver [13]. The effect of castration was explained as the removal of a negative feedback of androgen on the release of a pituitary factor, an effect which cannot be seen in a hypophysectomized animal. The feedback was thought in previous reports to be directed towards a pituitary 'feminizing factor', a concept that will be further discussed below. Another possible explanation for these results is the antagonism at the level of the liver of a pituitary hormone by androgens. Removal of the testes allows free expression of the 'feminizing' effect while hypophysectomy removes both the androgen and the pituitary hormone, therefore giving no effect. Lidocaine 3-hydroxylase was seen to be unaffected by castration or hypophysectomy alone but significantly elevated by the combined operation. These data indicate a dual suppression of 3-hydroxylase activity in the intact animal by a testicular and a pituitary mechanism.

In experiment 2, ovariectomy was seen to have no

Table 2. The effect of ovariectomy and hypophysectomy on the metabolism of lidocaine and imipramine by female rat liver

	Imipramine				
	N-Oxidase	*N*-Demethylase	2-Hydroxylase	*N*-Deethlyase	3-Hydroxylase
Control females (A)	0.37 ± 0.06 (5)*	0.66 ± 0.08	0.63 ± 0.10	0.21 ± 0.05	0.32 ± 0.06
Ovarectomized females (B)	0.35 ± 0.07 (4)	0.58 ± 0.14	0.70 ± 0.08	0.27 ± 0.09	0.38 ± 0.18
Hypophysectomized females (C)	0.75 ± 0.02 (4)	0.96 ± 0.03	0.64 ± 0.20	0.66 ± 0.08	0.35 ± 0.11
	B A C †	B A C	A C B	A B C	A C B

* See Table 1.

† See Table 1.

effect on the hepatic metabolism of imipramine or lidocaine, indicating that estrogens have no effect on liver mono-oxygenase activity. Hypophysectomy of female animals, however, led to a significant increase in imipramine *N*-oxidase and *N*-demethylase and lidocaine *N*-deethylase activities. All of these changes can be construed as a move towards a more masculine pattern of metabolism. In fact, the values obtained for the hypophysectomised females are similar to those for the castrated males except in the case of the imipramine 2-hydroxylase which is little affected by any of the treatments studied, indicating a possible genetic determination of this enzyme activity. These results indicate the dominant role of the pituitary in the control of hepatic drug metabolism in the female.

Overall, the results presented in this study indicate the existence of at least two types of control of hepatic drug metabolism in the rat. In the male, the testes are the dominant factor acting either directly or indirectly on the liver. At least part of the testicular effect appears to be indirect via the pituitary gland. In the female the pituitary gland is the dominant controlling influence and the ovaries are not involved, although the involvement of other endocrine organs under the control of the pituitary gland cannot be ruled out. Removal of the testes in the male and the pituitary gland in the female leads to a nondifferentiated pattern in metabolism.

This is a similar situation to the metabolism of steroids by the endoplasmic reticulum in the rat liver [7–16]. Evidence exists that steroid metabolism in the rat is controlled by a pituitary 'feminizing factor', which is under tonic inhibitor control from the hypothalamus in the male and thus normally secreted only in the female, and that gonadal steroids have their effects via an effect of 'feminizing factor' secretion [13]. It has been shown using both a hepatoma tissue culture assay system and *in vivo* techniques that 'feminizing factor' is not one of the known pituitary hormones [31–34]. The data presented in this study suggest that this hypothesis may also be applied to drug metabolism, although for the *N*-oxidation and *N*-demethylation of imipramine there would appear to be a gonadal control not acting via the pituitary gland.

It would seem, therefore, that steroid and drug metabolism are controlled in a similar way—via a gonadal influence in the male and a pituitary influence in the female. The possibility that 'feminizing factor' or other pituitary hormones may be active on drug metabolism is under investigation. Further work is also necessary to establish whether androgens are the gonadal influence operating in this study and, in the case of lidocaine *N*-deethylase activity, if the gonadal effect is mediated via the pituitary gland.

Acknowledgements—This work was supported by grants from the Swedish Medical Research Council (13X-2819) and the University of Glasgow Medical Research Fund.

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