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On developmental changes in pulmonary and hepatic monooxygenase activities of the rat

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Evidence accumulated from several studies suggests that the controls of monooxygenase activities in the various organs of the rat are probably tissue-differentiated. Among these studies are those of Oppelt *et al.* [1], Lake *et al.* [2], and Litterst *et al.* [3] which demonstrated that phenobarbital pretreatments of rats increase hepatic and nephric monooxygenase activities but produce no change in the corresponding pulmonary activities. Histologically differentiated effects similar to those produced by phenobarbital pretreatments of rats have also been shown for pretreatments with methyltestosterone, spironolactone or pregnenolone-16 α-carbonitrile [4]. More lately, Al-Turk *et al.* [5–8] have shown that gonadectomy, estrogen-pretreatments, hypophysectomy or streptozotocin-induced diabetic states produce histologically differentiated effects on monooxygenase activities in rats.

In the present communication, we provide data which seem consistent with these earlier observations. Monooxygenase activities of liver and of lung microsomes from rats aged 1–10 weeks were studied using DME*, ethylmorphine and aniline as substrates in order to compare temporal changes in hepatic and pulmonary activities. This study was stimulated by the apparently striking observation that the ability of female rat liver enzymes to form an oxidation product, designated M_2, from DME retrogresses to undetectable limits before adulthood while the corresponding activity for lungs is retained. Earlier reports upon this substrate (DME) have shown that the metabolite M_2 is the major product formed by adult male rat liver enzymes and that the ability of the female rat liver to form the same product appears to be lost during adolescence [9–11].

Methods

Sprague–Dawley rats (C.D. Strain) aged 1–10 weeks were killed by decapitation. Liver and lung microsomes were prepared as described by Litterst *et al.* [12] except that we used KCl where the previous authors used sucrose. Microsomal protein was determined by the method of Lowry *et al.* [13].

Ten microlitres of ethanolic solution containing 250 nmoles DME were incubated with shaking (0.8 c/s) at 36° for 4 min (liver) or 6 min(lung) in 2.5 ml aqueous medium (pH 7.2) containing 200 μmoles sodium phosphate buffer, 300 μmoles KCl, 20 μmoles G-6-P, 2 μmoles NADP, 1.6 units glucose-6-phosphate dehydrogenase and 0.4 mg liver microsomal protein or 1 mg lung microsomal protein. The reactions were stopped by the addition of 1 ml acetone. The mixtures were then extracted twice with *n*-hexane and the amount of each product formed was estimated by g.l.c. [10].

Assays involving aniline were performed as follows: 15 μmoles aniline were incubated at 36° with shaking (0.8 c/s) for 10 min (liver) of 30 min (lung) with 3 mg microsomal protein in 3 ml of aqueous reaction mixture containing 300 μmoles sodium phosphate buffer (pH 7.2), 300 μmoles KCl, 30 μmoles G-6-P, 3 μmoles NADP, and 2 units glucose-6-phosphate dehydrogenase. Aniline hydroxylase activity was determined by the method of Imai *et al.* [14] in which *p*-aminophenol production is monitored.

Incubation mixtures for the assay of ethylmorphine demethylase activity contained the following components in a final volume of 6 ml: 60 μmoles ethylmorphine, 5 mg microsomal protein, 600 μmoles sodium phosphate buffer (pH 7.2), 600 μmoles KCl, 50 μmoles semi-carbazide, 30 μmoles G-6-P, 3 μmoles NADP and 2 units glucose-6-phosphate dehydrogenase. Incubations at 36° with shaking (0.8 c/s) were for 10 min (liver) or 20 min (lung). The demethylase activity was determined by estimation of formaldehyde formed using the method of Nash [15].

In all the assays described above, the reactions are linear with time for the periods stated.

Results and discussion

Temporal changes in hepatic formation of *p*-aminophenol and metabolite M_1 from aniline and DME respectively are broadly similar in pattern, and they show no marked sex differences (Table 1). Developmental patterns in the corresponding pulmonary activites also compare well between the reactions and between the sexes. However,

* Abbreviations: 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. M_1, M_2: two monohydroxylated derivatives formed from DME.

Table 1. Monooxygenase activities (nmole product formed /min/mg microsomal protein)

Age of rat (weeks)	Sex of rat	Liver				Lung			
		M_1*	M_2*	PAP†	HCHO‡	M_1	M_2	PAP†	HCHO‡
1	F	2.9 ± 0.3	1.8 ± 0.3	1.1 ± 0.2	1.6 ± 0.2	0.3 ± 0.0	0.2 ± 0.0	(n.d)	(n.d)
	M	3.2 ± 0.4	1.5 ± 0.4	0.9 ± 0.3	1.8 ± 0.2	0.3 ± 0.0	0.2 ± 0.0	(n.d)	(n.d)
4	F	14.8 ± 1.1	2.8 ± 0.4	1.5 ± 0.2	6.4 ± 0.5	1.6 ± 0.4	0.7 ± 0.1	<0.1	0.4 ± 0.0
	M	10.4 ± 0.8	5.2 ± 0.7	1.4 ± 0.2	9.2 ± 1.0	1.5 ± 0.3	0.6 ± 0.0	<0.1	0.5 ± 0.2
7	F	10.6 ± 0.8	(n.d)	1.3 ± 0.1	5.2 ± 0.3	2.4 ± 0.4	0.8 ± 0.3	~0.1	0.9 ± 0.1
	M	8.8 ± 0.6	10.9 ± 1.0	1.3 ± 0.2	12.7 ± 0.8	1.8 ± 0.6	0.7 ± 0.2	~0.1	0.7 ± 0.3
10	F	11.2 ± 1.1	(n.d)	1.2 ± 0.1	5.0 ± 0.3	2.9 ± 0.5	0.7 ± 0.3	~0.2	1.3 ± 0.3
	M	8.6 ± 0.7	11.2 ± 0.8	1.3 ± 0.6	12.4 ± 0.6	3.2 ± 0.4	0.7 ± 0.2	~0.2	1.5 ± 0.2

The data represent mean ± S.D. for three independent experiments using 3–11 rats per age group.
F = female rat.
M = male rat.
* = monohydroxylated derivative formed from DME.
† = *p*-aminophenol formed from aniline.
‡ = formaldehyde formed from ethylmorphine.
(n.d.) = not detectable.

an organ difference in developmental programme is apparent for each of the reactions. The hepatic activities undergo a temporal decline between the fourth and the tenth weeks *post partum*, but the corresponding pulmonary activities increase during the same period.

Although the rate of pulmonary formation of formaldehyde and metabolite M_2 from ethylmorphine and DME respectively are sexually identical at all ages, corresponding hepatic activities show profound sex differences as from 4 weeks of age (Table 1). These hepatic sex differences are in general characterized by age-related increase in activities for male rats but a decrease or no change in the case of the females. It is particularly striking that while the ability of female rat liver to form the metabolite M_2 is apparently lost before the age of 7 weeks, the corresponding pulmonary activity remains stable with age at the level attained in the fourth week from birth.

These results demonstrate both histological and sexual differentation in the post natal development of monooxygenase activities. For each of the four reactions studied, a histological difference in developmental programme occurs, but the age-dependent appearance of sex difference is reaction-specific and liver-directed. The pattern in hepatic sex difference in the rat is thought to be underlined by two physiological phenomena: (i) imprinting of masculine patterns by androgens and (ii) feminization of the same pattern by a process requiring a hypophyseal feminizing factor [16]. Although it is not conclusive from our data that these particular hormonal controls are primarily liver-specific, it is reasonable to suspect that they are. This suspicion is augmented by the fact that sex hormones or their analogues produce histologically differentiated effects upon hepatic and pulmonary monooxygenase activities [4, 6] as does hypophysectomy [8] or gonadectomy [5].

Summary

Organ-differentiated pattern in the effects of (i) chemical pretreatment of rats and (ii) *in vivo* perturbance of endocrine functions on monooxygenase activities of hepatic and extra-hepatic tissues are reviewed. The pattern in these effects together with our data, which demonstrate organ differences in the pattern of postnatal development of hepatic and pulmonary monooxygenase activities, would indicate that physiological regulations of these activities differ between organs.

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