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DRUG MONOOXYGENASE ACTIVITY IN THE HARDERIAN GLAND

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Abstract—Drug monooxygenase activity with 7-ethoxycoumarin as a substrate was found in homogenates of the Harderian gland in mice and hamster, but not in rabbits and rats. The inhibition by carbon monoxide, the induction by phenobarbital and the high inhibition by metyrapone suggests the presence of a cytochrome P450-system similar to that of liver after pretreatment with phenobarbital. Pretreatment with 3,4-benzo(a)pyrene did not increase the activity in two strains of mice.

The size of the Harderian gland (glandula palpebrae tertiae) varies in different animals (review see [1]). The largest glands are found in the orbita of rodents, whereas in primates and humans this organ is completely absent. Although there are many reports in discussing the biological role of the gland, its physiological function is still obscure. A predominant feature is the high content of porphyrins [2, 3] and enzyme activities concerning the metabolism of steroids have been reported [4–8]. After application of labelled drugs rather high concentrations of metabolities can be detected by autoradiography (A. Garbe, Fa. E. Merck, unpublished) and for this reason we have studied the occurrence of drug metabolizing activities in the Harderian gland. Since the gland is phyllogenetically quite different from the liver we were interested whether drug metabolism in this organ also involves the cytochrome P450-dependent drug monooxygenase activity [9] which so far has been found in small intestine, lung, skin and kidney. This system is part of the endoplasmic reticulum and consists of a NADPH-dependent reductase and several cytochrome P450 species [10–15] which are characterized by a different but overlapping substrate specificity. The level of each cytochrome P450 is regulated by the rate of synthesis and the rate of breakdown, both of which can be affected by a spectrum of lipophilic compounds acting as inducers [16]. This paper reports the existence of a drug monooxygenase activity in the Harderian glands of mice and hamsters and describes the use of inhibitors and inducers to characterize the system in comparison to the well-known activity in the liver.

MATERIALS AND METHODS

Female adult animals of the following species were used: NMRI mice, black mice (strain C57 BL/6N), Wistar rats, New Zealand rabbits and Syrian hamsters. NADPH was purchased from Boehringer, Mannheim, GFR, 7,8-benzoflavone from EGA-Chemie KG, Steinheim, GFR and tetrahydrofurane from Merck, Darmstadt, GFR, Metyrapone was a gift of Ciba–Geigy, Basle, Switzerland.

For the induction experiments phenobarbital (sodium salt) and 3.4-benzo(a)pyrene were dissolved in water and sunflower oil, respectively, and administered intraperitoneally (phenobarbital once daily at a dose of 80 mg/kg over 3 days, 3.4-benzo(a)pyrene at a dose of 20, 40 and 80 mg/kg over 2 days). The animals were killed by decapitation, the livers were removed immediately and the Harderian glands were carefully prepared. The organs of five mice and two of the other species were pooled for each preparation. All producers were carried out at 4°. The Harderian glands and the livers were washed in icecold 0.1 M Tris–HCl buffer pH 7.6 and homogenized in icecold 0.01 M Tris–HCl buffer pH 7.6. Protein was determined according to Lowry [17]. The O-dealkylating activity of the homogenates was measured with 7-ethoxycoumarin as a substrate as described by Ullrich and Weber [18]. A Zeiss PMQ II spectrophotometer with fluorescence attachment was used.

RESULTS AND DISCUSSION

The fluorometric test for umbelliferone formation from 7-ethoxycoumarin allows a direct recording of the monooxygenase activity even in tissue homogenates with high turbidity. No activity was found in the Harderian glands of rabbits and rats, however, both strains of mice and the Syrian hamsters showed O-dealkylating activities in this organ (Table 1).

Carbon monoxide inhibits the activity to the same extent as in liver indicating the involvement of the cytochrome P450 system. In view of the various species of cytochrome P450 it was interesting to characterize the activity with respect to some biochemical parameters which have been used for liver preparations [13, 19, 20]. The pH optimum in mice was around 8.0, but 7.4 in the Syrian hamster. A rather high K_m-value of 2–3 × 10^{-4} M was found in

Table 1. 7-ethoxycoumarin O-dealkylation activity in the Harderian glands of various animals. The K_m values were obtained by Lineweaver–Burk plots. Metyrapone was added as a 10^{-5} M solution in water, 7.8-benzoflavone (10^{-5} M) was dissolved in a medium containing 30 mg albumin/ml. The inhibition by carbon monoxide was performed by gassing the cuvette with a CO–O_2-mixture (2:1, v/v) for 20 sec. The values are averages of three experiments from a preparation containing the glands of five mice or two of the other species

Species	Harderian gland (wet weight mg)	Specific activity (nMole umbelliferone × mg protein^{-1} × min^{-1})	pH-optimum	K_m (mM)	Inhibition (in % of control) CO/O_2 (2:1, v/v)	Metyrapone (10^{-5} M)	Tetrahydrofurane (10^{-3} M)	7.8-Benzoflavone (10^{-5} M)
Mice (NMRI/Han 20)	33	0.037 ± 0.005	8.0	0.21	98 ± 8	69 ± 7	25 ± 4	2 ± 2
Mice (C57 BL/6N)	22	0.11 ± 0.004	8.2	0.26	84 ± 5	74 ± 4	19 ± 3	4 ± 6
Syrian hamster	35	0.043 ± 0.006	7.4	0.31	75 ± 10	10 ± 3	46 ± 7	58 ± 7
Rabbit (New Zealand)	750	0	—					
Rat (Wistar)	60	0	—					

Table 2. O-dealkylation activity in NMRI mice before and after induction by phenobarbital (80 mg/kg/day) over a period of 24, 48 or 72 hr. The values represent means (±S.E.) of triplicates from a pooled preparation of five organs

Organ	Time of induction (hours) 0	24	48	72
Harderian Gland	0.04 ± 0.005	0.04 ± 0.006	0.09 ± 0.01	0.12 ± 0.01
Liver	0.29 ± 0.03	1.91 ± 0.23	1.79 ± 0.15	2.42 ± 0.36

nmole umbelliferone × mg protein^{-1} × min^{-1}

Table 3. Influence of different inhibitors on the O-dealkylation activities in NMRI mice treated with phenobarbital (80 mg/kg/day) over a period of 3 days. Values represent means (±S.E.) of triplicates from one preparation obtained from five pooled organs

Inhibitors	% Inhibition Harderian gland Controls	Pb*-treated	Liver Controls	Pb*-treated
Metyrapone (10^{-5} M)	69 ± 7	98 ± 5	18 ± 3	95 ± 4
Tetrahydrofurane (10^{-3} M)	25 ± 4	0 ± 2	17 ± 4	20 ± 3
7.8-Benzoflavone (10^{-5} M)	2 ± 2	1 ± 3	0 ± 0	1 ± 3
CO/O_2 (2:1, v/v)	98 ± 8	100 ± 3	98 ± 5	93 ± 6

* Pb = Phenobarbital.

all three preparations. A clear differentiation between the two species, however, was possible by the use of the inhibitors metyrapone, 7.8-benzoflavone and tetrahydrofurane. Metyrapone at rather low concentrations was an effective inhibitor for the activity in mice whereas only a weak inhibition was observed in the hamster. The opposite was true for 7.8-benzoflavone.

According to our results obtained with rat liver microsomes the large variation in these parameters suggests the presence of different species of cytochrome P450 which, however, could all react with 7-ethoxycoumarin as a substrate. Metyrapone had been characterized as a potent inhibitor for the phenobarbital-inducible O-dealkylating activity and 7.8-benzoflavone for the 3.4-benzpyrene-inducible activity [19, 20]. The pH-optimum of the latter was around 8.2 and that of the phenobarbital-inducible activity at 7.4. That a system similar to the drug monooxygenases of liver was present in the Harderian gland could be confirmed by induction experiments. Table 2 shows that pretreatment of NMRI-mice with phenobarbital increased the O-dealkylating activity in liver and in the Harderian gland.

In liver the induction was about 8-fold and in the Harderian gland about 3-fold. The inhibition pattern after phenobarbital-induction shifted to the metyrapone-sensitive activity in both organs (Table 3).

The complete inhibition by carbon monoxide proved that the activity was still mediated by cytochrome P450. In earlier experiments we had found that the NMRI-strain of mice did not respond to an induction by 3.4-benzo(a)pyrene. This could be reconfirmed. Also no increase of the specific activity was

Table 4. O-dealkylation activities in black mice (C57 BL/6N) of liver and Harderian gland after 48 hr induction by 3.4-benzo(a)pyrene: 20, 40 and 80 mg/kg/24 hr compared to controls. Values represent means (±S.E.) of three preparations from five pooled organs

			% Inhibition				
		Spec. activity (nmole/mg protein × min)	Metyrapone (10^{-5} M)	Tetrahydrofurane (10^{-3} M)	7.8-Benzoflavone (10^{-5} M)	CO/O_2 2:1 (v/v)	K_m (mM)
Liver							
controls		0.68 ± 0.14	41 ± 3	53 ± 10	30 ± 6	87 ± 3	
induced							
BP*	20 mg	0.93 ± 0.10	38 ± 10	14 ± 6	59 ± 3	71 ± 6	
	40 mg	1.87 ± 0.32	0	0	61 ± 3	66 ± 4	
	80 mg	2.11 ± 0.63	0	0	56 ± 7	85 ± 5	
Harderian gland							
controls		0.11 ± 0.04	74 ± 4	19 ± 3	4 ± 6	84 ± 5	0.26
induced							
BP*	20 mg	0.09 ± 0.03	67 ± 2	29 ± 4	12 ± 5	100 ± 0	
	40 mg	0.10 ± 0.07	84 ± 3	28 ± 3	21 ± 2	88 ± 4	
	80 mg	0.08 ± 0.02	78 ± 5	8 ± 14	33 ± 8	90 ± 2	0.14

* 3.4-benzo(a)pyrene in mg/kg/24 hr after 48 hr.

found in the Harderian gland of this strain of mice. On the other hand the strain of black mice was fully inducible by 3.4-benzpyrene in liver, however, no induction of the activity in the Harderian gland could be measured (Table 4).

From the inhibitor studies it is seen, however, that a significant increase in 7.3-benzoflavone inhibition occurs, which may indicate a small induction by benzpyrene followed by a concomitant decrease of the cytochrome P450 forms present in normal untreated animals. An explanation for the small response of the Harderian gland to benzpyrene could be either an insufficient accessibility of the gland to the hydrocarbon or an insufficient activity of the receptor [21]. For the hamster this could be different as indicated by the high relative inhibition of the O-dealkylation activity by 7.8-benzoflavone in controls. Induction experiments with this species may clarify this hypothesis. At present we would like to only stress the existence of drug monooxygenase activity in the Harderian gland. Certainly, no significance for the overall metabolism of drugs in these animals can be derived, since the total activity in the gland is negligible in comparison to liver. However, it may be that the monooxygenase activity plays a role in the metabolism of endogenous compounds. The rather low affinity for 7-ethoxycoumarin would be in agreement with this assumption. Steroids could be likely candidates, however, preliminary results on progesterone 16α-hydroxylase and on testosterone metabolism gave no indication for a monooxygenation of these steroids. Thus the role of cytochrome P450 in the Harderian gland awaits further clarification.

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