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Expression of rat aldehyde reductase AKR7A1: influence of age and sex and tissue-specific inducibility☆

Anne Grant[a], Louise Staffas[b], Louise Mancowiz[b], Vincent P. Kelly[c], Margaret M. Manson[d], Joseph W. DePierre[b], John D. Hayes[c], Elizabeth M. Ellis[a,*]

[a]*Department of Bioscience & Biotechnology and Department of Pharmaceutical Sciences, University of Strathclyde, 204, George Street, Glasgow G1 1XW, Scotland, UK*

[b]*Unit for Biochemical Toxicology, Department of Biochemistry Wallenberg Laboratory, Stockholm University, Stockholm S-10691, Sweden*

[c]*Biomedical Research Centre, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY, Scotland, UK*

[d]*Centre for Mechanisms of Human Toxicity, Hodgkin Building, University of Leicester, PO Box 138, Leicester LE1 9HN, UK*

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Abstract

The regulation of the aldo-keto reductase AKR7A1 was examined in the livers of male and female rats during development by using Western blots, and its contribution to carbonyl metabolism was assessed by using enzyme assays. Hepatic levels of AKR7A1 are low in fetal rats and rise to a peak at around 6 weeks of age in animals of both sexes. Higher levels of the enzyme are found in adult male rat liver than in adult female rat liver. The reductase, therefore, appears to be subject to sex-specific regulation. The effect of growth hormone in mediating this difference in expression was examined by using hypophysectomized animals whose serum growth hormone levels had been feminized by continuous administration. Results demonstrate that such treatment leads to a reduction in AKR7A1 expression. AKR7A1 was found to be constitutively expressed in rat tissues such as liver, kidney, small intestine, and testis, but it was not detected in nasal mucosa, skeletal muscle, heart, adrenal gland, brain, or spleen. However, AKR7A1 was inducible by the synthetic antioxidant ethoxyquin in liver, kidney, and small intestine, but not in the other tissues examined. These results show that levels of this important detoxication enzyme vary considerably according to age and sex and that dietary antioxidants can also influence its level in several tissues.

Keywords: Aldehyde reductase; Developmental regulation; Fetal expression; Sex-specific expression; Growth hormone

1. Introduction

Aldehydes and ketones are ubiquitous in the environment, and some of these compounds have been shown to cause damage to protein, DNA, and membranes, leading to toxicity and mutagenicity [1]. Most organisms have evolved protective mechanisms to detoxify carbonyl-containing compounds. Enzymatic defences include the glutathione S-transferases, which can conjugate glutathione (GSH) to reactive carbonyls [2]; aldehyde dehydrogenases, which can oxidize aldehydes to acids [3]; and enzymes, which can reduce aldehydes and ketones to alcohols [4]. This latter biotransformation is catalysed by enzymes that fall into three separate families: the aldo-keto reductases [4]; the alcohol dehydrogenases, and NAD(P)H:quinone oxidoreductase (NQO1; also called DT-diaphorase). The aldo-keto reductases comprise a large superfamily of NAD(P)H-dependent reductases, members of which have been isolated from a variety of species and tissues [5,6]. Several members of this family have been identified in the rat, including aldehyde reductase (AKR1A3) [7], aldose reductase (AKR1B4) [8], and 3α-hydroxysteroid dehydrogenase (AKR1C9) [9,10]. In addition to their potential roles in detoxification, aldo-keto reductases can reduce sugars, ste-

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* Corresponding author. Tel.: +44-141-548-2122; fax: +44-141-553-4124.

E-mail address: elizabeth.ellis@strath.ac.uk (E.M. Ellis).

Abbreviations: 2-CBA, 2-carboxybenzaldehyde; 4-NBA, 4-nitrobenzaldehyde; 9,10-PQ, 9,10-phenanthrenequinone; NQO1, NAD(P)H:quinone oxidoreductase; GH, growth hormone; GSH, glutathione; and SDS, sodium dodecyl sulfate.

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roids, and prostaglandins, but because of their overlapping substrate specificity, definitive functions have been difficult to ascribe.

We have previously identified and characterized an aldehyde reductase from rat liver (AKR7A1) that is capable of detoxifying a dialdehyde metabolite of aflatoxin B_1 [11, 12]. This enzyme is also capable of reducing a range of cytotoxic and mutagenic aldehydes and ketones, including products of lipid peroxidation such as 4-hydroxynonenal and hexanal [13]. We have shown that the hepatic level of this enzyme, unlike most other members of the aldo-keto reductase family, is increased in rats after dietary exposure to certain xenobiotics. Such inducing agents include the phenolic antioxidants butylated hydroxyanisole and ethoxyquin, as well as naturally occurring compounds including coumarin [12,14]. Levels of AKR7A1 are also elevated in the liver of rats raised on a selenium-deficient diet [15]. Modulation of the level of AKR7A1 correlates with profound differences in susceptibility to chemical carcinogenesis: animals with high levels of AKR7A1 in the liver appear to be more resistant to the hepatocarcinogen aflatoxin B_1 than are animals with low levels of AKR7A1 [11].

This study was designed to investigate whether factors such as age and sex also influence the expression of AKR7A1, as well as to examine the tissue-specific inducibility of this enzyme. Such information can provide insights into factors that may affect susceptibility to the effects of toxic aldehydes and ketones, as well suggesting additional biological roles for this important enzyme in normal cellular processes.

2. Materials and methods

2.1. Animals

Developmental expression of AKR7A1 was examined in male and female Fischer 344 rats that were purchased from Harlan Olac Ltd. (Oxon, England). Such animals were fed *ad lib* a control diet comprising RM1 diet (SDS Ltd., Edinburgh, Scotland) [15]. Livers were prepared from fetal animals (17 days gestation) or animals at the ages of 2, 4, 6, 8, 10, and 12 weeks. Tissue-specific expression was examined in the same animals. For the enzyme induction experiments, 10-week-old animals were fed on a RM1 diet containing 0.5% (w/w) ethoxyquin for the 10 days immediately before being killed.

The growth hormone regulation study was carried out using Sprague-Dawley rats obtained from Mollegaards Avslaboratorium (Denmark). Sham-operated animals were used as controls, and hypophysectomy was performed at 6 weeks of age. Hormone treatment was initiated 4 weeks later, as described previously by Staffas *et al*. [16]. To compensate for thyroid and adrenal gland deficiency in the hypophysectomized rats, thyroxine and cortisone acetate (Sigma-Aldrich, Poole, Dorset, UK) were administered daily, at doses of 10 μg and 500 μg/kg body weight respectively, as described previously, starting 2 days before growth hormone treatment [16]. Growth hormone (a gift of Pharmacia and Upjohn, Stockholm, Sweden) was administered at a rate of 50 m-units/hr and kg body weight for 7 days [16].

Animals were killed by decapitation; livers were removed and placed on ice. Tissue extracts were either prepared immediately, or tissues were frozen at -70°C for further analysis. Thin slices (1–2 mm) of various tissues were fixed in ice cold acetone before sectioning for immunohistochemistry.

2.2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and western blotting

Cell extracts were prepared from livers by homogenization in 20 mM sodium phosphate buffer, pH 7.0. Soluble fractions were obtained by centrifugation at $9{,}000 \times g$ for 20 min (4°C). Sodium dodecyl sulfate-polyacrylamide gel electrophoersis (SDS-PAGE) was performed by the method of Laemmli [17], and proteins were subsequently transferred to nitrocellulose membranes (Merck, Poole, Dorset, UK). Immunoreactive proteins were identified by using antisera that had been raised previously in female New Zealand White rabbits using recombinantly expressed aldo-keto reductases or NQO1 as immunogens [14,18]. Immune complexes were detected by enhanced chemiluminescence and exposure to X-ray film. After scanning, relative amounts of each antigen were quantitated by measuring pixel densities by using National Institutes of Health image analysis software (*http://rsb.info.nih.gov/nih-image/*).

2.3. Immunohistochemistry

Immunohistochemistry, using the polyclonal antisera raised to AKR7A1 at a dilution of 1:500, was carried out on acetone-fixed tissues as described previously [19].

2.4. Protein and enzyme assays

Protein concentrations were measured by using the method of Bradford [20]. Aldehyde and ketone reductase activity was measured as described previously [13] by measuring the rate of disappearance of the cofactor [either NADPH or NADH] at 340 nm and at 25°C. Assays were carried out in 0.1 M sodium phosphate buffer, pH 6.8, by using a Beckman DU650 UV spectrophotometer with a light path of 1 cm.

2.5. Statistical analysis

Statistical analysis was carried out by using InStat software (GraphPad, San Diego, CA). Pairwise sets of data

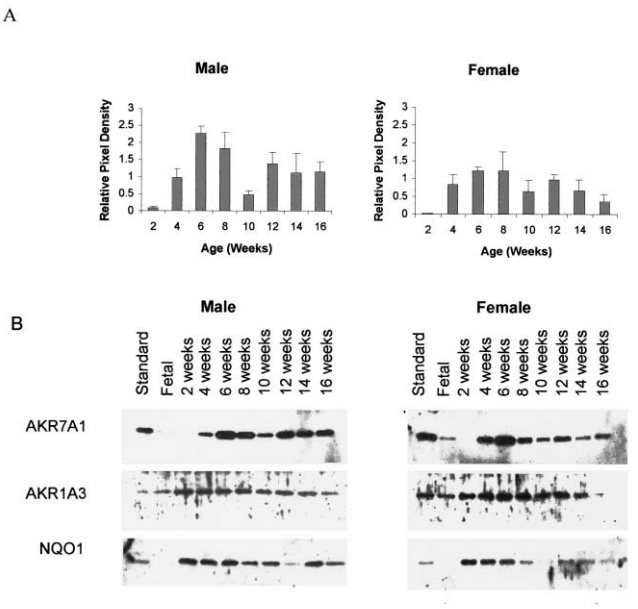

Fig. 1. Ontogenic regulation of AKR7A1. Extracts from 200 mg frozen liver tissue from male or female animals aged 2, 4, 6, 8, 10, 12, 14, or 16 weeks or from fetal (F; 17 days gestation) livers were prepared as described in Materials and Methods in 20 mM sodium phosphate buffer pH 7.0. Protein (10 μg) from each extract was separated by SDS-PAGE and transferred to nitrocellulose membranes. Western blots were probed with antisera and were processed employing enhanced chemiluminescence. Quantitation for each blot was standardized against controls, and the average pixel density of each band determined. (A) Mean values of AKR7A1 levels in 3 different animals. (B) Representative western blots probed with antisera raised against AKR7A1, AKR1A3, or NQO1.

were analysed by applying an unpaired *t* test. Three or more comparisons were made by using one-way ANOVA (analysis of variance) and the significance determined for each result by using Dunnett's multiple comparison post-test.

3. Results

3.1. Ontogenic regulation of AKR1A7 expression in the liver

Previous studies have shown that, whereas some carbonyl-reducing enzymes are expressed in the livers of adult rats, others, such as aldose reductase (AKR1B4), are only expressed in fetal liver [21]. These levels may influence sensitivity to toxic aldehydes and ketones. To investigate whether there is any variation in the expression of AKR7A1 during development, liver extracts prepared from male and female Fischer 344 rats of different ages were examined by using western blots probed with a polyclonal antibody toward purified AKR7A1. Fig. 1 shows that AKR7A1 was detectable in fetal rats and that levels were comparatively low in 2-week-old male and female animals. In both sexes, levels of AKR7A1 rose dramatically in the weeks after birth and peaked just before puberty at around 6 weeks, before decreasing in adult rats. Levels in adult male animals remained consistently higher than in female animals. These data are in contrast with the findings for aldehyde reductase (AKR1A3), the level of which increased only slightly in pubescent rats. NQO1 was undetectable in fetal rat liver, but was increased after birth in both male and female animals. In females, the level of this protein decreased significantly in adulthood.

To assess the impact of the levels of AKR7A1 on the detoxication capacity of rat liver, extracts were assayed for the ability to reduce a range of aldehydes and ketones, some of which are toxic and known to be substrates of AKR7A1. The results, documented in Table 1, show that levels of reductase activity toward 2-CBA (a substrate that is relatively specific for AKR7A family members when measured by using NADH as cofactor [18]) reach a maximum at 6

Table 1
Levels of the activities of carbonyl reducing enzymes in rat liver during development[a]

Cofactor:	NADH				NADPH			
Substrate:	2-CBA (nmol min^{-1} mg^{-1})		2-CBA (nmol min^{-1} mg^{-1})		4-NBA (nmol min^{-1} mg^{-1})		9,10-PQ (nmol min^{-1} mg^{-1})	
Age (weeks)	Male	Female	Male	Female	Male	Female	Male	Female
Fetal	2.3 ± 0.2[b]		4.8 ± 2.5[b]		9.9 ± 1.9[b]		13.3 ± 3.7[b]	
2	6.5 ± 1.7*	5.6 ± 1.0**	4.8 ± 1.1	3.6 ± 0.9	35.0 ± 4.7	37.8 ± 4.1	57.6 ± 6.7**	60.7 ± 4.5
4	8.8 ± 0.4	13.9 ± 3.9*	6.8 ± 1.4	4.4 ± 0.2	35.0 ± 11.5	39.0 ± 4.1	54.7 ± 5.4**	57.5 ± 3.7
6	16.0 ± 1.9*	12.8 ± 1.8	7.4 ± 0.1	4.7 ± 0.2	34.1 ± 8.0	36.8 ± 1.4	51.6 ± 1.0**	58.7 ± 2.7
8	14.6 ± 1.1	11.2 ± 1.8	8.3 ± 1.3	4.7 ± 0.4	34.3 ± 6.5	41.6 ± 3.6	48.0 ± 1.9	60.8 ± 8.3
10	8.3 ± 1.7	9.5 ± 2.1	5.6 ± 0.4	4.2 ± 0.3	28.0 ± 10.9	38.5 ± 6.5	39.2 ± 6.2	61.4 ± 3.2*
12	12.9 ± 2.3	11.8 ± 1.3	7.9 ± 0.3	4.9 ± 0.2	32.1 ± 12.6	37.4 ± 1.8	43.3 ± 6.6	51.0 ± 0.9
14	11.0 ± 1.0	9.2 ± 1.1	6.2 ± 0.7	4.5 ± 0.5	26.8 ± 10.3	37.1 ± 4.5	38.9 ± 3.6	49.3 ± 2.6
16	11.6 ± 2.6	8.6 ± 1.5	6.0 ± 0.7	4.9 ± 0.1	25.3 ± 11.1	36.4 ± 4.5	36.3 ± 5.7	49.7 ± 8.4

[a] Cytosols were prepared from the livers of Fischer 344 rats at the developmental stages indicated and assayed as described in Materials and Methods using the substrates indicated. Values represent the means ± SEM, for 3 animals.

[b] Fetal samples were not sexed.

* Activities are significantly different, $P < 0.05$, from 16 week animals of the same sex; ** $P < 0.01$.

2-CBA, 2-carboxybenzaldehyde; 4-NBA, 4-nitrobenzaldehyde; 9,10-PQ, 9,10-phenanthrenequinone.

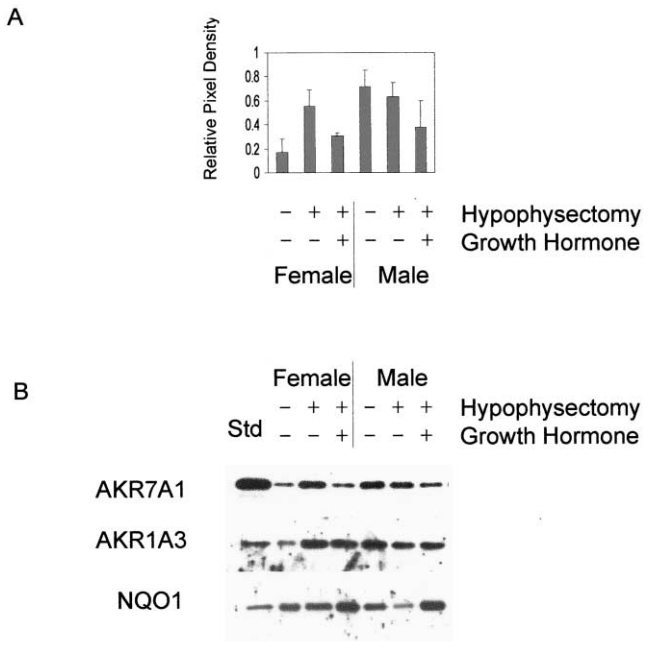

Fig. 2. Sex-specific regulation of AKR7A1 expression by GH. Extracts from 200 mg frozen liver tissue from male or female animals treated as described were prepared as described in Materials and Methods in 0.25 M sucrose. Protein (10 μg) from each extract was separated by SDS-PAGE and transferred to nitrocellulose membranes. Western blots were probed with antisera and processed by using enhanced chemiluminescence. Quantitation for each blot was standardized against controls, and the average pixel density of each band determined. (A) Mean values of AKR7A1 levels in 3 different animals. (B) Representative western blots probed with antisera raised against AKR7A1, AKR1A3, or NQO1.

weeks of age in male animals. This correlates well with the levels of AKR7A1 detected on western blots.

3.2. Sex-specific regulation of AKR7A1

During this and previous studies, the levels of AKR7A1 protein in the liver have consistently been found to be higher in males than in female rats. To establish the basis for this sex-specific regulation, we examined the expression of AKR7A1 in Sprague-Dawley rats that had been hypophysectomized and subsequently treated with Growth Hormone (GH). The continuous administration of GSH to hypophysectomized animals of either sex was designed to feminize the pattern of expression, as has been described previously [16].

Extracts from the livers of treated animals were analyzed on western blots by using antibodies raised against AKR7A1 protein. The results in Fig. 2 confirmed that the hepatic expression of AKR7A1 is higher in male rats than in female rats by approximately 3-fold. Hypophysectomy of female animals led to an increase in the amount of AKR7A1 to levels that are equivalent to those seen in male animals. Continuous administration of GH to hypophysectomized females led to a reduction in AKR7A1 to levels that are similar to those seen in the control females. Hypophysectomy of male animals led to a small (20%) reduction in the levels of AKR7A1. However, continuous administration of GH hypophysectomized male rats led to a 60% reduction in the level of AKR7A1 in the liver and appeared to mimic the female pattern of expression. Levels of aldehyde reductase (AKR1A3) are also lower in female animals than in males. Similarly, hypophsyectomy of females leads to increased levels of expression, but in this case, administration of GH had little effect on these increased levels. Levels of NQO1 appeared more similar between males and females. There a slight decrease in hypophysectomised males, and animals of both sexes showed increased levels of expression after the administration of GH.

Enzyme assays on rat liver cytosols from control, hypophysectomized, and treated animals (Table 2) show that the capacity of the liver to metabolize aldehydes and ketones varies according to sex. In these Sprague-Dawley rats, the aldehyde and ketone reductase activities measured were higher than in the Fischer 344 rats. Activities were also in general higher in females than in males, with the notable exception of 2-carboxybenzaldehyde (2-CBA) reductase activity, measured with NADH as cofactor. In this case, activity was significantly higher in males than in females, and this difference mirrored the differences in AKR7A1 levels observed by western blotting. Levels of 4-nitrobenzoldehyde (4-NBA) reductase activity increased significantly in male animals after hypophysectomy and were still high, although slightly reduced, after GH treatment. Similarly, 9,10-phenanthre-nequinone (9,10-PQ) reductase activities increased slightly in hypophysectomized male animals and increased further with GH treatment. In contrast, 2-CBA reductase activity decreased in hypophysectomized male rats and was decreased yet further on treatment with GSH. In female rats, 4-NBA and 9,10-PQ reductase activities were higher than in males, but there was a reduction in 9,10-PQ reductase activity in hypophysectomized female animals, which increased on GH treatment. 2-CBA reductase activity increased in hypophysectomized female rats to levels approaching those found in the male rat, but was decreased by treatment with GH. These results suggest that feminization of GH patterns leads to a decrease in NADH-dependent 2-CBA reductase activity, thus mirroring the western blotting data for AKR7A1.

3.3. Tissue-specific expression of AKR7A1

Previous studies have examined the expression of AKR7A1 in a variety of rat tissues [18,22], but there is little information presently available on the regulation of AKR7A1 expression by chemoprotectors in tissues other than the liver and kidney. To determine the effect of chemoprotectors on the capacity to metabolize carbonyls and their effects on the levels of members of the AKR superfamily, we examined their expression in different tissues from ethoxyquin-treated and control rats. Immunoblotting

Table 2
Effects of hypophysectomy and treatment with growth hormone on hepatic carbonyl reducing activity[a]

	Cofactor:	NADH				NADPH			
	Substrate:	2-CBA (nmol min^{-1} mg^{-1})		2-CBA (nmol min^{-1} mg^{-1})		4-NBA (nmol min^{-1} mg^{-1})		9,10-PQ (nmol min^{-1} mg^{-1})	
Treatment	Sex:	Male	Female	Male	Female	Male	Female	Male	Female
Sham operated (control)		28.7 ± 3.6	8.2 ± 2.2	3.6 ± 2.0	4.8 ± 2.0	86 ± 4	133 ± 19	116 ± 5	238 ± 56
Hypopysectomy		16.1 ± 5.5*	19.8 ± 5.3*	1.2 ± 0.8	4.6 ± 0.9	142 ± 25**	158 ± 16	133 ± 16	177 ± 4**
Hypophysectomy + growth hormone		8.8 ± 2.0**	9.2 ± 5.7	3.7 ± 1.1	7.0 ± 3.5	133 ± 12*	148 ± 7	176 ± 14**	196 ± 11

[a] Cytosols were prepared from the livers of Sprague-Dawley rats given the treatments indicated and assayed as described in Materials and Methods using the substrates indicated. Values represent the mean of assays ± SEM, carried out on livers from 3 separate animals.

* Activities are significantly different, $P < 0.05$, from control animals of the same sex; ** $P < 0.01$.

2-CBA, 2-carboxybenzaldehyde; 4-NBA, 4-nitrobenzaldehyde; 9,10-PQ, 9,10-phenanthrenequinone.

(Fig. 3) confirmed that AKR7A1 is expressed constitutively at relatively high levels in liver, kidney, small intestine, and testis. This tissue distribution differs from that of either of the two related human AKR7A enzymes, which are expressed at high levels in liver, kidney, small intestine, and pancreas (both AKR7A2 and AKR7A3); skeletal muscle, heart, testes, and ovary (AKR7A2); and in thyroid and adrenals (AKR7A3) [23,24]. A novel rat enzyme that is highly related to AKR7A1 has recently been identified [18, 25] and exhibits a tissue distribution similar to that of human AKR7A2 and AKR7A3.

The rat AKR7A1 is inducible in liver, as previously documented, and is slightly inducible in kidney. Important, the data in Fig. 3 show that AKR7A1 is also inducible in the small intestine. The reductase does not appear to be inducible in the testes or any other tissue we have examined to date.

Enzyme assays on cytosols prepared from tissues isolated from ethoxyquin-treated and control rats were also carried out (Table 3). These show that there was significant increase in 2-CBA reductase activity in the liver, kidney, and testis of animals treated with ethoxyquin, when measured by using NADH as cofactor. There was also an increase in 2-CBA reductase activity in the small intestine, adrenals, and brain, although these were not statistically significant. Treatment with ethoxyquin also appears to lead to a significant increase in 4-NBA reductase activity in the heart, although this does not correlate with increased AKR7A1 levels as determined by western blots.

Immunohistochemical staining of kidney, small intestine, and testis revealed those areas of these tissues where AKR7A1 is expressed (Fig. 4). In the kidney, staining for AKR7A1 was pronounced in the collecting ducts (Fig. 4a). In the small intestine, staining in the villi (distinct from basal alkaline phosphatase) was observed. Staining was also observed in the testis. In addition, although AKR7A1 is not detectable in skeletal muscle when using western blots, we were able to identify discrete areas of staining in the nerve bundles, suggesting that the enzyme may fulfill a function in these highly specialized cells.

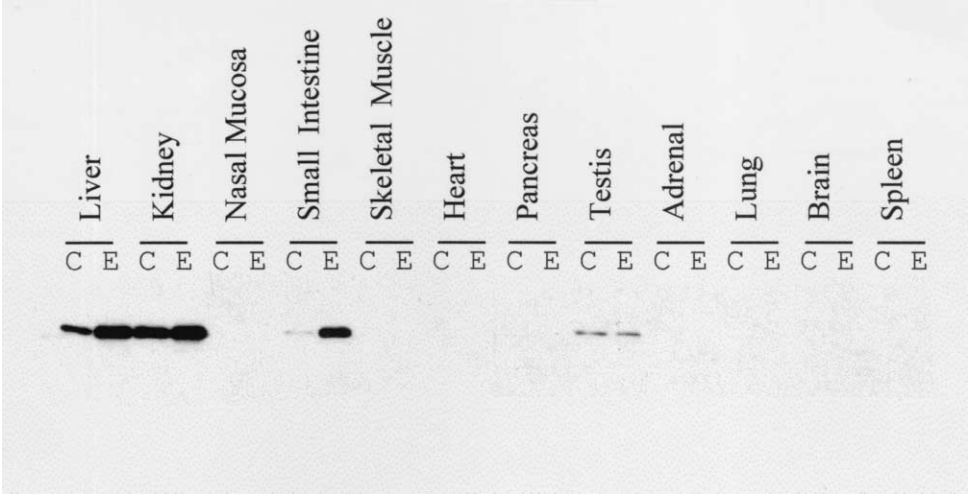

Fig. 3. Ethoxyquin induction of AKR7A1 in rat tissues. Extracts of the frozen tissues indicated from adult male rats aged 10 weeks were prepared as described in Materials and Methods in 20 mM sodium phosphate buffer, pH 7.0. Protein (10 μg) from each extract was separated by SDS-PAGE and transferred to nitrocellulose membranes. Western blots were probed with antisera raised against AKR7A1, AKR7A2, or NQO1 and were processed employing enhanced chemiluminescence. Blots were carried out on extracts from 3 animals; representative blots are shown. (C) Control animals. (E) Ethoxyquin-treated animals.

Table 3
Inducibility of carbonyl reducing enzyme activity by ethoxyquin treatment in various male rat tissues[a]

Cofactor:	NADH				NADPH			
Substrate	2-CBA (nmol min^{-1} mg^{-1})		2-CBA (nmol min^{-1} mg^{-1})		4-NBA (nmol min^{-1} mg^{-1})		9,10-PQ (nmol min^{-1} mg^{-1})	
Tissue	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Liver	19.4 ± 0.9	40.8 ± 7.9**	5.2 ± 2.5	9.0 ± 2.9	39.1 ± 11.0	42.0 ± 6.8	60.3 ± 6.3	74.2 ± 3.6*
Kidney	25.8 ± 2.1	33.9 ± 1.1**	5.8 ± 0.8	9.8 ± 1.6*	71.6 ± 6.6	80.4 ± 15.3	43.3 ± 4.9	52.6 ± 2.7*
Nasal	2.6 ± 0.7	2.0 ± 0.8	2.0 ± 0.8	2.5 ± 0.4	5.35 ± 3.9	7.1 ± 2.9	46.8 ± 4.4	40.8 ± 5.6
Small intestine	1.7 ± 0.6	4.8 ± 2.3	1.2 ± 0.7	3.2 ± 1.5	15.4 ± 6.3	21.8 ± 12.5	29.0 ± 7.4	28.7 ± 3.4
Skeletal muscle	3.5 ± 2.7	2.4 ± 1.1	1.1 ± 0.5	1.8 ± 0.7	4.0 ± 1.2	5.9 ± 5.5	6.5 ± 2.7	6.3 ± 1.1
Heart	9.0 ± 1.9	8.7 ± 4.2	2.4 ± 1.0	3.0 ± 0.5	7.1 ± 2.3	14.2 ± 0.9**	19.2 ± 3.9	19.9 ± 6.1
Pancreas	1.9 ± 0.3	2.7 ± 0.7	1.1 ± 1.6	5.2 ± 2.3	6.1 ± 4.8	8.5 ± 4.7	6.3 ± 1.4	9.7 ± 2.9
Testis	2.1 ± 0.3	3.7 ± 0.8*	1.6 ± 0.5	2.6 ± 1.6	16.9 ± 12.1	17.1 ± 6.7	13.6 ± 3.3	15.1 ± 1.6
Adrenal	4.0 ± 1.8	10.1 ± 3.8	4.3 ± 1.8	5.2 ± 2.0	19.5 ± 11.4	25.4 ± 11.6	49.8 ± 13.3	77.6 ± 16.7
Lung	2.0 ± 0.9	1.9 ± 0.7	2.5 ± 0.3	2.9 ± 0.6	12.2 ± 5.7	7.7 ± 2.5	29.8 ± 5.8	22.3 ± 9.6
Brain	2.6 ± 0.8	4.0 ± 1.1	2.0 ± 1.3	3.0 ± 0.9	8.1 ± 0.9	10.0 ± 3.5	15.0 ± 3.0	19.4 ± 7.1
Spleen	1.8 ± 0.4	2.4 ± 1.2	2.4 ± 0.2	3.2 ± 1.4	9.5 ± 1.8	10.7 ± 4.2	10.1 ± 2.3	8.3 ± 3.3

[a] Cytosols were prepared from tissues of Fischer 344 rats at 10 weeks of age, after treatment with ethoxyquin for 2 weeks. Extracts were assayed as described in Materials and Methods using the substrates indicated. Values represent the means ± SEM, for 3 animals.

* Activities are significantly different, $P < 0.05$, from control animals; ** $P < 0.01$.

2-CBA, 2-carboxybenzaldehyde; 4-NBA, 4-nitrobenzaldehyde; 9,10-PQ, 9,10-phenanthrenequinone.

4. Discussion

The important role played by AKR7A1 in the metabolism of aflatoxin B_1 metabolites has been clearly demonstrated [11,24,26]. Its regulation by dietary constituents has been shown to contribute to the detoxication of the hepatocarcinogen in exposed animals [11,27]. In the present study, we have shown that AKR7A1 is also regulated by other factors, including age and sex, and that is inducible in the small intestine.

Age-specific regulation of hepatic enzymes including other drug-metabolizing enzymes, such as cytochromes P-450 [28], has been observed previously. Another aldo-keto reductase known to be regulated ontogenically in liver is aldose reductase, which is expressed in fetal, but not in adult, rat liver and in certain tumors [21]. Aldose reductase is also regulated ontogenically in rat kidney, where its levels increase after birth [29]. The results presented here show that AKR7A1 is elevated approximately 8-fold in 6-week-old rats compared to 2-week-old animals. This affects the ability of the liver to metabolize aldehydes and ketones. Table 1 shows clearly that hepatic activity toward 2-CBA, which is a relatively specific substrate for AKR7A subfamily members, is much lower in fetal rats than in prepubescent rats; interestingly, this effect is most obvious when NADH is employed as a cofactor.

Levels of GH are known to vary dramatically in animals of different ages [30]. In female rats, GH secretion is episodic in 4-week-old rats, but in 13-week-old animals, this secretion is more irregular. In male rats, the episodic nature of secretion is established at 4 weeks of age, (with higher peak serum levels of GH than in female animals of the same age and between peaks, periods of negligible levels), and this pulsatile pattern of expression is maintained through to maturity. Only at 6 weeks of age is the pattern of secretion in male and female rats similar [30].

These short absences of GH are required to obtain the male-specific expression of certain hepatic enzymes such as cytochromes P450, whereas the more continuous secretion of GH induces the female expression pattern [28,31]. This GH pulse-regulated gene expression is mediated by the STAT5 family of transcription factors [32]. We have shown here that rats in which GH secretion is pulsatile express higher hepatic levels of AKR7A1 than do those in which GH secretion is irregular and/or results in lower peak serum levels.

To investigate whether it is the pattern of GH secretion or the peak levels of GH that affect AKR7A1 expression, we examined levels of AKR7A1 in male and female adult rats in which the pituitary had been surgically removed and to which GH was administered continuously to mimic the female pattern of expression [16].

Hypophysectomy was shown to lead to higher levels of AKR7A1 expression in female rats, and continuous administration of GH lead to a reduction in these levels, suggesting that continuous administration of GH can repress the expression of AKR7A1. Hypophysectomy of male animals has very little effect on the higher level of AKR7A1 expression. It is possible that the pulsatile nature of GH secretion normally present in males is unable to elicit the same degree of repression as is the continuous but lower level of GH secretion seen in females. In support of this hypothesis, continuous administration of GH to hypophysectomized male rats was found to lower the expression of AKR7A1.

This hypothesis based on our present data cannot explain the low levels of AKR7A1 expression observed in 2-week-old rats, where GH secretion is known to be episodic in both male and female rats, although resulting in much lower

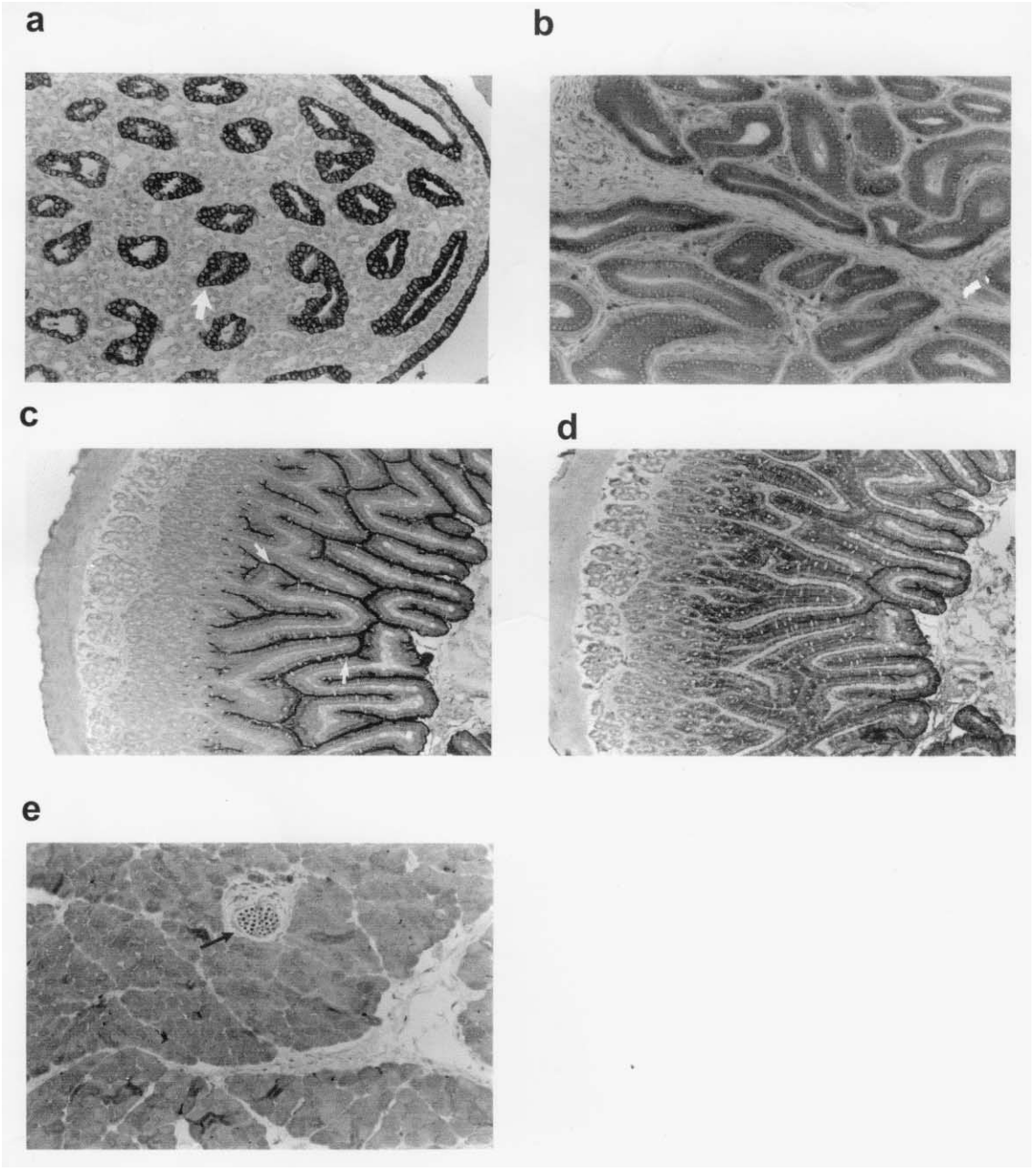

Fig. 4. Immunohistochemical localization of AKR7A1 in rat tissues. Tissues from rats were sectioned and probed with antibodies raised against rat AKR7A1. Detection was achieved by using alkaline phosphatase conjugated secondary antibody. (a) Kidney (arrows indicate collecting ducts). (b) Testis. (c) Small intestine without antibody (arrow indicates endogenous alkaline phosphatase). (d) Small intestine with antibodies to AKR7A1. (e) Skeletal muscle (arrows indicate nerve bundles).

serum levels [30]. It is likely that additional regulatory mechanisms influence AKR7A1 expression in neonatal rats.

The effect of growth hormone on the expression of another important detoxication enzyme, glutathione S-transferase GSTA5, has been examined previously. Although AKR7A1 and GSTA5 are co-induced in the liver by ethoxyquin, GSTA5 shows a pattern of regulation by GH distinct from that of AKR7A1 [16]. In this case, levels of GSTA5 are normally much higher in females than in males. Hypophysectomy increases the levels of GSTA5 in both sexes. Administration of GH to hypophysectomized animals decreases the levels of GSTA5 in both male and female rats, although the downregulation in males is not as marked as that in females. This further illustrates the complexity of enzyme regulation by GH, which can both upregulate and downregulate the levels of enzymes, for example, cytochrome P450 (for review see [33] and for GST isoenzymes [16,34].

The expression of rat 3-α hydroxysteroid dehydrogenase/dihydrodiol dehydrogenase (AKR1C9), another mem-

ber of the aldo-keto reductase family, has also been reported to exhibit sex-specific regulation in the liver and other tissues [35–37]. Female animals express 2-fold higher levels of AKR1C9 in the liver than did males, and hypophysectomy leads to a reduction in expression in both male and female animals, although this reduction is not apparent immediately after surgery, indicating an indirect effect. Treatment of male rats with estrogen leads to increased levels of AKR1C9 expression, suggesting that this enzyme is directly regulated in the liver by estrogen.

Mechanisms of regulation of protein levels in rat liver often involves elevated levels of mRNA which may be due to increased rates of transcription. We have found that the level of AKR7A1 mRNA is higher in male rat liver (data not shown), and we will now investigate whether this reflects a higher rate of transcription of the *AKR7A1* gene. By using mice in which the genes for *Stat5a* and *Stat5b* have been disrupted, it has been possible to demonstrate that the levels of these two transcription factors are important in the regulation of certain GSH-regulated cytochromes P-450 [38]. Identification and analysis of 5′-flanking regions of the *AKR7A1* gene may reveal whether the promoter contains consensus binding sites for these factors.

Among the other liver enzymes subject to sex-specifc regulation, many are involved in steroid metabolism [39, 40]. It is not clear why such expression would be observed for an enzyme whose only known role to date has been assumed to be in detoxication. To understand more about the role of AKR7A1 in the whole animal, we examined its expression in a range of different tissues from both control and ethoxyquin-treated rats. As expected, AKR7A1 is inducible in the liver and kidney, but unexpectedly it is also inducible in the small intestine. Surprisingly, this protein is also expressed at a high level in the testis, but is not inducible in this tissue. These data support our hypothesis that AKR7A1 is a key defensive enzyme in the rat: all of the tissues where this protein is either highly expressed and/or inducible represent locations where exogenously-derived toxic aldehydes or ketones are expected to be encountered (e.g. the liver, kidney, and small intestine) or where the ability of the cell to detoxify endogenous aldehydes is critical (as in the testis). It is also possible that AKR7A1 plays some other endogenous role in the testes, possibly in connection with the metabolism of hormones. AKR7A1 is not inducible by ethoxyquin in the testis. There is substantial evidence that ethoxyquin regulates transcription in the liver via an antioxidant response element in the promoter of regulated genes [41–43]. Possibly, ethoxyquin does not reach the testis or is not metabolized to a form that leads to the induction of gene expression or in this tissue, the signalling pathways or transcription factors required for such induction are not present.

In conclusion, we have shown that levels of AKR7A1 are regulated in rat liver by age and sex and that GH may in part explain aspects of this regulation. These differences have consequences for the metabolism of aldehydes and ketones by the liver in animals of different ages and sex. In addition, expression of AKR7A1 appears to be regulated only in liver, kidney, and testes, supporting its role as an inducible detoxication enzyme.

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