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ESTRONE SULFATASE ACTIVITY IN GUINEA PIG TISSUES

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SUMMARY

Estrone sulfatase activity is widespread in guinea pig tissues. Whole homogenates of adult testis, uterus, lung, adrenal, amnion, ovary, chorion, small intestine, placenta, spleen, kidney and liver exhibit approximately descending order of specific activity. Certain properties, including pH requirement, lack of inhibition by inorganic sulfate and magnitude of estimated K_m values, are similar to that for arylsulfatase C of rat liver. Of the subcellular fractions prepared from guinea pig tissues, microsomes exhibit the highest specific activity although considerable enzyme activity remains associated with large cellular fragments sedimenting at 750 *g*. The sulfatase activity is readily inhibited by inorganic phosphate even when substrate concentration satisfies zero order kinetics. Rat liver arylsulfatase C is not inhibited under these conditions. Sensitivity of the guinea pig enzyme activity to inhibition by a variety of steroids and related compounds, is markedly less than for rat liver. Diethylstilbestrol (DES) strongly inhibits the rat liver enzyme but has little effect on the guinea pig liver system. Guinea pig testicular activity is suppressed to a degree intermediate between these extremes by increasing DES concentration. In guinea pig lung, kidney, and possibly liver, elevated fetal enzyme activities decrease from neonatal to adult life. Testicular activity appears to follow the opposite trend. Uterine enzyme activity is not markedly affected by pregnancy.

INTRODUCTION

Since the establishment of certain steroid sulfates as major circulating forms in blood [1], the role of tissue sulfatases in the deconjugation process has emerged as an area for investigation. The widespread occurrence of estrone sulfate sulfohydrolase (also termed estrogen sulfatase [2] or estrone sulfatase [3]), apparently synonymous with arylsulfatase C in rat liver [2], has been demonstrated. This enzyme, or a similar one, has been implicated in the release of potentially active estrogen in target sites such as uterus [4] and pituitary [5]. Thus its tissue distribution may be of importance. Data are available for some rat tissues [2] but it has been claimed that guinea pig testis, though not liver, contains arylsulfatase C activity [6]. However, estrone sulfate does undergo hydrolysis *in vivo* in the guinea pig [7] as well as *in vitro* with liver [8, 9] and kidney [10] systems from this species.

Studies in our laboratory concerning hydroxylation of estrone sulfate by guinea pig liver systems [7, 8], and the synthesis of this conjugate by guinea pig uterus [11], prompted us to look at tissue distribution of estrone sulfate sulfohydrolase (termed hereafter estrone sulfatase for convenience). In addition, we have investigated some of the properties of the guinea pig enzyme activity relative to the abundant activity in rat liver.

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Abbreviations: SA = specific activity; Miranol H2M is a dicarboxylated derivative of lauryl imidazoline; DHA = dehydroepiandrosterone; DHAS = the 3-sulfate of DHA; DES = diethylstilbestrol.

MATERIALS AND METHODS

Animals

Guinea pigs of the English Shorthair variety were bred in our animal quarters, Fetal and maternal tissues were obtained near term (about 65 days of gestation). Neonates were classified as 1–2 days of age, immature animals ranged from 5 to 21 days of age and mature animals were more than 2 months old (generally upwards of 450 g body weight). Mature rats were of the Wistar strain.

Chemicals and reagents

[6,7-^{3}H]-Estrone-3-sulfate (Na^+ salt), of SA approx. 50 Ci/mmol, was purchased from New England Nuclear (Canada) Ltd, Dorval, Quebec. Unlabelled sodium estrone sulfate was synthesized as described previously [12]. Both forms were stored and checked periodically for possible breakdown [12]. Dehydroepiandrosterone-3-sulfate (DHAS) and diethylstilbestrol (DES) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. All unlabelled free steroids were purchased from Sigma or Steraloids Inc., Wilton, NH, U.S.A. All of the above were tested for purity and purified where necessary [12]. Miranol H2M was obtained from the Miranol Chemical Co., Irving, NJ, U.S.A. DEAE Sephacel and Sephadex G-200 were products of Pharmacia (Canada) Ltd, Montreal. ACS liquid scintillation fluid was obtained from Amersham, Oakville, Ontario, Canada. All other reagents were of suitable analytical grade.

Tissue preparation

Animals were sacrificed by a blow to the neck area and tissues were rapidly excised and washed in cold

saline. After blotting, and removing any extraneous material, each tissue was finely chopped with sharp scissors and homogenized in 10 vol/g of 0.25 M sucrose containing 10 mM Tris–acetate buffer, pH 7.6. This was performed either in a glass tube using a motor-driven nylon pestle (clearance 0.1–0.15 mm), or a Brinkman Polytron (PT 10-35) for 2 × 10 s at setting 6. These operations were conducted on ice.

Homogenates were spun at 760 *g* for 10 min in a Sorvall RC2B refrigerated centrifuge using a SS-34 rotor. Supernatants were further centrifuged at 10,000 *g* for 20 min. Supernatants from the latter step were spun at 100,000 *g* for 60 min in a Beckman L3-50 centrifuge employing a 50 Ti rotor. Each pellet was reconstituted in a volume of sucrose–Tris which allowed appropriate aliquots to be taken for enzyme assay. Guinea pig liver nuclei were isolated as described by others [13].

Enzyme assay

Routine incubations, in duplicate, employed 0.1 mM estrone sulfate containing 2 × 10^5 d.p.m. ^3H in 0.1 M Tris–acetate buffer, pH 8.0, total volume 1.0 ml. These conditions ensured zero order substrate kinetics. Incubation time was 15 min at 37°C. Blanks without enzyme or with boiled enzyme were included with each batch. These generally yielded <1% hydrolysis except when detergent was present which increased the blank to an apparent 4% hydrolysis. In test samples, reaction rates were linear up to approx. 20 min provided that substrate hydrolysis was limited to <30%. Linearity with respect to protein concentration was ascertained for each tissue system. On completion of incubation 2 ml of cold 3 M acetate buffer, pH 4, were added to each tube and extraction was immediately performed with 6 ml of diethyl ether in the cold. Triplicate 0.5 ml volumes of the upper phases were transferred to counting vials, evaporated, and submitted to liquid scintillation spectrometry [14]. Replicate counts differing by not more than ±5% were utilized to calculate the degree of substrate hydrolysis. The unit of activity was expressed as nmol substrate hydrolyzed/h. Protein was measured by the method of Lowry *et al.*[15]. Sodium acetate buffer was used to obtain pH 5.0 and 6.0 in constructing pH/activity curves.

Substrate concentration/activity experiments were performed up to 0.5 mM and the range 2 to 40 μm was utilized to obtain K_m values from double reciprocal plots of data subjected to regression analysis.

A substrate concentration of 10 μM was employed to study the inhibitory effects of free steroids, DHAS and DES. Solubility of DES and free steroids was facilitated by the addition of 20 μl of methanol.

Solubilization of testicular sulfatase

Guinea pig testicular microsomes were treated with imidazole buffer containing 1% (w/v) Miranol H2M exactly as described by Iwamori *et al.*[3]. The high speed supernatant was chromatographed on DEAE-Sephacel and then on a Sephadex G-200 column precalibrated with dextran blue [3]. Removal of excess detergent was achieved by passage through a column of Amberlite XAD-2 prior to the G-200 step. Protein elution was monitored at 280 nm and sulfatase activity was determined as described above.

RESULTS

Tables 1 and 2 contain data for the sulfatase activity in whole homogenates of guinea pig tissues and rat liver. Since no attempt has been made to establish statistical significance of differences between the various tissues, only mean values are shown. All guinea pig tissues studied contained estrone sulfatase activity, the adult testis being highest with SA somewhat similar to rat liver. Guinea pig liver in every individual case, was lower in activity than any other tissue from the same animal. In adult animals, lung and uterine activities were second only to testis. In lung, kidney, and possibly liver, sulfatase activity decreased from fetal to adult state (Table 1). This was particularly prominent in female kidney where the range for the fetus was 16.6–24.8 units/mg protein while for the adult it was 1.8–4.5. This trend seemed

Table 1. Estrone sulfatase activity (nmol substrate hydrolyzed/h/mg protein) in whole homogenates of tissues*

Tissue source		Tissue			
		Liver	Lung	Kidney	Small intestine
G.P. male	—fetus (2)	1.5	28.1	13.1	n.d.†
	—neonate (2)	3.1	24.7	9.8	n.d.
	—immature (3)	1.6	19.3	4.2	4.8
	—mature (3)	1.2	14.0	4.9	4.6
G.P. female	—fetus (3)	3.6	29.2	20.7	n.d.
	—neonate (2)	3.4	27.4	13.2	n.d.
	—immature (2)	2.0	19.7	3.6	7.2
	—mature (5)	1.3	18.8	3.8	8.2
	—pregnant (3)	2.9	25.8	3.4	n.d.
Rat	—mature (3)	70.0	n.d.	n.d.	n.d.

* Values are means for No. of animals shown in parentheses.
† Not determined.

Table 2. Estrone sulfatase activity (nmol substrate hydrolyzed/h/mg protein) in whole homogenates of guinea pig tissues

Tissue source		Activity	Tissue source		Activity
Uterus	—fetus (6)*	23.3	Testis	—fetus (2)	41.0
	—neonate (2)	15.1		—neonate (2)	58.0
	—immature (6)	15.2		—immature (3)	65.0
	—mature (15)	20.8		—mature (6)	87.0
	—pregnant (15) (horn with fetus)	25.7			
			Placenta (17)		9.6
	—pregnant (5) (horn without fetus)	26.1	Amnion (14)		15.0
			Chorion (18)		8.3
	—endometrium (2) (pregnant)	22.9			
	—myometrium (2) (pregnant)	19.6			

* Values are means for No. of animals in parentheses.

to be reversed in testis (Table 2). The latter Table also shows that pregnancy did not materially alter the SA of uterine sulfatase and that the activity was similar in myometrium and endometrium. There was considerable variation in uterine activity between individual animals but no clear pattern emerged over the estrus cycle or during gestation.

Not shown in the Tables are values for mature ovary (mean = 9.6 units/mg protein for a pool of 6); mature female adrenal (16.0 for a pool of 6); mature male adrenal (15.0 for a pool of 6); mature female spleen (5.3 for 5 individual animals); mature male spleen (4.7 for 5 individual animals).

Regardless of the homogenizing procedure utilized, a rather high percentage (40–65) of activity remained associated with the 750 *g* pellet from guinea pig lung, uterus and kidney. This was not altered by simple washing with buffers. For testis, the corresponding value was 25–30%. When guinea pig liver was homogenized by pestle, approximately 50% of the sulfatase activity was in the 750 *g* pellet, with about 25% in the 100,000 *g* pellet. When the Polytron was utilized, the respective values were 25 and 55%. Subcellular distribution was independent of the homogenizing technique in rat liver where about 26% of the activity was in the 750 *g* pellet and about 51% in the 100,000 *g* pellet. No more than traces of activity could be detected in any high speed supernatant. Guinea pig liver nuclei, prepared after homogenization of tissue with the pestle, contained < 10% of the sulfatase activity in the 750 *g* pellet. The 100,000 *g* pellets possessed the highest sulfatase SA of all subcellular fractions and representative values, along with estimated K_m data, appear in Table 3.

The sulfatase in all three pellets (750, 10,000 and 100,000 *g*) from all tissues displayed generally similar properties. The pH/activity curves each had an optimum in the range 7.6–8.2 for guinea pig and at 8 for rat liver. K_m values were similar for the pellets from any one tissue. Sulfatase activity in all guinea pig systems was inhibited by phosphate ion at substrate concentrations of 10 μM and 0.1 mM but rat liver activity was inhibited only at the lower substrate level (Table 4). Sulfate ion, up to 0.1 M, did not inhibit the sulfatase from any source but tended to stimulate (approx. 15% increase) in common with a number of inorganic ions. Typical Michaelis–Menten curves were obtained with all guinea pig and rat liver systems, up to about 0.2 mM substrate concentration. Beyond that, substrate inhibition occurred.

Table 5 demonstrates the ease of inhibition of rat liver sulfatase by free steroids. DHAS and DES. Guinea pig activity was much less sensitive to such compounds, only the uterine enzyme being inhibited at concentrations equimolar to that of the substrate. At concentrations 5 times that of the substrate, estradiol-17β, DES and DHAS in particular, inhibited to an increased degree, although much less than for rat liver activity. However, DES did not inhibit guinea pig liver activity, even up to 0.25 mM (25 × substrate concentration), as shown in Fig. 1. Under these latter conditions guinea pig testicular and rat liver activities were respectively inhibited by 70 and 98%. At 50 μM DES the rat enzyme was already 90% suppressed.

Table 3. Specific activity of estrone sulfatase (nmol substrate hydrolyzed/h/mg protein) and K_m values for 100,000 *g* pellets from tissues of mature animals*

Tissue source		SA	K_m (μM)
G.P. liver	(3)	6.6	10
Rat liver	(2)	212	10
G.P. lung	(2)	60	11
G.P. uterus	(2)	48	25
G.P. testis	(3)	282	20
G.P. kidney	(2)	12.6	n.d.†

* Values are means for No. of animals shown in parentheses.
† Not determined.

Table 4. Percent inhibition by inorganic phosphate of estrone sulfatase activity in 100,000 *g* pellets from adult animal tissues*

Tissue source	Substrate concn. (μM)	Phosphate (mM) 50	100
Rat liver	10	30	41
Rat liver	100	2	5
G.P. liver	10	55	71
G.P. liver	100	38	52
G.P. testis	10	53	55
G.P. testis	100	12	33
G.P. lung	10	63	68
G.P. lung	100	27	37
G.P. kidney	10	57	77
G.P. kidney	100	42	53
G.P. uterus	10	45	73
G.P. uterus	100	28	49

* Means of at least 2 experiments in each case using tissues from different animals.

Figure 2 shows that estradiol-17β and DES powerfully inhibited the rat liver activity whereas estradiol-17α, estriol and estrone, in decreasing order, had smaller though significant effects. These same phenolic compounds were relatively poor inhibitors of guinea pig liver activity (not shown graphically).

Thus DES (Fig. 1), estriol, estradiol-17α and estrone each inhibited by 10% or less up to 0.25 mM. Estriol did not inhibit at all, even at 0.5 mM. Estradiol-17β was the strongest inhibitor of the guinea pig liver enzyme but even at 0.5 mM it suppressed by only 30% as compared to 95% for rat liver (see Fig. 1).

Solubilized guinea pig testicular enzyme was eluted from DEAE-Sephacel at approx. 0.25 M NaCl and was shown to be retained on Sephadex G-200 relative to dextran blue (Fig. 3). This material had an estimated K_m of 34 μM. As shown in Table 5, the solubilized activity was no more sensitive to inhibition, in an overall sense, than was the enzyme in intact microsomes.

DISCUSSION

It is evident that an estrone sulfatase activity is widely distributed in guinea pig tissues. This resembles an enzyme in rat liver in pH/activity relationship, K_m values and absence of inhibition by inorganic sulfate. Our K_m for the rat liver enzyme agrees with that of Dolly *et al.*[2]. One apparent difference between the two animal species resides in the failure of inorganic phosphate to inhibit the rat enzyme under zero order substrate kinetics. Indeed, this is part of the definition of arylsulfatase C [6]. This is not, however, absolute, since inhibition is seen at low substrate concentration.

The reported lack of arylsulfatase C in guinea pig liver [16] could be due to the non-identity of the latter enzyme with estrone sulfatase as claimed by others for human placental sulfatase [17] and rat kidney sulfatase [18]. Alternatively, if the very low guinea pig

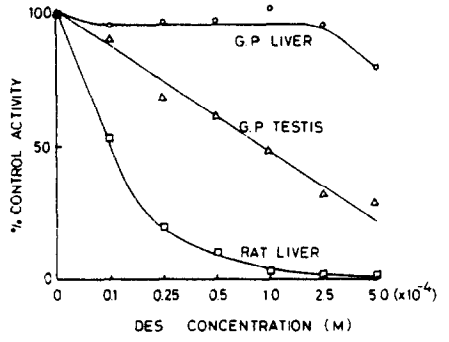

Fig. 1. Effect of increasing concentration of DES upon the microsomal estrone sulfatase activity of guinea pig liver and testis and of rat liver. Substrate concentration = 10 μM.

Table 5. Percent inhibition of estrone sulfatase activity in 100,000 *g* pellets of adult tissues by steroids and related compounds (substrate conc. = 10 μM)*

Potential inhibitor	Inhibitor concn. (μM) 10 RLI	LI	T	ST	LU	U	50 RLI	LI	T	ST	LU	U
DES	64	5	7	7	2	21	89	4	34	60	22	44
Estradiol-17β	46	0†	9	16	7	20	76	16	25	38	24	52
Estradiol-17α	33	0	0	0	4	8	49	0	6	0	7	17
Estrone	10	0	3	0	3	10	32	3	14	0	16	25
Estriol	14	0	0	0	5	11	32	0	0	0	16	8
DHAS	49	0	7	0	14	22	83	20	33	21	24	47
DHA	20	0	4	0	0	0	56	7	0	11	1	13
Testosterone	35	0	0	0	7	16	70	0	0	0	2	30
Δ^4-Androstenedione	15	0	5	0	2	8	40	0	0	0	4	18
Progesterone	18	3	0	0	0	0	59	1	0	0	5	6
Cortisol	2	0	4	0	4	12	9	0	0	0	0	16

* Values are means of at least 2 individual assays; LI = liver, T = testis, LU = lung, U = uterus, ST = solubilized testicular activity; all from guinea pig. RLI = rat liver.

† Zero indicates undetectable inhibition but note that values of 10% or less are unlikely to be meaningful.

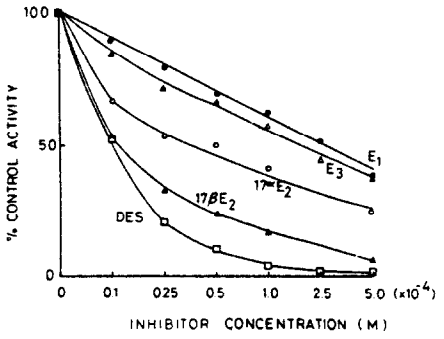

Fig. 2. Effect of increasing concentration of phenolic compounds upon the microsomal estrone sulfatase activity of rat liver. Substrate concentration = 10 μM. E_1 = estrone; E_3 = estriol; $17\alpha E_2$ = estradiol-17α; $17\beta E_2$ = estradiol-17β.

liver estrone sulfatase mirrors arylsulfatase C, perhaps assay of the latter is not sensitive enough to allow for detection. Strain of animals used may also be a factor.

Available information indicates that rat liver estrone sulfatase increases from the neonatal state to adulthood [2]. A similar increase appears to occur in the guinea pig testicular enzyme activity whereas, in lung and kidney, and perhaps liver, the opposite trend is evident. The absence of variation of enzyme activity in the uterus during the estrus cycle of the guinea pig appears to agree with published data for rat [19], pig [20] and human [21]. We cannot directly compare our results for guinea pig to those for rat and pig since the authors do not express them in terms of units. The values for the human are about 1 nmol/h/mg protein, i.e. very low in comparison to guinea pig and to other data for rat (14 units/mg according to Dolly *et al.*, Ref. [2]). Our failure to demonstrate any real effect of pregnancy on enzyme activity contrasts with the pig and sheep in which estrone sulfatase respectively increases and decreases with advancing gestation [22]. Units of activity were not provided in this last study. The considerable levels of enzyme activity in guinea pig testis, uterus and lung raise the question as to its real role and its physiological substrate.

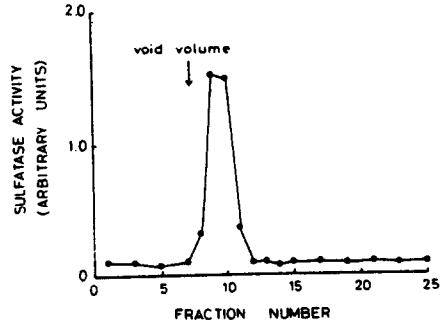

Fig. 3. Elution of solubilized estrone sulfatase activity from a Sephadex G-200 column using 0.02 M imidazole buffer, pH 7.4.

Others have speculated upon the possibility that naturally-occurring steroids might control estrone sulfatase activity via inhibition [2]. This could control release of potentially active hormones at, or near target sites. In this respect the rat liver enzyme seems quite non-specific in its sensitivity to free steroids, DHAS and DES. There is, nevertheless, some relationship between estrogenic potency and ability to inhibit (DES > estradiol-17β > estradiol-17α, estriol and estrone). Dolly *et al.*[2] also commented upon the difference between estradiol-17β and estrone in this respect. Estrone sulfatase activity of guinea pig liver, testis and lung are not so readily inhibited. That this is not merely due to a relationship between enzyme and certain microsomal membrane components, is suggested by a similar lack of sensitivity to inhibitors on the part of the solubilized enzyme from testis. We did not intend to extensively purify the enzyme but simply to separate it from its membrane environment. Testis was chosen for this approach because of its high enzyme activity.

Apart from differences between estrone sulfatase of rat liver and guinea pig tissues, a certain dissimilarity is apparent even between different tissues from the latter species. Particularly evident is the failure of DES to inhibit the liver activity while the testicular sulfatase is suppressed by increasing concentrations of the inhibitor. Also, guinea pig uterine sulfatase is more susceptible to inhibition by steroids, and DES, than is that from testis, lung or liver. Thus, an estrone sulfatase activity is widely distributed in the guinea pig. The enzyme bears a certain similarity to that occurring in rat liver but, for reasons outlined above, one cannot readily assign the term "arylsulfatase C" to it.

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