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Differences in the activity of human term placenta sulphatases for steroid ester sulphates

The role of the human placenta in the biogenesis and metabolism of various steroids has been established by different groups[1-3]. The presence of arylsulphatase (EC 3.1.6.1) and steroid sulphatase (EC 3.1.6.2) activities for $^5\Delta$-3β-hydroxy and phenol steroids has also been demonstrated *in vitro* and *in vivo*[4-6]. In this study the sulphatase activity of human term placenta was studied by perfusion *in vitro* with different radioactive steroid ester sulphates.

The technique of perfusion was the same as that previously described[7]. Steroid ester sulphates were synthesized by reacting chlorosulphonic acid with the respective tritiated steroid[8,9]. The radiochemical homogeneity of labelled steroid ester sulphates was established by successive crystallization in ethanol and ethanol–acetone mixtures. After hydrolysis or solvolysis of the different ester sulphates, the liberated steroids were chromatographed in different systems and crystallized with authentic reference compounds to demonstrate that the steroid moiety remained unchanged. The following labelled steroid ester sulphates were used: [1,2-$^3H_2$]corticosterone 21-sulphate (11β-hydroxypregn-4-ene-3,20-dione-21-yl sulphate), specific activity 0.1 μC/μg; [5,6-$^3H_2$]androsterone 3α-sulphate (5α-androstan-17-one-3α-yl sulphate), specific activity 6 μC/μg and [7α-^{3}H]dehydroepiandrosterone 3β-sulphate (androst-5-en-17-one-3β-yl sulphate), specific activity 0.2 μC/μg.

Two experiments were performed with each compound, using 10 and 45 μC, except for dehydroepiandrosterone sulphate where only one experiment was performed using a dose of 10 μC. Samples of perfusate were collected 15, 30, 60, 90 and 120 min after the beginning of the experiments. Following homogenization, the different samples and the placental tissue were precipitated with 2 vol. of ethanol; the whole extracts were left 48 h at 0° and centrifuged. The supernatants were evaporated to dryness, dissolved in 90% (v/v) ethanol, left for 3 days at −10°, centrifuged, evaporated to dryness and dissolved in water.

The radioactive material in the aqueous solution was extracted by dichloromethane (Extract I) and then by *n*-butanol (Extract IIa). A part of the radioactive material present in Extract I was re-extracted by washing with 9% Na_2CO_3 (w/v). The radioactive material was extracted again with *n*-butanol from the alkaline wash. Chromatography on paper in the butyl acetate–toluene–*n*-butanol–4 M NH_4OH–methanol (12:6:2:10:10, by vol.) and butyl acetate–toluene–*n*-butanol–acetic acid–water–methanol (10:8:2:1:9:10, by vol.) systems indicated that this material was still in conjugated form (dichloromethane-soluble ester sulphate fraction) (Extract IIb). The absence of conjugates in Extract I was established by chromatography in toluene–propanediol, isooctane–toluene–methanol–water (8:2:5:5, by vol.) and isooctane–propanediol systems. The absence of free steroids in Extract IIa was established in the same system as for Extract IIb. Radioactivity was measured in a liquid-scintillation counter (Packard 3002; efficiency for ^{3}H: 29%) and quenching was measured using tritiated toluene as an internal standard.

Table I gives the percentage of free and conjugated steroids in the perfusates at 15, 30, 60, 90 and 120 min after the beginning of the perfusion of the 3 different ester sulphates. As indicated in Table I the capacity for the hydrolysis of a 21-sulphate

TABLE I

PERCENTAGE OF HYDROLYSIS OF DIFFERENT STEROID ESTER SULPHATES BY HUMAN TERM PLACENTA AT DIFFERENT TIMES OF PERFUSION

The percentages of the radioactivity in each period are given. Doses: I, 10 μC; II, 45 μC. D.S.E.S., dichloromethane-soluble ester sulphate.—, fraction not studied.

Steroid ester sulphate	*Fractions*	*Time of perfusion (min)*										*Placental tissue*	
		15		*30*		*60*		*90*		*120*			
		I	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*
[^{3}H]Corticosterone 21-sulphate	Unconjugated	3	1	3.2	1.2	3.6	1.5	3.3	2	—	3	8	6
	Conjugated D.S.E.S.	7	5	4	6	4	8	21	16	—	46	30	50
	Conjugated *n*-butanol	75	90	84	90	70	76	75	75	—	50	50	30
[^{3}H]Androsterone 3α-sulphate	Unconjugated	2	2.5	1	2.8	1	3.3	3.5	3.8	7	6	15	10
	Conjugated D.S.E.S.	15	40	16	47	20	45	21	45	50	69	62	65
	Conjugated *n*-butanol	67	50	70	45	76	45	60	50	27	10	9	10
[^{3}H]Dehydroepiandrosterone 3β-sulphate	Unconjugated	5	—	13	—	28	—	28	—	—	—	77	—
	Conjugated D.S.E.S.	20	—	28	—	25	—	22	—	—	—	10	—
	Conjugated *n*-butanol	75	—	48	—	40	—	30	—	—	—	7	—

(corticosterone 21-sulphate) and a 3α-sulphate (androsterone 3α-sulphate) by the human term placenta was limited. However, a considerable quantity of 3β-ester sulphate (dehydroepiandrosterone sulphate) was hydrolyzed. These last results are in agreement with those of other authors[6,10].

It is of interest to note that a high percentage of the conjugated material was extracted by dichloromethane, especially from 120-min perfusion samples and the

TABLE II

DICHLOROMETHANE-EXTRACTABLE LABELLED STEROID ESTER SULPHATES RECOVERED FROM AQUEOUS AND BLOOD SOLUTIONS

D.S.E.S.,dichloromethane-soluble ester sulfate.

Steroid ester sulphates	*Fraction*	*Radioactive material extracted, after 15 min contact, from*	
		Aqueous solution (%)	*Blood solution (%)*
[^{3}H]Corticosterone 21-sulphate (4 μC)	Unconjugated	0	0
	Conjugated D.S.E.S.	0	7.5
	Conjugated *n*-butanol	99.5	91.2
[^{3}H]Androsterone 21-sulphate (4 μC)	Unconjugated	0	0
	Conjugated D.S.E.S.	0	21
	Conjugated *n*-butanol	100	78
[^{3}H]Dehydroepiandrosterone 3β-sulphate (4 μC)	Unconjugated	0	0
	Conjugated D.S.E.S.	0	17
	Conjugated *n*-butanol	99.5	82

tissue fractions following the corticosterone sulphate and androsterone sulphate perfusions. Parallel experiments carried out with the same labelled ester sulphates dissolved in water and in whole blood (human blood + Krebs–Ringer buffer solution, 1:5, v/v; pH 7.4) and extracted with dichloromethane show that no radioactive material was extracted from aqueous solution. However, a proportion of the radioactive conjugates was extracted from the blood. This is indicated in Table II. BURSTEIN has also demonstrated that some dehydroepiandrosterone sulphate can be extracted from an aqueous solution by toluene if the water contains phospholipids[11]. It was also shown that oestrone sulphate can be extracted with chloroform from aqueous human bile solution[12].

It is relevant to note that a 17β-hydroxy-C_{19} steroid sulphate (testosterone 17β-sulphate) is not hydrolyzed by human placenta[13] whereas arylsulphates are[14]. Experiments involving the injection of [^{3}H]corticosterone 21-sulphate into the intact foeto-placental unit at mid-pregnancy indicate that this ester sulphate is not hydrolyzed *in vivo* by the placenta[15]. Thus, it seems likely that the sulphatases of the human placenta attack mainly oestrogens and Δ^5-3β-hydroxy steroids 3-sulphates.

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