Development and characteristics of an oestrogen sulphotransferase in placenta and uterus of the pregnant mouse

Comparison between mouse and rat

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The mouse placenta possesses a soluble oestrogen sulphotransferase activity which increases markedly from at least 12 days of gestation until term. At about 16 days of gestation, a similar activity is found in the uterus. This activity also increases until term and disappears rapidly *post partum*. The uterine enzyme activity appears to require the presence of the foetal unit for its onset, since unoccupied horns, whether their endometrial stromal cells are differentiated to decidual cells or not, are essentially devoid of it. Uterine cytosols from non-pregnant mice are also inactive in this respect. In late gestation, the uterine sulphotransferase is confined to the decidua basalis, the areas to which the placentas are attached. The sulphotransferase(s) of placenta and uterus has an absolute requirement for 3'-phosphoadenosine 5'-phosphosulphate, and possesses little activity in the absence of exogenous thiol groups. Stimulation is also seen in the presence of Mn^{2+} , Mg^{2+} or Ca^{2+} . Oestrone and oestradiol, and to a lesser degree oestriol, are substrates for the enzyme(s), whereas testosterone, cortisol and dehydroepiandrosterone are not. Oestrone and oestradiol at higher concentrations (1.0–1.5 μM) completely inhibit the enzyme(s). These enzymes could play a role in altering tissue concentrations of active oestrogens during gestation in the mouse. Oestrogen sulphotransferase activity is low or absent in reproductive tissues of the pregnant rat.

A considerable literature has developed on the topic of steroid sulphotransferase enzymes, particularly those in liver (Singer, 1982). There is increasing evidence for the presence of oestrogen sulphotransferases in the reproductive tissues of several mammalian species. This includes the secretory uterine endometrium of pig (Pack & Brooks, 1974) and human (Burchell & Hahnel, 1975), as well as the uteri of the sheep and pig during pregnancy (Dwyer & Robertson, 1980) and the uterus and chorion of the pregnant guinea pig (Freeman *et al.*, 1983). There is evidence to suggest that oestrogen sulphotransferases may control the concentrations of free, active, oestrogens in the secretory endometrium of pig (Pack *et al.*, 1978) and human (Pack *et al.*, 1979), and thus play a role in controlling the biological action of these hormones in the uterus. Our own studies on the guinea pig (Freeman *et al.*, 1983) have indicated the presence

of a fairly specific oestrogen sulphotransferase, of relatively low K_m , in the uterus and chorion. The temporal relationships of this led us to speculate about its biological function and also about the possibility that it might be under foetal control. The considerable gestational length in the guinea pig, relative to other small laboratory animals, poses some difficulties in such studies. We have therefore turned our attention to the mouse and rat as potential models for investigation. The present paper reports our findings with regard to some of the characteristics and behaviour of oestrogen sulphotransferase activity in mouse placenta and uterus, as well as to the possible control of the onset of this activity in uterus.

Experimental

Materials

[6,7- 3H]Oestrone (sp. radioactivity 55.9 Ci/mmol), [6,7- 3H]oestrone 3-sulphate (sp. radio-

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activity 53 Ci/mmol), [6,7-³H]oestradiol (sp. radioactivity 56.4 Ci/mmol), [7(n)-³H]dehydroepiandrosterone (sp. radioactivity 25 Ci/mmol) and [1,2(n)-³H]cortisol (sp. radioactivity 42.2 Ci/mmol) were purchased from New England Nuclear (Canada), Dorval, Que., Canada. [6,7-³H]-Oestradiol (sp. radioactivity 55 Ci/mmol) and [1 α ,2 α (n)-³H]testosterone were purchased from Amersham, Oakville, Ont., Canada. These were purified and stored for use by established methods (Hobkirk *et al.*, 1969a). Unlabelled oestrone 3-sulphate was synthesized chemically (Fieser, 1948), purified, and stored as described elsewhere (Hobkirk *et al.*, 1969a). All other unlabelled steroids were obtained from Steraloids, Wilton, NH, U.S.A. 3'-Phosphoadenosine 5'-phosphosulphate was synthesized as described by Singer (1979). Purity was checked by high-performance liquid chromatography (Freeman *et al.*, 1983). α -Monothioglycerol and purified bovine serum albumin were purchased from Sigma Chemical Co., St Louis, MO, U.S.A. DEAE-Sephadex A25 was obtained from Pharmacia (Canada) Ltd., Montreal, Que., Canada. The scintillation fluid used was xylene-based ACS (Amersham). All other reagents and solvents were of acceptable analytical grade.

Albino mice of the Swiss CD-1 strain were purchased from Charles River (Canada) Inc., St. Constant, Que., Canada, or bred in our own animal facility. They were housed in a thermostatically and light-controlled environment (10 h dark, 14 h light). Evidence of mating was taken to be the presence of a vaginal plug, the date of which was considered to be day zero of pregnancy. These mice normally reach term on the early morning of day 19. Sprague-Dawley rats were purchased from Charles River (Canada) Inc., and housed as for mice. All animals were fed on Purina Chow and had free access to drinking water.

Surgical procedures on mice

To obtain a mouse model in which one uterine horn was unoccupied during pregnancy, non-pregnant females were unilaterally oophorectomized or subjected to unilateral oviduct resection under diethyl ether anaesthesia. Incisions were closed with surgical clips. After at least 2 weeks the animals were mated. Some of these mice were again submitted to surgery on day 4 after mating. On this occasion the uterine horn which was to remain unoccupied was traumatized by inserting a burred needle along its length and scratching the anti-mesometrial surface. Incisions were closed and pregnancy was allowed to proceed. Under these conditions the endometrial stromal cells of the unoccupied horn differentiated into decidualized cells, which constitute the maternal component of

the placenta in the absence of embryos (Psychoyos, 1973).

Preparation of tissue cytosols

Animals were killed by a blow to the head or by cervical dislocation. Tissues were rapidly removed, cleaned, washed in cold 0.9% NaCl, blotted, weighed and homogenized on ice in 0.25 M-sucrose containing 10 mM-Tris/acetate, pH 7.6 (up to 1.0 g of tissue/10 ml of buffer), for 2 $\times$ 15 s with a Polytron (model PT 10-35) at setting 6. In some unoperated animals the decidua basalis, underlying the sites of placental attachment, was carefully dissected out and homogenized separately from the uterine residue. Each homogenate was centrifuged at 105 000 g for 60 min at 4°C in a Beckman model L5-65 ultracentrifuge with a type 50 Ti rotor. High-speed supernatants were assayed for sulphotransferase activity on the day of preparation. In certain instances the pellets were reconstituted in 10 ml of homogenization buffer and assayed for sulphatase activity. Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Sulphotransferase assay

Tissue cytosols were diluted to a maximum of 1:100 with 0.25 M-sucrose containing 10 mM-Tris/acetate, pH 7.6, before incubation. The degree of dilution depended on the tissue involved and the stage of pregnancy. Protein concentrations incubated ranged from 30 μ g/ml for placenta and uterus from late pregnancy to 150 μ g/ml for uterus from early pregnancy. The routine assay was essentially that described by Freeman *et al.* (1983) for guinea-pig tissues. Cytosol samples were pre-incubated with 16.7 mM-monothioglycerol for 15 min at 20°C, and 0.6 ml volumes of this were transferred to tubes containing 0.1 ml of 1 M-Tris/acetate, pH 8.0, and 0.3 ml of 10 mM-Tris/acetate, pH 7.6, containing [³H]steroid, 3'-phosphoadenosine 5'-phosphosulphate and Mg²⁺ at 37°C. Blanks contained all components except 3'-phosphoadenosine 5'-phosphosulphate. Incubations were performed in duplicate at 37°C for times of 10 min to 1 h. Total volume was 1.0 ml, and final consns. were: steroid substrate, 0.1 μ M (approx. 2 $\times$ 10⁵ d.p.m. of ³H); 3'-phosphoadenosine 5'-phosphosulphate, 50 μ M; Mg²⁺, 10 mM; monothioglycerol, 10 mM. Incubations were terminated by the addition of 2.0 ml of 3 M-sodium acetate buffer, pH 4.0, at 4°C. The tubes were extracted with 2 $\times$ 6 ml of ether, and were kept on ice between extractions. The ether was removed by aspiration, and triplicate 0.2 ml volumes of aqueous phase were transferred to polyethylene mini counting vials, mixed with 5 ml of ACS scintillation fluid, and counted for radioactivity in a Nuclear-Chicago Unilux II A spectrometer for a

sufficient time to ensure an error of no more than $\pm 5\%$ for any sample. Quenching was corrected for by the channels-ratio method. Where duplicate incubations resulted in differences of more than $\pm 10\%$, these were repeated, wherever possible. One unit of enzyme activity is that amount of enzyme required to produce 1 pmol of oestrogen sulphate/h. Specific activity is defined as the number of units of enzyme activity/mg of protein.

Product identification in cytosol assays

Incubations were performed as described above, except that 2×10^6 d.p.m. of [^3H]oestradiol was used as substrate. Reactions were terminated by adding 15 ml of cold methanol, and the mixture was left overnight at -20°C . The filtrates from these were evaporated under reduced pressure at 40°C and transferred, in water, to columns ($0.9 \text{ cm} \times 60 \text{ cm}$) of DEAE-Sephadex. These were eluted with linear gradients of NaCl (500 ml each of water and 0.8 M -NaCl) and the eluates were analysed for radioactivity. The methods have been published in detail elsewhere (Hobkirk *et al.*, 1969b). Eluted peaks of oestrogen sulphates were identified by crystallization to constant specific radioactivity with pure labelled carrier before and after removal of the sulphate group by solvolysis (Hobkirk *et al.*, 1969a,b).

Incubation of whole tissues

Quartered mouse placentas and sections of uteri were each incubated in 2 ml of Krebs–Ringer phosphate buffer (Umbreit *et al.*, 1964), pH 7.4, supplemented with glucose, with [^3H]oestrone or [^3H]oestradiol as described by Freeman & Hobkirk (1978). Tissue weights were 125 mg and substrate concns. were 10 nM . Incubations were performed for 1 h at 37°C and terminated by the addition of methanol. Labelled metabolites were analysed and identified as described above for cytosol incubations.

Sulphatase assay

Sulphatase activity was assayed on 105 000 g pellets as described by Hobkirk *et al.* (1982). The unit of activity is that amount of enzyme required to hydrolyse 1 nmol of oestrone 3-sulphate/h to free oestrone.

Results

Reliability of the sulphotransferase assay

The rate of sulphate conjugation was linear with time, over the protein-concentration range incubated, up to about 1 h provided that less than 50% of substrate had been used up. Blank incubations resulted in an apparent oestrogen sulphate production of 1.5 pmol/h. This gave blank specific-activity

values of 25 and 50 units/mg of protein for early and late mouse placentas respectively. The corresponding blanks for early and late mouse uteri were 10 and 50 units/mg of protein. For mouse chorion, non-pregnant mouse uteri and all pregnant rat tissues, blank values were about 10 units/mg of protein. Fig. 1 shows that, when [^3H]oestrone was incubated with the placental cytosol system, the sole metabolite was [^3H]oestrone 3-sulphate. When [^3H]oestradiol was the substrate, 98% of the product was [^3H]oestradiol 3-sulphate and 2% was [^3H]oestrone 3-sulphate. These results, for placental cytosol, were similar to those with uterine cytosol. There was no evidence for attachment of sulphate at position 17 of oestradiol.

Sulphotransferase activity in mouse and rat

Fig. 2 shows the temporal behaviour of the enzyme activity in placental and uterine cytosols of the mouse during gestation. Measurable activity was present in placenta at 12 days, and this increased to very high values near term. The uterus was more or less devoid of activity until about 16 days, at a time when foetal weight averaged about 0.7 g. This latter activity increased sharply to term and fell rapidly after delivery, so that it was undetectable after 24 h *post partum*. Mouse chorion activity was 32 ± 10 units/mg of protein (S.E.M., $n = 15$) and showed no distinct temporal pattern. For the chorion, a value of 20 units/mg of protein differs significantly ($P < 0.05$) from blanks. The uteri of non-pregnant mice yielded

Fig. 1. Elution profile from a DEAE-Sephadex column of metabolites of oestrone from a placental cytosol incubation

[^3H]Oestrone ($0.1 \mu\text{M}$; 2×10^6 d.p.m.) was incubated with mouse placental cytosol under the conditions described in the Experimental section. An aqueous extract of the incubation products was transferred to a column ($60 \text{ cm} \times 0.9 \text{ cm}$) and eluted with a linear $0\text{--}0.8 \text{ M}$ -NaCl gradient. Abbreviations: OE₁, oestrone; OE₃S, oestrone 3-sulphate; OE_{2,3}S, oestradiol 3-sulphate (arrow indicates where this would be eluted, if present). O, ^3H radioactivity; ---- [NaCl].

Fig. 2. Uterine and placental cytosol oestrogen sulphotransferase activities during the last 7 days of pregnancy in the mouse

Uterine (hatched bars) and placental (open bars) oestrogen sulphotransferase activities are presented as mean values $\pm$ S.E.M. on individual days of pregnancy. Each bar represents the mean for 8–16 animals. These animals would normally deliver during the early morning of day 19. The values above each set of bars represent the average foetal wt. (g) on each day. All data are corrected for experimental blanks.

Table 1. Effect of foetal occupancy on mouse uterine oestrogen sulphotransferase activity

Activities are shown for cytosols of whole uterine horns which had been unoccupied, or occupied by foetuses, in 12 mice at various stages of pregnancy. All values are corrected for experimental blanks. For occupied horns bearing foetuses of less than 1.0 g each, and for all unoccupied horns, activities (20 units/mg of protein) differ significantly ($P < 0.05$) from blanks. For occupied horns bearing foetuses of over 1.0 g, activities (100 units/mg of protein) differ significantly ($P < 0.05$) from blanks. Average wet wt. of unoccupied horns = 95 mg. Average wet wt. of occupied horns (minus contents) = 690 mg. Abbreviation: N.D., not detectable.

Surgery (unilateral)	Average wt./ foetus (g)	Sulphotransferase (units/mg of protein)	
		Occupied horn	Unoccupied horn
Oophorectomy	0.28	N.D.	N.D.
	0.46	10	N.D.
	0.49	1	19
	0.57	9	N.D.
	1.17	189	54
	1.25	466	4
	1.53	809	103
	1.77	672	64
Oviduct resection	0.86	55	N.D.
	1.20	153	7
	1.26	286	2
	1.40	1140	44
	1.77	672	64

values which were not significantly different from blanks at the $P = 0.05$ level.

Table 1 demonstrates that unoccupied uterine horns at various times during gestation do not

Table 2. Effect of decidualization on mouse uterine oestrogen sulphotransferase activity

Activities are shown for cytosols of whole decidualized-unoccupied uterine horns, and occupied uterine horns, from six mice at various stages of pregnancy. All values are corrected for experimental blanks. For all decidualized-unoccupied horns and for occupied horns bearing foetuses of less than 1.0 g, activities (20 units/mg of protein) differ significantly ($P < 0.05$) from blanks. For occupied horns bearing foetuses of over 1.0 g, activities (100 units/mg of protein) are significantly different ($P < 0.05$) from blanks. Average wet wt. of decidualized-unoccupied horns = 302 mg. Average wet wt. of occupied horns (minus contents) = 782 mg. Abbreviation: N.D., not detectable.

Average wt./ foetus (g)	Sulphotransferase (units/mg of protein)	
	Occupied horn	Decidualized- unoccupied horn
0.51	3	N.D.
0.57	4	61
0.70	26	27
1.03	2105	43
1.23	1874	76
1.40	1018	64

possess much, if any, sulphotransferase activity. Table 2 further shows that, even when the unoccupied horn has developed the typical decidualized response secondary to mechanical traumatization, little enzyme activity develops.

The data in Table 3 demonstrate that essentially the entire uterine sulphotransferase activity in the mouse is localized in the decidua basalis to which the placentas are attached. The considerable concentration of enzyme activity in placenta and decidua

Table 3. *Localization of oestrogen sulphotransferase activity in pregnant mouse uterus*

Activities are shown for cytosols of decidua basalis and of residual uteri from 13 intact mice at various stages of pregnancy. All values are corrected for experimental blanks. For decidua basalis from uteri bearing foetuses of less than 1.0g, and for all residual uteri, activities (20 units/mg of protein) differ significantly ($P < 0.05$) from blanks. For decidua basalis from uteri bearing foetuses of over 1.0g, activities (100 units/mg of protein) are significantly different ($P < 0.05$) from blanks. Abbreviation: N.D., not detectable.

Average wt./ foetus (g)	Sulphotransferase (units/mg of protein)	
	Decidua basalis	Residual uterus
0.19	25	3
0.30	9	N.D.
0.51	6	3
0.59	1	N.D.
0.61	334	36
0.73	321	4
0.89	207	6
1.02	376	96
1.02 }	1219 (pool)	13
1.40 }		46
1.20	514	16
1.30	656	24
1.70	1569	36

basalis is particularly evident when it is expressed as units per entire organ or tissue and compared with liver. The following represent mean values for six mice between 17 and 18 days of gestation: a single placenta, 4100 units; decidua from a single attachment site, 1600 units; an entire liver, 980 units. Oestrone was the substrate in each case.

Attempted assay of oestrogen sulphotransferase in cytosols of placenta, uterus and foetal membranes from rats ($n=7$) at various stages of gestation yielded activities less than 20 units/mg of protein. There was no difference between the activity in the decidua basalis and that in residual uterine tissue.

Whole-tissue studies

Whole placental tissue from eight mice between 13 days of gestation and term converted [^3H]-oestrone into the sulphated form to an average extent of 9.4%. The corresponding value, with [^3H]oestradiol as substrate, was 20.4%. Whole uterine tissue from the same animals produced detectable oestrogen sulphate from the same two substrates only when the uterine cytosol sulphotransferase activity exceeded about 650 units/mg of protein.

Sulphatase activity

There was no obvious fluctuation of activity during the final 5–6 days of gestation. The values for

ten mice, in terms of nmol/h per mg of protein (mean $\pm$ S.E.M.), were: placenta, 1.93 ± 0.43 ; decidua basalis, 16.74 ± 2.28 ; residual uterus, 4.15 ± 0.76 .

Characteristics of the sulphotransferase

Activities in mouse uterine and placental cytosols displayed similar characteristics. The optimum pH range was 7.5–8.5. In the absence of added thiol groups, placental and uterine enzymes respectively possessed only 1.5% and 11% of the optimal activity reached in the range 15–50 mM monothioglycerol. Mercapthoethanol and dithiothreitol yielded similar results at these concentrations, and 25 mM-cysteine caused a stimulation equivalent to 75% of the optimum. The very low apparent activities in some individual rat tissues were the same with or without monothioglycerol. In the presence of sufficient monothioglycerol to ensure optimal activity of mouse uterine and placental cytosols, Mn^{2+} stimulated by a factor of 3-fold, whereas Ca^{2+} and Mg^{2+} each stimulated 1.5–2-fold. These ionic effects were constant between 10 mM and 25 mM. Phosphate and K^+ had no effect up to at least 25 mM.

Fig. 3 shows the inhibitory effect of increasing oestradiol concentration on enzyme activity of mouse uterine and placental cytosols. Oestrone displayed a similar effect and brought about essentially complete inhibition at $1.5 \mu\text{M}$. Plots of v against $[\text{S}]$ for various concentrations of 3'-phosphoadenosine 5'-phosphosulphate resulted in typical Michaelis–Menten curves.

Mouse placental and uterine cytosols each displayed similar activities towards [^3H]oestrone and [^3H]oestradiol. [^3H]Oestriol, under the same incubation conditions, was conjugated with sulphate to a relative degree of 43%. [^3H]Testosterone, [^3H]dehydroepiandrosterone and [^3H]cortisol, each at $0.1 \mu\text{M}$, did not act as substrates. Enzyme activity toward [^3H]oestrone or [^3H]oestradiol was inhibited by 60% and 95% by oestriol when present at concentrations 5-fold and 25-fold, respectively, that of either substrate. Neither dehydroepiandrosterone nor cortisol was an inhibitor under these conditions. Testosterone and diethylstilboestrol, each at concentrations 25-fold those of the substrates, inhibited by 25% and 37% respectively.

Discussion

The effects of thiol groups and bivalent cations on the oestrogen sulphotransferase activities of mouse uterine and placental cytosols, as well as the substrate specificity of the enzyme(s), are generally similar to those found for guinea-pig uterus and chorion (Freeman *et al.*, 1983). Substrate inhibition of certain steroid sulphotransferases is not uncommon.

Fig. 3. Effect of oestradiol substrate concentration on the oestrogen sulphotransferase activities of mouse uterine and placental cytosols

Cytosols of mouse uterus (O) and placenta (●) were assayed for oestrogen sulphotransferase activities as explained in the Experimental section. Increasing concentrations of [3 H]oestradiol were utilized as substrate. This represents one of three experiments performed with different mice, all of which gave similar results. Each experimental point has been corrected by subtraction of the blank.

mon (Singer, 1978). However, the marked inhibition of the mouse sulphotransferase(s) in the present study, together with the low substrate concentration at which it occurs, does not appear to have been demonstrated for other oestrogen sulphotransferases, e.g. bovine adrenal (Adams *et al.*, 1974), bovine placenta (Adams & Low, 1974), mammary tumour (Godefroi *et al.*, 1975), or rat liver (Green & Singer, 1983). A realistic estimate for K_m is thus not possible for the mouse enzyme(s). The nature of the system points to a possible regulatory role for the mouse sulphotransferase(s).

Progesterone is capable of inducing an oestrogen sulphotransferase in human uterine endometrial cells (Tseng & Liu, 1981; Clarke *et al.*, 1982). This steroid obviously does not act in this way in unoccupied horns, decidualized or otherwise, of the pregnant mouse. The possible role of progesterone in enzyme induction in the decidua basalis at about 16 days of gestation is yet to be explored. Available

evidence suggests that the decidua basalis in mouse, rat and guinea pig is a maternal structure (Pijnenborg *et al.*, 1981). A foetal factor (or factors) could conceivably be responsible for the onset of the enzyme activity, as speculated for an oestrogen glucuronyltransferase (Hobkirk & Cardy, 1980) and an oestrogen sulphotransferase (Freeman *et al.*, 1983), in the uterus of the pregnant guinea pig. However, in the guinea pig, these enzymes are distributed throughout the endometrium (P. Tziougras & R. Hobkirk, unpublished work).

An oestrogen sulphotransferase in the secretory endometrium of pig (Pack *et al.*, 1978) and human (Pack *et al.*, 1979) might, through formation of inactive water-soluble oestrogen sulphates, regulate the amount of active oestrogen available for binding to its receptor. Further work is necessary to prove whether the mouse sulphotransferase(s) plays a similar role in gestation. Our data from whole mouse uterus *in vitro* suggest that effective oestrogen sulphate formation may occur *in vivo* only very near parturition. This could be due to high sulphatase activity in the decidua basalis. In whole placental tissue, *in vitro*, oestrogen sulphate formation is seen as early as 13 days of gestation. The placental sulphotransferase could be a means of limiting the transfer of potentially injurious concentrations of oestrogen to the foetus. It is generally considered that the main source of oestrogen in the small rodent during gestation is the maternal ovary (Ryan, 1973), although we can find no definitive statement about the mouse.

The absence of oestrogen sulphotransferase activity from pregnant-rat reproductive tissues may cast doubt on the importance of the enzyme in the mouse. However, a sharp increase in blood oestrogen has been reported to precede parturition in the rat (Wilson *et al.*, 1982), suggesting a requirement for the active (free) hormone. Also, the rat foetus is known to possess α -foetoprotein, which is capable of binding oestrogens strongly (Swartz *et al.*, 1974), and thus possibly playing a protective role. In the guinea pig there is no good evidence for increased circulating oestrogen near parturition; indeed, excess oestrogen has been reported to prolong gestation (Illingworth *et al.*, 1974). Thus, in different species, there may be different requirements for control of oestrogen during gestation.

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