Biochemical Evidence for the Non-inactivation of the Steroid Sulfatase Locus in Human Placenta and Fibroblasts

Monique Bedin1, Dominique Weil1, Thérèse Fournier1, Lise Cedard1, and Jean Frezal2
1 Groupe de Recherches sur l'Endocrinologie de la Reproduction, INSERM U. 166, Maternité Port-Royal, 123, Bld de Port-Royal, 75014 Paris, France
2 Unité de Recherches INSERM U. 12, Hôpital des Enfants Malades, 149, rue de Sévres, 75730 Paris Cedex 15, France

Summary. Steroid sulfatase activities are significantly higher in placentas obtained after the birth of girls than after the birth of boys, and also in female fibroblasts compared to male strains. This constitutes biochemical evidence for the non-inactivation of the X-linked sulfatase locus. No hydrolytic activity is found in the fibroblasts of ichthyotic boys. Heterozygosity is demonstrated in the fibroblasts of the four mothers studied, as they have steroid sulfatase activity of less or equivalent to the normal male value.

Introduction

The enzymatic properties of the sterol sulfate sulfohydrolase (steroid sulfatase) have been under investigation for many years but the tightly membrane-bound nature of this enzyme hampered its purification and characterization. Despite these difficulties, the clinical importance of this hydrolytic activity appeared when the steroid sulfatase was shown to be deficient in the human placenta from pregnancies characterized by extremely low estrogen production (France and Liggins 1969). More recently this inborn error of metabolism has been shown to persist in the fibroblasts of the boys born of the affected pregnancies and to be associated with the appearance of a skin disorder, the so-called X-linked ichthyosis (Koppe et al. 1978; Shapiro et al. 1979).

Genetic studies indicate either that the steroid sulfatase and the X-linked ichthyosis are coded for by two closely linked loci or, and more likely, that X-linked ichthyosis is in some unknown way a consequence of the mutation at the steroid sulfatase locus, which has been assigned to the distal part of the short arm of the X chromosome, i.e., the Xp22.3 band (Mohandas et al. 1979; Muller et al. 1980b; Tiepolo et al. 1980).

Since 1970, we have demonstrated placental sulfatase deficiency in 22 pregnancies leading to the birth of 24 boys (first report, see Bedin et al. 1980a), three of the boys were triplets, and two others were born to sisters (Bedin et al. 1979). The dermatologic examination of 16 of these boys revealed the existence of X-linked ichthyosis in all of them except one.

The present work has been undertaken to investigate the problem of the inactivation of the steroid sulfatase locus, considering first the findings of Ropers et al. (1978) on the preferential paternal X inactivation in the placenta, and second the presumed linkage between the X-linked ichthyosis and the blood group Xg' loci (Race and Sanger 1975). We studied the expression level of the steroid sulfatase activity in placentas and fibroblasts obtained from normal individuals of both sexes as well as from ichthyotic boys and their mothers. In the meantime, other studies have provided strong evidence for the non-inactivation of the steroid sulfatase locus (Mohandas et al. 1979; Shapiro et al. 1979).

Materials and Methods

1) Cultures of Fibroblasts

Skin biopsies were taken from normal individuals and from ichthyotic boys with or without a proven placental defect and from their carrier mothers. Fibroblast cultures were performed from these biopsies with the RPMI-1640 medium plus 10% fetal calf serum. The cells were harvested by trypsinization and washed three times with 90% saline solution. Pellets were kept frozen at -80°C until the day of experiment.

2) Placentas

Placentas were obtained from normal pregnancies immediately after vaginal delivery or cesarian section, freed of vessels and connective tissue, and homogenized in 0.25 M sucrose, 0.002 M Tris-HCl buffer at pH 7.4. The homogenates were kept frozen at -80°C until the day of experiment.

3) Steroid Sulfatase Titration

Incubations were carried out in duplicate according to the method of Burstein and Dorfman (1963) at 37°C in the presence of labeled dehydroepiandrosterone sulfate (1H-DHA-S, S.A. 24 Ci/mM, NEN Chemicals) at a final concentration of 4 × 10⁻³ M; 0.1 ml of placental homogenate (1–2 mg protein/ml) was incubated with 0.4 ml of 0.02 M Tris-HCl pH 7.4 and 0.5 ml of substrate solution; 0.1 ml of fibroblast suspension was incubated with 0.05 ml of 0.1 M Tris-HCl and 0.1 ml of substrate solution. The incubations were stopped by addition of 1 N NaOH and the yield of liberated unconjugated steroid was measured after direct extraction by the scintillation mixture (Toluène—POPOP-PPO); the results were corrected for methodologic losses. The reaction rates were linear within the incubation time and the protein concentration range employed.
Fig. 1. Sex different gene dosage for the steroid sulfatase in normal placentas and fibroblasts. \( \bullet \) \( \square \) represent the mean values determined for the four series of "n" samples each. • and • indicate the range of values for the different series. The value in brackets is eliminated from final calculations (see Results)

**Results**

The hydrolysis rate of DHA-S was determined in placentas obtained after the birth of either a boy \((n = 30)\) or a girl \((n = 24)\). The results, expressed as nmoles DHA-S hydrolyzed per minute per mg of protein, are shown in Fig. 1(A). Preliminary results reported elsewhere \((\text{Bedin et al. 1980b})\) were confirmed: a 1.38 higher activity exists in "female placentas" compared with "male placentas," and the \( t \)-test applied to the two means is significant \((P < 0.001)\). We have verified that the mode of delivery has no influence on the placental sulfatase activity.

The steroid sulfatase activity in fibroblasts was tested on 13 female and eight male normal cell cultures. The titrations are reported in Fig. 1(B) and expressed as nmoles of DHA-S hydrolyzed per hour per mg of protein. The highest value observed in the female group has been omitted from the final calculations: indeed it falls outside the 95% confidence limit determined from the 13 values namely \(0.916 \pm 0.325\) nmol/protein/min. Nevertheless and despite large individual variations, a highly significant difference appears between the eight normal males \(\text{(mean value} = 0.476\text{nmol hydrolyzed/hour/mg protein)}\) and the 12 normal females \(\text{(mean value} = 0.784\text{nmol hydrolyzed/hour/mg protein)}\) as shown by the Wilcoxon \( U \)-test \((P < 0.005)\): the 1.64 multiplication factor existing between the two means suggests that a linear gene dosage relationship exists for this enzyme activity.

As well as our study using normal tissues, we have checked the steroid sulfatase expression in skin fibroblasts from ichthyotic boys and their mothers considered to be obligatory heterozygotes in keeping with the hypothesis of an X-linked trait transmission. The results are reported in Fig. 2. Five ichthyotic boys, for whom we had already proven the placental defect, and two other affected boys had zero values, thus con-