Unmasking by neuraminidase of specific chorionic gonadotrophin binding activity of human placental syncytiotrophoblast

S. Paul* and B. L. Jailkhani†

*Department of Biochemistry and †Neurosciences Center, All-India Institute of Medical Sciences, New Delhi, India

Summary. Purified human placental syncytiotrophoblast consistently failed to bind specifically to ^{125}I -labelled hCG. Treatment of the syncytiotrophoblast with neuraminidase resulted in the ability to bind ^{125}I -labelled hCG that was displaceable by excess of unlabelled hCG. Neuraminidase treatment removed 73.8% of the total neuraminic acid of syncytiotrophoblast. The specific binding of ^{125}I -labelled hCG increased linearly with increasing amount of neuraminidase-treated syncytiotrophoblast, was saturable and had a $K_a = 1.6 \times 10^7 \text{ M}^{-1}$. Excess of GH, prolactin, placental lactogen or insulin did not inhibit the binding, whereas LH did so completely and FSH partly.

Introduction

The hormone human chorionic gonadotrophin (hCG) is reported to stimulate a number of metabolic activities in the human placenta, including steroid synthesis (Demers, Gabbe, Villee & Greep, 1973; Menon & Jaffe, 1973; Gnanaprakasam & Talwar, 1975; Guichard, de Ikonicoff & Cedard, 1975). If this represents a direct effect of hCG on the syncytiotrophoblast, the steroid-producing tissue in the placenta (Baillie, Cameron, Griffiths & Hart, 1965), it should be mediated by association of hCG to specific receptor binding sites on the syncytiotrophoblast, as in other hormone–target tissue interactions. HCG is synthesized by the syncytiotrophoblast, and is detectable on the surface of this tissue (Paul, Jailkhani & Talwar, 1978). Whether part or all of the hCG present on the syncytiotrophoblast surface is receptor bound is not known. We have therefore investigated the expression of receptors for hCG on human syncytiotrophoblast.

Materials and Methods

Full-term human placentae from normal pregnancies were collected in tissue culture Medium 199 (Gibco, Paisley, Scotland) from the Department of Gynaecology & Obstetrics, AIIMS, New Delhi, or the Frauenklinik of the University of Kiel. The central chorionic villus-rich tissue was obtained by dissection and processed for isolation of syncytiotrophoblast as described in detail elsewhere (Paul *et al.*, 1978). Purified syncytiotrophoblast was maintained in tissue culture in a viable and metabolically active state (Paul *et al.*, 1978; Paul, Jailkhani, Gupta & Talwar, 1979). The preparations used for the hCG binding experiments had been maintained in culture for 2–11 days. The DNA content of syncytiotrophoblast was estimated according to Burton (1956) using calf thymus DNA (Sigma, Type I) as standard. For the neuraminidase treatment,

* Present address: University Frauenklinik, Hegewischstrasse 4, 2300 Kiel 1, West Germany.

syncytiotrophoblast with a DNA content of 300–350 μg was washed twice with 10 ml Medium 199, resuspended in 3 ml Medium 199, pH 6.0, containing 0.3 units neuraminidase (Sigma, Type VI)/ml and 0.03% bovine serum albumin (BSA), incubated for 2 h at 37°C, and finally washed exhaustively with Medium 199 to remove neuraminidase. The sialic acid content of untreated and neuraminidase-treated syncytiotrophoblast was measured according to Jourdin, Dean & Roseman (1971), following its release from the cells by treatment with 0.1 N-sulphuric acid (Warren, 1959). The crude particulate fractions of syncytiotrophoblast were obtained by homogenization of the tissue in 10 volumes of 50 mM-Tris-HCl buffer, pH 7.2, with a Brinkman Polytron, centrifugation of the homogenate at 600 *g* for 30 min at 4°C, and resuspension of the pellet in 50 mM-Tris-HCl, pH 7.2, containing 0.3% BSA (Tris-BSA buffer). The particulate fractions were stored at -20°C until used. Purified hCG (10^4 i.u./mg) was iodinated with Na^{125}I (Radiochemical Centre, Amersham, U.K.) according to Saxena, Hasan, Haour & Schmidt-Gollwitzer (1974). The specific activity of ^{125}I -labelled hCG was 40–50 $\mu\text{Ci}/\mu\text{g}$ as determined by radioimmunoassay. The particulate fraction was incubated with ^{125}I -labelled hCG (10^5 c.p.m.) in a total volume of 0.5 ml Tris-BSA buffer for 2 h at 37°C or 24 h at 4°C, in the absence and presence of 10 μg non-radioactive hCG (Sigma, 3×10^3 i.u./mg). At the end of the incubation, the assay tubes were centrifuged at 1000 *g* for 15 min, the pellets washed once with 2 ml Tris-BSA buffer, and counted in a gamma spectrometer. The specific binding was calculated as c.p.m. bound in the absence of non-radioactive hCG minus c.p.m. bound in the presence of 10 μg non-radioactive hCG. Ovine LH (S-19), ovine FSH (S-11), ovine GH (S-8) and ovine prolactin (S-12) were gifts from NIAMDD, NIH, Bethesda, U.S.A. Insulin was purchased from Boots, India, and human placental lactogen from Nutritional Biochemicals Company, Cleveland, Ohio, U.S.A.

Results

The syncytiotrophoblastic particulate fractions exhibited a low level of binding to ^{125}I -labelled hCG that was not significantly different in the absence and presence of excess (10 μg) of non-radioactive hCG (Table 1), indicating that the syncytiotrophoblastic particulate fractions did not bind hCG specifically. When the particulate fractions were pretreated with neuraminidase in Medium 199, pH 6, there was a 9-fold increase in the binding of ^{125}I -labelled hCG (Table 1): 78.1% of the binding was displaceable by excess non-radioactive hCG, this value thus representing the specific ^{125}I -labelled hCG binding component. The binding after pretreatment at pH 6 without neuraminidase was indistinguishable from that with untreated

Table 1. Unmasking of ^{125}I -labelled hCG binding activity of syncytiotrophoblast by neuraminidase

Treatment	N	c.p.m. bound/ μg DNA	
		Total	Non-specific
None	8	68	57
Neuraminidase, pH 6	8	556*	122
Medium 199, pH 6	3	58	80

Details of the treatments that syncytiotrophoblast received are given in the text. The particulate fractions of the treated syncytiotrophoblast preparations (7.9–15.0 μg DNA) were used for the ^{125}I -labelled hCG binding assays. Non-specific binding was determined in the presence of 10 μg non-radioactive hCG. N is the number of different syncytiotrophoblast preparations treated, each in triplicate.

* $P < 0.001$ compared to non-specific binding.

particulate fractions (Table 1). Colorimetric determination of sialic acid yielded values of 0.42 μg sialic acid/ μg DNA for untreated syncytiotrophoblast, and 0.11 μg sialic acid/ μg DNA for neuraminidase-treated syncytiotrophoblast, treatment with neuraminidase liberating $73.8 \pm 9.4\%$ of the syncytiotrophoblast-associated sialic acid.

Incubation with progressively increasing quantities of ^{125}I -labelled hCG revealed the saturability of the syncytiotrophoblast binding sites (Text-fig. 1a). A linear increase of specific ^{125}I -labelled hCG binding was obtained with increasing amount of the neuraminidase-treated syncytiotrophoblast (Text-fig. 1b). Non-radioactive human placental lactogen, prolactin, GH and insulin failed to compete with ^{125}I -labelled hCG for binding to the syncytiotrophoblast binding sites, whereas LH and FSH, hormones possessing considerable amino acid homology with hCG (Ross, 1977), inhibited the binding completely and partly respectively (Table 2).

Text-fig. 1. (a) Saturation of specific ^{125}I -labelled hCG binding to neuraminidase-treated syncytiotrophoblast, and (b) specific ^{125}I -labelled hCG binding to increasing amount of neuraminidase-treated syncytiotrophoblastic particulate fraction. Values are mean $\pm$ s.d. for 3 replicates.

Table 2. Ligand specificity of binding of ^{125}I -labelled hCG to neuraminidase-treated syncytiotrophoblast

Hormone	% inhibition of specific binding
hCG	100 (3590 c.p.m.)
LH	96
FSH	47
Human placental lactogen	1
Prolactin	5
GH	0
Insulin	3

The particulate fraction of neuraminidase-treated syncytiotrophoblast (DNA content: 8.5 μg) was incubated with ^{125}I -labelled hCG in the absence and presence of 10 μg each of the indicated hormones for 24 h at 4°C . Values for inhibition of ^{125}I -labelled hCG binding were computed relative to the inhibition obtained with 10 μg hCG (100%). Values are means of triplicate determinations.

Scatchard plots of binding of hCG to two preparations of neuraminidase-treated syncytiotrophoblastic particulate fractions revealed linear curves (Text-fig. 2). The mean affinity constant (K_a) was $1.6 \times 10^7 \text{ M}^{-1}$, and the hCG binding capacity was $1.5 \times 10^{-7} \text{ mol}/\mu\text{g DNA}$ of the syncytiotrophoblast.

Neuraminidase activity in the neuraminidase-treated syncytiotrophoblast was undetectable by colorimetric sialic acid estimations using *N*-acetylneuramin-lactose as substrate, as well as by histochemical means. Therefore, the ability of the neuraminidase-treated syncytiotrophoblast to bind hCG specifically was not due to desialylation of the hormone by the cells, analogous to increased binding of desialylated hCG, compared to native hCG, to gonadal receptors (Catt & Dufau, 1975).

Text-fig. 2. Scatchard plots of ^{125}I -labelled hCG binding to particulate fractions of neuraminidase-treated syncytiotrophoblast from 2 placentae (○—○, ●—●), in the presence of various quantities of unlabelled hCG (10–500 ng; 10^4 i.u./mg). The molecular weight of hCG used for computation was 47 000 (Bahl, 1969).

Discussion

The method used in this study for isolation of the syncytiotrophoblast yields preparations composed of large syncytial portions, free of contamination with other cell types, as judged microscopically; the only contaminants are acellular connective tissue fragments (about 5%) (Paul *et al* 1978, 1979). Expression of the trophoblastic markers hCG, placental lactogen, oxytocinase and pregnancy-specific β_1 -glycoprotein was demonstrated by a sandwich immunofluorescence test (Paul *et al.*, 1978). Syncytiotrophoblast isolated from full-term placentae and maintained in culture retained the capacity to synthesize hCG and progesterone, and the output of hCG did not diminish during the period of culture (Paul *et al.*, 1978; Paul, Das, Jaikhani & Talwar, 1981). Based on these observations, we conclude that the syncytiotrophoblast preparations investigated in this study for expression of hCG receptors are adequately representative of placental syncytiotrophoblast *in vivo*.

Untreated syncytiotrophoblast may have failed to bind hCG specifically because its hCG receptors were already saturated by endogenous hCG, and neuraminidase, by stripping away receptor-bound hCG, could have unmasked the receptors. However, immunohistochemical localization studies failed to reveal any difference in the intensity of reaction for hCG on untreated and neuraminidase-treated syncytiotrophoblast, and there was no detectable release of hCG during treatment with neuraminidase (Paul, 1979). It is therefore more likely that the neuraminidase-mediated removal of sialic acid from the syncytiotrophoblast surface exposes previously masked receptors for hCG, as postulated in relation to histocompatibility antigens (Currie, van Doorninck & Bagshawe, 1965). The significantly lower affinity of the

syncytiotrophoblast receptors for hCG, compared to testicular and ovarian receptors (Catt & Dufau, 1975; Paul, 1979), may be due to the incomplete unmasking action of neuraminidase, and persistence of steric constraints for the association of hCG to the receptors.

The absence of any overt hCG binding activity of untreated syncytiotrophoblast argues against a direct effect of hCG on the metabolism of this tissue, and is in concordance with the failure of hCG to stimulate conversion of cholesterol/pregnenolone to progesterone by purified syncytiotrophoblast *in vitro* (Paul *et al.*, 1981). The stimulatory effect of hCG on steroidogenesis, noted in studies with unfractionated placental tissue (Villee & Gabbe, 1972; Guichard *et al.*, 1975), may represent a secondary effect of the hormone on syncytiotrophoblastic metabolism; the placenta consists of a number of cell types, and hCG may act on a cell type such as cytotrophoblasts, or cells of the villous matrix, to increase production of cofactors/steroidal precursors that are essential for synthesis of oestrogens and progesterone by syncytiotrophoblast. The physiological significance of the neuraminidase-exposed hCG receptors on syncytiotrophoblast is not immediately obvious. However, the existence of these masked hCG receptors does indicate that the syncytiotrophoblast is potentially capable of expressing hCG receptors in overt form. For example, it is possible that the hCG receptors are overtly expressed at early developmental stages, analogous to their reported presence on mouse blastocyst (Sengupta, Gupta, Manchanda & Talwar, 1978), and later are sterically masked by surface glycoproteins.

This study was supported by the Family Planning Foundation, India, and the Alexander von Humboldt Foundation, Federal Republic of Germany. We thank Professor G. P. Talwar for making available purified hCG and for discussion, and Professor L. Mettler and Mr A. Czuppon for help with neuraminic acid determinations.

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Received 15 December 1981