Proteases in the human full-term placenta

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Summary. Aminopeptidase A, not yet defined aminopeptidases and endopeptidases, dipeptidyl peptidase I, II and IV, γ-glutamyl transferase and oxytocinase were investigated in the normal human full-term placenta using qualitative (catalytic) cytochemistry, isoelectric focusing, immunocytochemistry and kinetic fluorometry. Aminopeptidase A could be visualized cytochemically in the smooth muscle cells of the chorionic plate, stem villi and basal plate blood vessels. Aminopeptidases were found in connective tissue fibres of the chorionic plate, villous stroma, basal plate and paraplacenta. Dipeptidyl peptidase IV was detected at the same sites as the aminopeptidases and, in addition, in amniotic epithelial cells, fibroblasts of the villous stroma, endothelium of chorionic plate and villous blood vessels as well as in the basophilic cytotrophoblast cells (x-cells) of the basal plate and paraplacenta, and it possibly also occurred in some domains of the plasma membrane of the syncytiotrophoblast and cytotrophoblast cells. The x-cells surrounded the fetus in the form of a dipeptidyl peptidase IV-positive shell at the border to the mother. The enzyme represented the first specific marker for x-cells. Dipeptidyl peptidase I and II were primarily found in Hofbauer cells (macrophages) of the villous stroma, but also in the syncytiotrophoblast, other villous stromal cells and cells of the chorionic and basal plate. γ-Glutamyl transferase was present in some connective tissue elements of the chorionic plate. Oxytocinase and endopeptidases were not detected. Isoelectric focusing of proteases revealed different molecular forms of dipeptidyl peptidase IV in the paraplacenta and villous tree, while the aminopeptidases shared the same pattern in both regions. Immunoocytochemical staining of dipeptidyl peptidase IV in the villous tree resembled the pattern obtained by catalytic cytochemistry except for the blood vessel endothelium and the x-cells of the basal plate. Fluorometrically, all proteases were more active in the villous tree than in the paraplacenta. The kinetic measurements revealed the highest hydrolysis rates for dipeptidyl peptidase IV followed by the aminopeptidases. In contrast to catalytic cytochemistry all proteases were detectable when using fluorometry.

Introduction

Cytochemical studies of proteases in the mature mouse, rat, guinea-pig and marmoset placenta revealed striking species differences (Graf and Gossrau 1985; Gossrau and Graf 1986) even in placenta with identical ultrastructure of all placental zones (mouse, rat; Kirby and Bradbury 1965; Franke 1969) or of the placental barrier which is the functionally most important unit of this organ (guinea-pig, marmoset; Kaufmann and Davidoff 1977; Bremer 1983). Proteases of the human full-term placenta have not been studied in this respect. So far either biochemical data exist for aminopeptidases, oxytocinase, cathepsin B, renin and some not yet further classified proteinases (for references see Oya et al. 1974; Hiwada et al. 1977, 1980; Morton 1977; Unger and Struck 1978; Lampelo and Vanha-Perttula 1980; Mizutani et al. 1981; Lampelo 1982; Lampelo et al. 1982, 1983) which do not report on the localization of these enzymes in this complex organ in man; or the few cytochemical studies about aminopeptidases, oxytocinase and dipeptidyl peptidase IV mostly suffer from the use of inadequate methods and thus from less satisfactory information about the localization of these enzymes (for references see Becker et al. 1983; Duenas et al. 1984; Heymann et al. 1984). Furthermore, comparative cytochemical studies dealing with the different parts of the placenta, i.e. chorion and basal plate and the villous tree and with the paraplacental proteases are not available either.

Therefore, we wish to describe in the present paper proteases relevant for placental function of the chorionic and basal plate, villous tree and paraplacenta in man with complementary techniques, i.e. catalytic and immunocytochemistry, isoelectric focusing and kinetic fluorometry. Moreover, dipeptidyl peptidase IV will be described as a new and highly specific marker enzyme for the basophilic cytotrophoblast cells (x-cells).

Materials and methods

Normal human full-term placentas were obtained from the Department of Perinatal Medicine (Head: Prof. Dr. E. Saleng)1, samples of the paraplacenta and central and peripheral parts of the chorionic and basal plate and villous tree of the placenta were mounted on cork plates, wrapped with plastic foil and frozen in liquid nitro-

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gen (N₂) for cytochemistry and immunocytochemistry. For analytical isoelectric focusing (IEF) and fluorometry the samples were directly frozen in N₂ within 5 to 10 min after delivery and transported under N₂ to the Department of Anatomy.

**Tissue pretreatment. Cytochemistry. Immunocytochemistry.** 10 µm thick sections were cut with Reichert-Jung (model 2700 or 2800, Nussloch, FRG) or Dittes-Duspinia (model 808 T, Heidelberg, FRG) cryostats at −25 °C and mounted on room temperature on glass slides. The sections were either air-dried (C sections), treated with an acetic:chloroform mixture (1:1) at −25 °C for 5 min (CAC sections) or freeze-dried according to Lojda et al. (1979) with Edwards (model EPD 3 and ETD 4; Edwards, Crawley, UK) or Leybold-Hereaus (model GT 001; Köln, FRG) freeze-dryers, equipped with an ED 12 vacuum pump (Edwards) and mounted on albuminized glass slides with 0.5% cellodio (FDC sections).

**Fluorometry.** 20% (w/v) homogenates were prepared in ice-cold 0.05 M imidazole buffer, pH 7.4 with 2% Triton X-100 with a Potter Elvelheim homogenizer (Braun, Melsungen, FRG; 20 strokes, 1,000 or 1,500 rpm). The homogenates were centrifuged with a bench centrifuge (Labofuge B or Biofuge A; Hereaus-Christian, Osterode/Harz, FRG) at 4,000 rpm and room temperature and the supernatants were frozen in N₂ as 1 ml aliquots and stored at −25 °C in Eppendorf cups (Hamburg, FRG) until use. Before use the supernatants were thawed once. For isoelectric focusing homogenates were prepared as for the fluorometric protease determinations. The homogenates were treated with 6.5 U/1 ml homogenate papain (No. P-3125; Sigma, Munich, FRG) at 37 °C for 3 h. Afterwards, the homogenates were centrifuged with a Beckman cooling centrifuge (Munch, FRG) at 15,000 rpm and 4 °C. Then, the supernatant was dialyzed with Serva pure dialysing tubings (diameter 7 mm; Serva, Heidelberg, FRG) against 0.05 M imidazole buffer, pH 7.4 for 24 h with a two times change of the buffer.

**Protease methods for catalytic cytochemistry were performed, if not otherwise stated according to the procedures of choice given by Lojda et al. (1979), Gossrau (1981), Gossrau (1985) and Gossrau and Graf (1986b). CAC sections and Fast Blue B (FBB) or hexazotized new fuchsin (HNF) for simultaneous coupling were used. Controls were performed by parallel incubation with preimmune serum. Photographs were taken with a Leitz fluorescence microscope (Leitz, Wetzlar, FRG) and an automatic camera (Wild MPS 50, Heerbrugg, Switzerland).

**Analytical isoelectric focusing was carried out with 10 or 20 µl of dialysed supernatant on agarose gels with ampholines in the range of pH 3–6 or 3–10 according to the procedure given by Sinha and Gossrau (1984) with an LKB (Bromma, Sweden) 2117 multiphor apparatus lasting 90 min. Bands were developed for AP and DPP IV multiple forms using the assays as described above for catalytic cytochemistry with Ala- and Gly-Pro-MNA and FBB. Gels were incubated for 10 to 30 min at room temperature.

**Kinetic fluorometry of AP, APA, DPP I, II and IV, GGT and oxtocinase was performed with 50 µl of thawed supernatant kept in an ice bath at 4 °C from the villous tree and paraplacenta and 1 ml 1.5 mM substrate solution in 0.1 M cacodylate buffer whereby the assays for fluorometry corresponded to those for catalytic cytochemistry, but instead of MNA the 2NA substrates were used and FBB was omitted. Kinetic measurements were carried out 30 s after thorough mixing of the supernatant and substrate solution for 5 min at room temperature with a quinine sulfate calibrated Farrand Foci Ratio fluorometer (New York, USA; primary filter 350 mm, secondary filter 450 mm) connected with a Euromak computer for continuous data monitoring and calculations. Activities referred to 10 µg wet weight and were given in fluorescence units (FU/10 µg ww).

**Source of chemicals.** The 2NA and MNA substrates for the proteases were obtained from Buchem (Bubendorf, CH) and Novabiochem (Laufelfingen, Switzerland). FBB purity grade, Gly-Gly, Cys and 2-NA peptides from Serva, NaCl, Tris, EDTA, ethanol, acetone and chloroform from Merck (Darmstadt, FRG), cacodylate and phosphate salts from Roth (Karlsruhe, FRG), the chemicals for IEF from LKB and Serva, new fuchsin and cellodio from Chroma (Stuttgart, FRG) and the IEF chemicals from LKB or Serva.

**Results**

**Catalytic cytochemistry**

AP detection yielded identical localization with all amino acid MNA substrates among which Ala and Met delivered the heaviest staining. EDTA, NaCl and Cys enhanced the DPP I reaction. Double incubation of AP and DPP IV reliably answered the question of identical or different localization of both proteases. Oxytocinase and endopeptidase I and II could not be detected cytochemically. Differences

**Figs. 1 and 2.** Chorionic plate, dipeptidyl peptidase IV reaction. **Fig. 1.** Staining of amniotic and chorionic connective tissue elements (arrow heads). Arrows site of the amniotic epithelium, double arrow heads compact amniotic connective tissue, F fibrinoid. **Fig. 2.** Staining of x-cells (arrows). Arrow heads stained fibres of the chorionic connective tissue, F fibrinoid, MBS maternal blood space. **Figs. 3–5.** Villous tree, MBS maternal blood space, arrow heads syncytiotrophoblast. **Fig. 3.** Aminopeptidase reaction in connective tissue fibres (arrows). **Fig. 4 and 5.** Dipeptidyl peptidase IV reaction. **Fig. 4.** Staining of connective tissue elements (arrows). **Fig. 5.** Staining of connective tissue elements (double arrows) and arterial endothelial cells (arrows). **Fig. 6.** Basal plate, aminopeptidase reaction in connective tissue cells (arrows). x-Cells are unstained. Arrow heads syncytiotrophoblast, double arrow connective tissue fibres in anchoring villi. Magnification of all figures × 500.