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Localization of CAI and CAII Isoenzymes in Normal Term Human Placenta by Immunofluorescence Techniques

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INTRODUCTION

Carbonic anhydrase (CA) (EC.4.2.1.1), which classically catalyses the hydration of dissolved CO_2 to form bicarbonate ions and the dehydration of bicarbonate ($CO_2 + H_2O \rightleftharpoons H^+ + HCO^-_3$), is widely distributed in mammalian tissue (Maren, 1967; Carter, 1972; Carter and Jeffery, 1985). It is thought to regulate a variety of cellular functions such as CO_2 exchange, pH balance, secretion of ions, calcification, and has some role in intermediary metabolism (Maren, 1967; Coulson and Herbert, 1984; Carter and Jeffery, 1985).

Carbonic anhydrase can be localized in tissue sections by histochemical and immunocytochemical techniques. The latter is the method of choice, because in addition to its specificity and sensitivity, it has the advantage of identifying the individual isoenzymes of CA (Kumpulainen, 1984). Previous studies using histochemical techniques have demonstrated the presence of CA in human placenta (Kurata, 1953; Bleyl and Masch, 1964), but did not distinguish different isoenzymes. The aim of the present study is to compare the distribution of two CA isoenzymes, the low activity CAI and the high activity CAII, in the normal term human placenta by immunocytochemical techniques.

MATERIALS AND METHODS

Tissues

Three placentae were obtained immediately after vaginal delivery at term from women with uncomplicated pregnancies and labour. They were divided into small pieces (1 mm diameter) which were washed in ice-cold 0.15 M saline and fixed overnight at 4°C in acid alcohol solution (95 per cent ethanol + 5 per cent acetic acid) or 4 per cent paraformaldehyde in 0.1 M phosphate buffered saline (PBS). The tissues were then washed in ice-cold PBS containing 5 per cent sucrose and frozen on solid CO_2. The frozen tissue was cut in a cryostat (Frigocut 2800-

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Cambridge Instruments GmbH) into 10 μm thick sections. These were collected on gelatine-coated slides, dried in air and stored at $-20°C$.

Immunofluorescence

The frozen sections were dried at room temperature and incubated in appropriately diluted primary antisera [rabbit antiCAI 1:50 and goat antiCAII (The Green Cross Corporation, Japan) 1:50] for 45 min at room temperature in a plastic box lined with wet paper to maintain a moist atmosphere. For control experiments normal rabbit serum and normal goat serum were substituted for the primary antisera at equivalent dilution. All antibodies and normal sera were diluted in PBA containing 1 per cent bovine serum albumen (BSA) with 0.02 per cent sodium azide. The slides were washed in PBS (three changes for 15 min each) and appropriate second antibodies [sheep antirabbit-FITC 1:100 and donkey antigoat-FITC (ICN Biomedicals Ltd) 1:100] were applied for 45 min as before. The sections were washed again in PBS and mounted in a solution of glycerol containing 2.5 per cent (w/v) 1,4-diazabicyclo(2.2.2)octane (Aldrich Chemical Co. Ltd.) to reduce quenching, and sealed with nail polish. They were photographed using a Leitz Ortholux II microscope.

Specificities of the CAI and CAII antisera were tested by the double immunodiffusion method of Ouchterlony (1964) and enzyme-linked immunosorbent assay (ELISA), and were found to be monospecific.

RESULTS

Tissues fixed in acid alcohol showed bright fluorescence with low non-specific background staining. Although morphological features were preserved better using paraformaldehyde fixation, the antigenicity of CAI and CAII in the tissues was markedly reduced. In acid alcohol-fixed placental tissue, strong fluorescence was seen in the syncytial trophoblastic layer of the chorionic villi for CAI [Figure 1(a)] and CAII [Figure 1(b)]. The pattern and intensity of fluorescence was similar for both CAI and CAII isoenzymes. Fetal erythrocytes inside the placental capillaries also gave strong positive staining with both CAI and CAII antibodies. No staining of the syncytial trophoblasts were seen in the controls [insert Figure 1(a) and 1(b)], where appropriately diluted normal rabbit and goat sera were substituted for CAI and CAII antisera, respectively.

DISCUSSION

The placentae of several mammalian species (rabbit, rat, hamster, guinea-pig, pig and sheep) have been extensively studied in the past and documented as rich sources of CA activity (Lutwak-Mann, 1955; Leder and Groh, 1965; Szenci, 1986). However, according to our knowledge there are only two previous reports on the presence of CA in human placenta (Kurata, 1953; Bleyl and Masch, 1964). The latter studies demonstrated the presence of CA in syncytial trophoblasts of human placenta by detecting the total CA activity in the tissue by histochemical methods. The present study using immunofluorescence confirms their findings. Furthermore, it indicates that both low activity CAI and high activity CAII isoenzymes are present in syncytial trophoblasts. The specificity and sensitivity of the method was confirmed by the absence of staining of the syncytial trophoblasts in the control experiments and by the presence of both CAI and CAII isoenzymes in fetal erythrocytes.

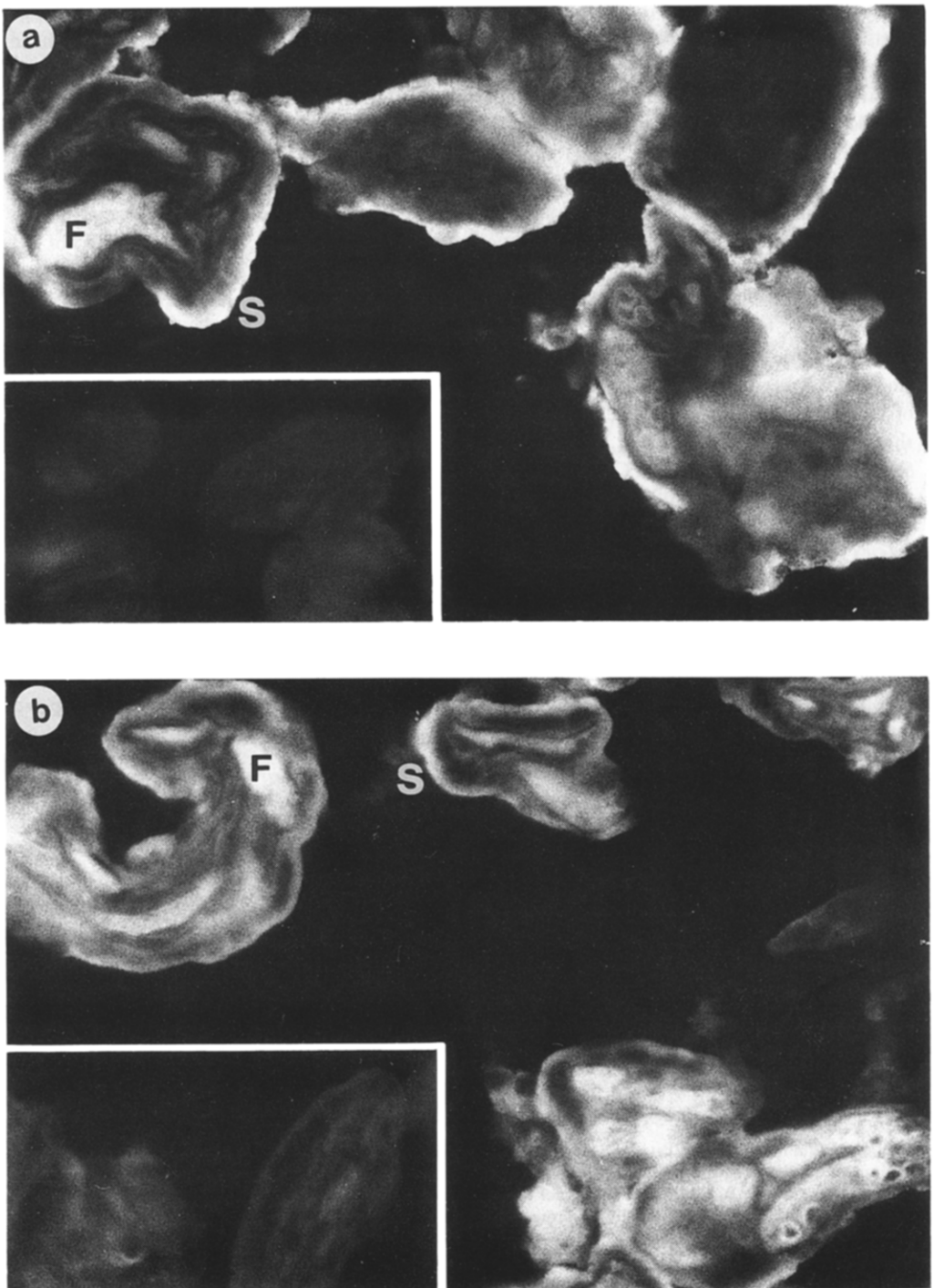

Figure 1. Normal term human placenta (acid-alcohol fixation) showing strong positive immunofluorescence staining for CAI (a) and CAII (b) in syncytial trophoblasts. Fetal erythrocytes inside placental capillaries also show strong positive staining for both isoenzymes. Inserts are appropriate normal serum controls. S-*syncytial trophoblasts*, F-*fetal erythrocytes*. Magnification × 336.

The presence of CA in the placenta of several mammalian species (including human) suggests that the enzyme has an important role in feto–placental physiology. Previous studies have shown that CA facilitates diffusion of CO_2 in tissues and across membranes (Longmuir, Forster and Woo, 1966; Enns, 1967; Gros et al, 1976; Gutknecht, Bisson and Tosteson, 1977). It has also been suggested that CA facilitates diffusion of CO_2 across the alveolar-capillary membrane in the lung (Swenson, 1984) and facilitates CO_2 transport out of the cell in skeletal muscle and lens (Enns and Hill, 1980; Friedland and Maren, 1981). Some other highly vascular tissues in animals involved with gas exchange, such as fish gill and swim bladder, also have CA. It has been shown, by selective inhibition of gill CA in the shark, that gill CA, and not red cell CA, is involved with both CO_2 exchange and HCO_3 excretion (Swenson, 1984). Some other groups also concluded from their studies on sheep and rat placental CA that the enzyme promotes the elimination of CO_2 from the fetus in these animals (Leder and Groh, 1965; Szenci, 1986). It is, therefore suggested that placental CA in human also facilitates the diffusion of CO_2 across the placental membrane by promoting rapid production and consumption of bicarbonate, especially as the fetus itself has a low blood concentration of CA compared to the adult (Kleinman, Petering and Sutherland, 1967; Shepherd, Spencer and Hulse, 1985). Such facilitated diffusion of CO_2 may be even more important under the stress of unusual demands such as placental insufficiency.

Hopping (1937) identified the calcium deposits in the mammalian placentae as chiefly $CaCO_3$. On the basis of a characteristic time relationship between embryonic development and appearance of the calcium deposit in the placenta, he suggested that placental calcification is intimately linked with the calcium metabolism of the fetus. The involvement of CA in the formation of the avian egg shell was first suggested by Meldrum and Roughton (1933), and later confirmed by Benesch, Barron and Mawson (1944). Tuan (1984) has confirmed the functional involvement of CA in calcium transport across the chick embryonic chorio-allantoic membrane. It is, therefore possible that placental CA has a physiological role in the calcium metabolism of the feto–placental unit.

SUMMARY

The carbonic anhydrase isoenzymes, low activity CAI and high activity CAII, were localized in normal term human placenta by immunocytochemical techniques. Both CAI and CAII isoenzymes were present in the syncytial trophoblasts. Fetal erythrocytes in the placental capillaries also showed positive staining for both CAI and CAII isoenzymes. The possible physiological roles of CA in human placenta are also discussed.

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