Measurement of carbonic anhydrase isoenzymes in early human placental tissues

S. ALIAKBAR,*† P. R. BROWN,* E. JAUNIAUX,† D. E. BIDWELL‡ and K. H. NICOLAIDES†

**Department of Biochemistry, King's College London, Strand, London WC2R 2LS, †Harris Birthright Research Centre for Fetal Medicine, Department of Obstetrics and Gynaecology, King's College Hospital, Denmark Hill, London SE5 8RX, and ‡Nuffield Laboratories of Comparative Medicine, The Zoological Society of London, Regent's Park, London NW1 4RY, U.K.*

Carbonic anhydrase (CA; EC 4.2.1.1), which reversibly catalyses the hydration of carbon dioxide to bicarbonate and hydrogen ions, is widely distributed in mammalian tissues and has an important role in gas transport, acid/base regulation, calcification and various secretory functions in tissues [1, 2]. The two CA isoenzymes, low-activity CAI and high-activity CAII, are clearly differentiated immunologically [3].

Although the placentae of several mammalian species (rabbit, rat, hamster, guinea-pig, pig and sheep) have been documented as rich sources of CA activity, we know of only two previous reports of the presence of CA in human placenta [4–6]. In these studies total CA activity was detected using histochemical methods. We have recently demonstrated the presence of CAI and CAII in the normal-term placenta by immunocytochemical techniques [7].

To quantify the amount of CAII in the placenta, we have developed a modified double-antibody-sandwich enzyme-linked immunosorbent assay (ELISA) for CAII. The polyvinyl microplate was coated with goat anti-(human CAII) IgG (Green Cross Corporation, Osaka 541, Japan). Rabbit anti-(human CAII) IgG (Dr N. Carter, London) was used as second-layer antibody. Goat anti-rabbit IgG) was conjugated with peroxidase and human CAII (Binding Site, Birmingham, U.K.) was used as standard. The method was found to be specific, simple, economical, reliable and precise. For CAI estimation the original ELISA method described by Shepherd *et al.* [8] was modified. Rabbit anti-(human CAI) was used as a first layer antibody and sheep anti-(human CAI) as a second layer. Peroxidase-labelled donkey anti-(sheep IgG) was used as a conjugate.

Twenty-five normal placentae from 8 to 16 weeks of gestation were obtained immediately after abortion. They were thoroughly washed in Krebs–Ringer bicarbonate glucose (1%, w/v) solution (pH 7.4) containing heparin (5000 i.u./l) and frozen in liquid nitrogen. Before assay these frozen tissues were thawed, washed again and tiny blood clots were removed under a dissecting microscope. These tissues were then homogenized in a small glass homogenizer. After spinning for 10 min, the supernatants were removed and used for assay. The total protein concentration was calculated from Lowry's method, using bovine serum albumin as standard.

Because of the very high levels of CAI and CAII in adult blood compared with fetal blood [9, 10], when estimating CAI and CAII in placental samples, possible contamination by maternal blood-derived enzymes had to be assessed. The degree of contamination of maternal blood was estimated by measuring adult haemoglobin (HbA) by a new double-antibody-sandwich ELISA method. We used sheep anti-(human HbA) (ICN) as a first-layer antibody and peroxidase-labelled sheep anti-(human HbA) as a conjugate to calculate the amount of HbA in placental samples. It was found that the HbA concentration in all samples was between 50 and 140 μg/g of total protein. From these data it was calculated that the contamination of CAI and CAII in these samples from maternal blood was negligible. The concentrations of CAI and CAII in early placental tissues are shown in Table 1.

Table 1. *Concentrations of CAI and CAII in placental tissues from 8 to 16 weeks of gestation*

Sample no.	Gestation (weeks)	No. of cases	CAI (μg/g of total protein)	CAII (μg/g of total protein)
1	6	2	169.5	52.5
2	9	6	386.8	64.5
3	10	2	34.5	29.4
4	11	5	69.8	30.7
5	12	6	100.6	24.9
6	13	3	168.6	42.6
7	16	1	78	14

From these results low levels of CAI and CAII appear to be present in the placenta very early in embryonic life. However, we have not yet completely eliminated the possibility that CAI and CAII derived from contamination by fetal blood might account for a part of our data. There are difficulties in obtaining fetal blood very early in gestation and the earliest reported gestational age at which both isoenzymes have been detected in fetal blood is 22 weeks [9, 10]. We are therefore seeking supporting evidence for the synthesis of the two isoenzymes in the placenta by detecting mRNA using Northern blot and hybridization *in situ* techniques.

Abbreviations used: CA, carbonic anhydrase; ELISA, enzyme-linked immunosorbent assay; HbA, adult haemoglobin.

1. Maren, T. H. (1967) *Physiol. Rev.* **47**, 595–781
2. Carter, N. & Jeffery, S. (1985) *Biochem. Soc. Trans.* **13**, 531–533
3. Funakoshi, S. & Deutsch, H. F. (1968) *J. Biol. Chem.* **243**, 6474
4. Lutwak-Mann, C. (1955) *J. Endocrinol.* **13**, 26–38
5. Kurata, Y. (1953) *Stain Technol.* **28**, 231–233
6. Bleyl, U. & Masch, B. (1964) *Klin. Wochenschr.* **42**, 402–405
7. AliAkbar, S., Brown, P. R. & Nicolaides, K. H. (1990) *Placenta* **11**, 35–39
8. Shepperd, J. N. *et al.* (1982) *Clin. Biochem.* **15**, 248–251
9. Wehinger, H. (1973) *Blut* **27**, 172–185
10. Tashian, R. E. & Carter, N. D. (1976) *Adv. Hum. Genet.* **7**, 1–56

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