Placental and Plasma Cystine Aminopeptidase in Pregnant Animals

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ABSTRACT. The placental and plasma cystine aminopeptidase (CAP) in pregnant animals was examined on stability after the treatment with L-methionine, ethylene diamine tetra-acetic acid (EDTA) and heat. Inhibitory effects of these treatments on enzyme activities were different among CAPs from the animal species, however, significant correlation in those effects between placental and plasma CAPs was observed. These results suggested that plasma CAP might reflect placental CAP and seemed to be available for estimating maternal gestational conditions.—Key words: cystine aminopeptidase, placenta, plasma.


Cystine aminopeptidase (CAP) [EC 3.4.11.3] synthesized in placental syncytiotrophoblast has been widely accepted to play an important role in the maintenance of gestation by inactivation of oxytocin in pregnant women [1, 4]. Since the enzymatic characteristic of plasma CAP is the same as those of placental CAP, plasma CAP levels are considered to be closely related with placental weight and functions, especially amino acids uptake, and also with fetal growth [11]. Plasma CAP activities in pregnant women increased remarkably at the late gestational period and these values were also found to be available for estimating maternal gestational conditions [4]. However, there are few informations on CAP in pregnant animals in spite of few markers for evaluation of gestational conditions [3, 4]. This note deals with enzymatic characteristics of placental and plasma CAP in pregnant animals with regard to their placental structures.

Samples: The full term placentas were obtained from the pregnant animals at delivery. Maternal blood were collected into heparinized tubes from clinically healthy pregnant women, cynomolgus monkeys, marmosets, dogs, pigs, goats, cows and horses just before the full gestational period. The plasma was separated by centrifugation at 600 g for 10 min and stored at −80°C until assay.

Sample preparation: The placental CAP was extracted by Oya’s method [11]. All procedures were carried out at 4°C. Briefly, the placentas were washed with cold physiological saline, followed by removal of the fetal membrane and umbilical cord. Then, they were minced in small pieces and added with 5 volumes of Tris-HCl buffer (5 mM, pH 7.4) containing 0.25 M sucrose. The homogenates prepared with Potter-Elvejem homogenizer were filtrated through gauze and centrifuged at 600 g for 10 min. The supernatants were stored at −80°C until use.

Enzyme activities: The CAP activities were measured by CAP color test Sankyo (Sankyo Co., Ltd.) using S-benzyl-L-cystein-p-dimethylaminoanilide as a substrate. The protein concentration was determined by Lowry’s method [8].

Inhibition test: Inhibitory effects of ethylene diamine tetra-acetic acid (EDTA, 0.1 and/or 10 mM) and L-methionine (10 and/or 20 mM) on placental and plasma CAP activities, and their heat stability (60°C, 30 min) were examined.

As shown in Fig. 1-a, EDTA revealed a significant inhibitory effect on both placental and plasma CAP in women, cynomolgus monkeys, marmosets, whereas it showed no inhibitory effect in the other animals. Many investigators reported that placental and plasma CAP from pregnant women was completely inhibited by EDTA [6, 7]. Furthermore, Hayashi and Oshima [2] reported a significant inhibitory effect of EDTA on placental CAP from monkeys (Macaca fuscata fuscata). Since such an inhibitory effect on CAP activity was considered to be associated with metal ions on its biologically active site [6], our results suggested that characteristics of CAP as a metalloenzyme were different among animal species. On the other hand, the L-methionine was reported to have no competitive inhibitory effect on neither placental nor plasma CAP in women, whereas it showed a complete inhibitory effect on leucine aminopeptidase [2, 5–7, 9–11]. In our study, L-methionine also exerted a remarkable inhibitory effect on both placental and plasma CAP from all of the pregnant animals except for only human (Fig. 1-b). These results also suggested that there was a difference in the competitive status of the biologically active site of CAP among animal species. The remaining activities of placental CAP were very low in human, cynomolgus monkeys, marmosets, pigs, and horses after incubation at 60°C for 30 min, whereas those of plasma CAP varied among animal species (Fig. 1-c). Placental and plasma CAP in pregnant women had been shown to lose completely their activities by preincubation at 60°C for 30 min [5, 9–10]. In this study, an inhibitory effect of heat treatment on placental and plasma CAP was observed in human, cynomolgus monkeys, marmosets, and pigs.

The correlation of inhibitory effects by EDTA, L-methionine, and heat treatment on placental and plasma CAP in pregnant animals is shown in Fig. 2. The significant correlation (p<0.001, n=24, r=0.843) was observed between them. Oya et al. [9–11] suggested plasma CAP in pregnant women may be originated from the placental lysosomal CAP according to the fact that they were not inhibited by L-methionine, but by heat treatment, and that they had the same electrophoretical mobility. Kleiner et al. [5] also reported that plasma CAP reflected placental CAP which might be released from...
Fig. 1. Inhibitory effects of EDTA, L-methionine, and heat treatment on placental and plasma CAP activities in pregnant animals (human: O, cynomolgus monkey: △, marmoset: ▽, dog: ◦, goat: ●, cow: ■, pig: ▲, horse: ▼).

Fig. 2. The correlation of inhibitory effects by EDTA (▲), L-methionine (●), and heat treatment (■) on placental and plasma CAP in pregnant animals.

placenta.

In conclusion, plasma CAP activities in pregnant animals might reflect placental CAP activities and seemed to be available for estimating maternal gestational conditions.

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REFERENCES