Elution Profiles and Molecular Weights of Placental Cystine Aminopeptidase in Animals

Hiddenori IKENAGA, Kenichiro ONO^{1)*} and Isamu TOMODA

Department of Veterinary Clinical Pathology, School of Veterinary Medicine, Nippon Veterinary and Animal Science University, 1-7-1 Kyonan-cho, Musashino, Tokyo 180 and ¹⁾Department of Veterinary Clinical Pathobiology, Faculty of Agriculture, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

(Received 5 July 1994/Accepted 8 September 1994)

ABSTRACT. Gel filtration was performed for cystine aminopeptidase (CAP) [EC 3.4.11.3] on full term placental extracts from human, cynomolgus monkey, dog, goat, pig and horse. The enzymatic profiles of CAP were examined and compared with that of CAP in pregnancy plasma on the basis of inhibitory effects. Elution profiles of placental extracts exhibited 2 CAP activity peaks in human and pig, 3 peaks in cynomolgus monkey, dog and goat, and 4 peaks in horse. The molecular weights of placental CAP that showed identical inhibitory effects to that of pregnancy plasma CAP were estimated to be approximately 325,000 in human, 350,000 in the cynomolgus monkey, 140,000 in the dog, 140,000 in the goat, 128,000 in the pig, and 115,000 in the horse. These molecular weights tended to decrease in accordance with the increase of barrier layers present between maternal blood and placental syncytiotrophoblasts in which CAP is synthesized.—**KEY WORDS:** cystine aminopeptidase, elution profile, molecular weight, placenta

J. Vet. Med. Sci. 56(6): 1139–1142, 1994

Cystine aminopeptidase (CAP) [EC 3.4.11.3] synthesized in placental syncytiotrophoblast has been widely accepted to play an important role on the maintenance of gestation by inactivation of oxytocin in pregnant women [8, 12]. 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 acid uptake, and also with fetal growth [16]. 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 [8]. In our previous studies, CAP primarily originated from placenta. Plasma CAP in pregnant animals showed identical inhibitory effects by certain conditions to those of placental CAP, and those activities might reflect placental CAP activities [10]. On the other hand, the gel filtration of human placental extract has been reported to give 2 distinct CAP activity peaks. These 2 forms differed in their enzymatic properties and also in their molecular weights [17].

Therefore, the elution profiles by gel filtration, the inhibitory effects by ethylene diamine tetra-acetic acid (EDTA), L-methionine and heat treatment on placental CAP were examined and their molecular weights were estimated.

MATERIALS AND METHODS

Samples: The full term placentas were obtained from human, cynomolgus monkey, dog, goat, pig and horse (one sample for each animal) at delivery and stored at -80°C until use.

Sample preparation: The placental CAP was extracted by Oya's method [11]. The placental extracts were added with solid ammonium sulphate at 60% saturation and stood for 24 hr at 4°C . The precipitates collected after centrifugation at 400 g for 30 min were dissolved in 20 mM Tris HCl buffer (pH 7.4), and the solution was dialysed for 24 hr against the same buffer using seamless cellulose tubing 20/32 (Sankou Junyaku Ltd., Tokyo, Japan). Dialysed materials were concentrated at 4°C . Samples from human (105 mg/ml), cynomolgus monkey (21 mg/ml), dog (130 mg/ml), goat (58 mg/ml), pig (14 mg/ml) and horse (29 mg/ml) were stocked at -80°C until gel filtration.

Gel filtration: Gel filtration was carried out using FPLC system (Pharmacia LKB Biotechnology, Sweden). Two hundred microliters of samples were individually eluted through Superose 12 HR 10/30 (Pharmacia LKB Biotechnology, Sweden) column (1×30 cm; total volume: 24 ml) previously equilibrated with 20 mM Tris HCl buffer (pH 7.4). Elutions were performed at a flow rate of 0.2 ml/min. Three hundred microliter fractions were collected from each sample by FRAC-200 (Pharmacia LKB Biotechnology, Sweden). Molecular weights were estimated from the absorption at 280 nm using high molecular weight gel filtration calibration kit (Pharmacia LKB Biotechnology, Sweden), consisting of thyroglobulin (MW 669,000), ferritin (MW 440,000), catalase (MW 232,000) and aldolase (MW 158,000) as molecular weight markers.

Enzyme activities: The CAP activities were measured using S-benzyl-L-cysteine-*p*-dimethylaminoanilide as a substrate [10, 11].

Inhibition test: Inhibitory effects of EDTA (10 mM), L-methionine (20 mM) and heat treatment (60°C , 30 min) on placental CAP activities were examined as described previously [10].

* CORRESPONDENCE TO: ONO. K., Department of Veterinary Clinical Pathology, Faculty of Agriculture, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan.

Fig. 1. Elution profiles of placental CAP (●—●) and protein concentration (—) in animals.

RESULTS

Elution profiles of placental extract: Figure 1 shows elution profiles of placental extract in animals. In human, 2 peaks of CAP activity were separated on Superose 12 HR 10/30, the large one (Peak 1) eluting in Fraction 17 and the small one (Peak 2) in Fraction 26 (Fig. 1-a). In the cynomolgus monkey, CAP activity was detected as a large one (Peak 1) eluting in Fraction 16 and 2 small peaks (Peak 2 and 3) in Fraction 21 and 26, respectively (Fig. 1-b). Peaks of 3 CAP activity in the dog and goat were detected in Fraction 20 (Peak 1), 27 (Peak 2) and 36 (Peak 3) (Fig. 1-c), and in Fraction 16 (Peak 1), 27 (Peak 2) and 31 (Peak 3) (Fig. 1-d), respectively. In the pig, CAP activity was detected as 2 peaks (Peak 1 and 2) eluting in Fraction 18 and 29 (Fig. 1-e). Four peaks of CAP activity in the horse were detected, a large one (Peak 1) eluting in Fraction 16, one (Peak 2) in Fraction 26, another one (Peak 3) in Fraction 32 and the last one (Peak 4) in Fraction 37 (Fig. 1-f).

Enzymatic profiles of placental CAP: The results of the inhibition test on pregnancy plasma CAP and placental CAP fractionated is shown in Table 1. In human, EDTA inhibited the placental CAP of both Peak 1 and 2. L-methionine inhibited Peak 2 while it had no effect on that of Peak 1. In addition, although heat treatment inhibited placental CAP of Peak 2 only slightly, it exerted a remarkable inhibition on that of Peak 1, resembling the effect observed on pregnancy plasma CAP. In the cynomolgus monkey, EDTA showed the same inhibitory effect on all placental CAP of peaks. L-methionine also inhibited the enzymes of 3 peaks equally. On the other hand, heat treatment showed no inhibitory effect on placental CAP of Peak 2 and 3, while it strongly inhibited that of Peak 1, resembling the effect observed on pregnancy plasma CAP. In the dog, EDTA showed no inhibitory effect on all placental CAP of peaks, however, the inhibitory effect of L-methionine on placental CAP of Peak 2 was observed. In addition, the inhibitory effect of heat treatment on placental CAP of Peak 1 and 2 more closely resembled that of plasma CAP, as compared with Peak 3. In the goat, EDTA showed no inhibitory effect on all placental CAP peaks, however, the inhibitory effect of L-methionine and heat treatment on placental CAP of Peak 2 was identical to that observed in plasma CAP. In the pig, EDTA slightly inhibited placental CAP of both Peak 1 and 2. L-methionine showed the inhibitory effects almost equally on the both enzyme Peaks. On the other hand, heat treatment exerted inhibition on placental CAP of Peak 2, which was almost identical to that on pregnancy plasma CAP. In the horse, EDTA showed no inhibitory effect on all placental CAP Peaks, while L-methionine exerted a strong inhibition on only placental CAP of Peak 3. Additionally, the inhibitory effect of heat treatment on placental CAP of Peak 3 was similar to that of pregnancy plasma CAP.

Molecular weights: The approximate molecular weights were determined on placental CAP that showed the

Table 1. Inhibitory effects of EDTA, L-methionine and heat treatment on fractionated placental CAP activities by Superose 12 gel filtration

		Human			Cynomolgus monkey			Dog			Goat			Pig			Horse		
		E	M	H ^{a)}	E	M	H	E	M	H	E	M	H	E	M	H	E	M	H
Plasma		+++	-	+++ ^{b)}	+++	+++	+++	-	+	±	-	+++	±	±	+++	++	-	+++	+
Placenta	Peak 1	+++	-	+++	+++	+++	+++	-	-	±	-	-	-	+++	+	-	-	-	-
	Peak 2	+++	++	±	++	++	-	-	±	±	-	+++	+	±	++	+++	-	-	-
	Peak 3				+++	+++	-	-	-	±	-	-	-				-	+++	+
	Peak 4																-	-	-

a) Inhibitory effects by 10 mM EDTA (E), 20 mM L-methionine (M) and heat treatment (H).

b) -, ±, +, ++, and +++ represent percent inhibition of the CAP activity at 0, 20-34, 35-54, 55-74, and 75-100, respectively.

enzymatic profile identical to that of plasma CAP in each animals (Fig. 2). The molecular weights were 325,000 (Peak 1) in human, 350,000 (Peak 1) in the cynomolgus monkey, 140,000 (Peak 2) in the dog, 140,000 (Peak 2) in goat, 128,000 (Peak 2) in the pig and 115,000 (Peak 3) in the horse.

DISCUSSION

Oya [18] and other investigators [13, 14] have studied CAP in placenta and plasma from pregnant and non-pregnant women. The gel filtration of pregnancy plasma was shown to give 2 peaks of aminopeptidase activity. The peak eluting faster (Peak 1) had activities of both CAP and arylamidase, while the other one (Peak 2) had only arylamidase activity. In plasma from non-pregnant women, on the other hand, only a single peak that is identical to Peak 2 in its elution profile was detected. Enzymatic profiles also indicated that the CAP activity (Peak 1) increasing in length of pregnancy reflected the peak observed in the placental fraction [18].

In this study, elution profiles were examined using Superose 12 in term placental extracts of various animals. The enzymatic profiles of CAP were also determined and compared with that of CAP in pregnancy plasma on the basis of their inhibitory effects by EDTA, L-methionine and heat treatment. The human placental extract had 2 peaks of CAP activity as has been reported by Oya [18]. The inhibitory effects on the enzyme of Peak 1 was identical to that on CAP appearing in the pregnancy plasma. In addition, the molecular weight of the enzyme of Peak 1 (325,000) was comparable to that determined by other workers [18, 21]. The molecular weights of CAP that showed identical enzymatic profiles to that of pregnancy plasma CAP varied between the other animals examined; 350,000 in the cynomolgus monkey, 140,000 in the dog, 140,000 in the goat, 128,000 in the pig and 115,000 in the horse. The placenta has been classified into 4 types on the basis of barrier layers that exist between maternal blood and placental syncytiotrophoblasts in which CAP is synthesized [19]. Although no barrier is present in human and the cynomolgus monkey (hemocho-

Fig. 2. Estimation of molecular weight of placental CAP peak that had identical inhibitory effects to that of pregnancy plasma CAP by gel filtration on Superose 12 in animals (○: CAP peak, ●: standard protein).

rial placenta type), capillary epithelium in the dog (endotheliochorial placenta type), capillary epithelium and connective tissue in the goat (syndesmochorial placenta type), capillary epithelium, connective tissue and endometrial epithelium in the pig and the horse (epitheliochorial placenta type) intervenes maternal blood and syncytiotrophoblasts. Interesting remarks were that the molecular weights of CAP with the enzymatic profiles identical to that of pregnancy plasma CAP tended to decrease in accordance with the increase of the barrier layers present in placenta. However, molecular weights of placental CAP might also vary within the same species as reported in Japanese monkey [9]. The cynomolgus monkey, which shares the placental structure with human, closely resembled human in its molecular weight of placental CAP, as shown in result, however, no changes of CAP activities related with gestational conditions was observed in pregnant cynomolgus monkey (data not shown). Further, plasma CAP activities in the horse that showed remarkably low molecular weights among animals

did not change with length of gestation [11].

On the other hand, oxytocin level in maternal blood elevates during pregnancy only in primates [5, 22]. The maternal blood oxytocin is also known to be produced in fetuses at the early period of gestation [2, 4, 20] and diffuse into the maternal blood through placenta in human and sheep [6], however, no increase of oxytocin level related to pregnancy is observed in the goat, pig and horse [1, 3, 7]. These animals only show the rapid elevation of oxytocin level in maternal blood at the time of delivery by Ferguson reflex. Therefore, transfer of placental CAP to the maternal blood may not be necessary in these animals, since oxytocin level in maternal blood is kept low during pregnancy. Moreover, Malinowska and Gajewski [15] demonstrated the transfer of placental CAP not into maternal but fetal circulation in the pig. These observations suggest that placental CAP in the animals, except for human, acts as an inactivator of fetus-originated oxytocin rather than maternal oxytocin in maternal circulation.

From these results, molecular weights of placental CAP tended to decrease in accordance with the increase of barrier layers present between maternal blood and placental syncytiotrophoblasts in which CAP is synthesized, however, further studies were necessary for understanding of the maternal blood CAP in pregnant animals.

REFERENCES

- Allen, W. E., Chard, T., and Forsling, M. L. 1973. Peripheral plasma levels of oxytocin and vasopressin in the mare during parturition. *J. Endocrinol.* 57: 175-176.
- Benirshlke, K. and McKay, D. G. 1953. The antidiuretic hormone in fetus and infant. Histochemical observations with special reference to amniotic fluid formation. *Obstet. Gynecol.* 1: 638-649.
- Chard, T., Boyd, N. R. H., Forsling, M. L., Mcneilly, A. S., and Landon, J. 1970. The development of a radioimmunoassay for oxytocin: the extraction of oxytocin from plasma, and its measurement during parturition in human and goat blood. *J. Endocrinol.* 48: 223-234.
- Chard, T., Hudson, C. N., Edwards, C. R. W., and Boyd, N. R. H. 1971. Release of oxytocin and vasopressin by the human foetus during labor. *Nature (Lond.)* 234: 352-353.
- Dawood, M. Y., Lauersen, N. H., Trivedi, D., Ylikorkala, O., and Fuchs, F. 1979. Studies on oxytocin in the baboon during pregnancy and delivery. *Acta Endocrinol.* 91: 704-718.
- Dawood, M. Y., Khan-Dawood, F. S., Ayromloo, J., and Tobias, M. 1983. Maternal and fetal plasma oxytocin levels during pregnancy and parturition in sheep. *Am. J. Obstet. Gynecol.* 147: 584-588.
- Forsling, M. L., Taverne, M. A. M., Parvizi, N., Elsaesser, F., Smidt, D., and Ellendorff, F. 1979. Plasma oxytocin and steroid concentrations during late pregnancy, parturition and lactation in the miniature pig. *J. Endocrinol.* 82: 61-69.
- Hashimoto, T. 1961. Studies on 1-cystine-aminopeptidase part 1. biochemical aspect. *J. Jpn. Obstet. Gynec. Soc.* 8: 87-104.
- Hayashi, M. and Oshima, K. 1976. Purification and properties of oxytocinase (cystine aminopeptidase) from monkey placenta. *J. Biochem.* 80: 389-396.
- Ikenaga, H., Mizuta, Y., Ono, K., Sawazaki, T., Suzuki, N., and Tomoda, I. 1993. Plasma and placenta cystine aminopeptidase in pregnant animals. *J. Vet. Med. Sci.* 55: 479-480.
- Ikenaga, H., Shimada, T., Ono, K., Takagi, S., Suzuki, N., and Tomoda, I. 1993. Changes of Plasma cystine aminopeptidase activities in pregnant horses. *Jpn. J. Equine. Sci.* 4: 69-71.
- James, N. T. 1966. Histochemical demonstration of oxytocinase in the human placenta. *Nature (Lond.)* 210: 1276-1277.
- Kleiner, H., Dictus-Vermeulen, C., May-Cocriamont, C., Brouet-Yagre, M., Popowski, A., Mosselmans, R., and Graff, G. 1980. Human placental oxytocinase and its relationship to pregnancy plasma oxytocinase. *Clin. Chim. Acta* 101: 113-123.
- Lampelo, S. and Vanha-Perttula, T. 1980. Fractionation and characterization of cystine aminopeptidase (oxytocinase) and arylamidase of human serum during pregnancy. *J. Reprod. Fertil.* 58: 225-235.
- Malinowska, A. and Gajewski, Z. 1987. The effect of duration of pregnancy on the activity of cystine aminopeptidase (CAP) isoenzymes in biological fluid and tissue of sows and their fetuses. *J. Vet. Med.* 34: 740-748.
- Oya, M., Yoshino, M., Mizutani, S., and Wakabayashi, T. 1974. The origin of human pregnancy serum oxytocinase. *Gynecol. Invest.* 5: 276-283.
- Oya, M., Yoshino, M., and Mizutani, S. 1975. Electrophoretic studies on aminopeptidase isozymes in human placenta and pregnancy serum. *Jpn. J. Legal Med.* 29: 70-81.
- Ramsey, E. M. 1982. Grosser classification. pp. 47-56. In: *The Placenta. Human and Animals.* Praeger Publishers, Praeger.
- Schubert, F., Gorge, J. M., and Rao, M. B. 1981. Vasopressin and oxytocin content of human fetal brain at different stages of gestation. *Brain Res.* 213: 111-117.
- Sjoholm, I. and Yman, L. 1966. Preparation of highly purified oxytocinase (cystine aminopeptidase) from retroplacental serum. *Acta Pharm. Suecica* 3: 377-387.
- Vasicka, A., Kumaresan, P., Han, G. S., and Kumaresan, M. 1978. Plasma oxytocin in initiation of labor. *Am. J. Obstet. Gynecol.* 130: 263-273.