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Changes in Cystine Aminopeptidase (Oxytocinase) Activity in Mouse Serum, Placenta, Uterus and Liver during Pregnancy or after Steroid Hormone Treatments

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ABSTRACT—Changes in cystine aminopeptidase (CAP) activity in serum, placenta, uterus and liver in mice under various physiological conditions were examined by enzymatic analysis using S-benzyl-L-cysteine-4-methylcoumaryl-7-amide as a substrate. Changes in serum CAP activity during pregnancy were less remarkable than those reported in humans; the activity was highest at day 13 of pregnancy and lowest at day 17. During the estrous cycle, the serum CAP level was high at diestrus, as high as that at day 13 of pregnancy. The activity in the male serum was significantly low compared to that in the diestrous serum. Although the CAP level was especially high in the maternal placenta, levels were also high in the fetal placenta, uterus and liver as compared with the serum, suggesting CAP synthesis in these tissues. The CAP activity in the uterine tissue was lower in pregnant than normal cycling mice. Furthermore, the serum CAP activity was modulated by estrogen and progesterone both in females and males, and by androgen in males. The relevance of these findings to the physiological role of CAP was discussed.

INTRODUCTION

During human pregnancy, an aminopeptidase that can degrade oxytocin is present in the maternal serum. The first evidence that the serum of pregnant women inactivates oxytocin was provided by Fekete (1930), and the inactivating enzyme was named oxytocinase. Because the enzyme cleaves oxytocin and vasopressin between the N-terminal half-cystine residue and the penultimate tyrosine, it is also referred to as cystine or cystyl-aminopeptidase (CAP; EC 3.4.11.3) (Tuppy, 1968; Roy and Karim, 1983). In addition, it was reported that human placental leucine aminopeptidase (P-LAP) is identical to CAP and degrades angiotensin III (Tsujimoto *et al*., 1992) and somatostatin (Mizutani *et al*., 1996) as well as oxytocin and vasopressin. Therefore, this enzyme is an α-aminoacyl-peptide hydrolase with a broad specificity, hydrolyzing peptide bonds with aromatic neutral and basic N-terminal amino acid residues in the peptides (Ryden, 1966; Sakura *et al*., 1981; Roy *et al*., 1993). Moreover, a novel insulin-regulated membrane aminopeptidase cloned by Keller *et al*. (1995) from vesicles with a facilitated-diffusion glucose transporter, GLUT4, in rat adipocytes was homologous to the human P-LAP cloned by Rogi *et al*. (1996). These findings suggest that CAP (P-LAP) plays important roles in the hormonal control of uterine contraction, osmoregulation and blood glucose level via degradation of the bioactive peptides.

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Whereas the circulating CAP activity is extremely low in both men and non-pregnant women, it rises progressively after conception, reaches a maximum at or near term and then decreases rapidly after parturition (Babuna and Yenen, 1966a,b; Roy and Karim, 1983; Mizutani and Tomoda, 1992). Since serum CAP levels are related to the function of the placenta which is a main source of the circulating CAP (Page *et al*., 1961), the determination of the activity changes is clinically applied to predict the onset of labor, preeclampsia or pregnancy-induced hypertension and impending fetal death (Babuna and Yenen, 1966b; Wood and Durham, 1988; Mizutani and Tomoda, 1992).

Few studies of CAP have been carried out with experimental animals, and the physiological role of this enzyme has therefore not yet been fully clarified. To address this issue, CAP activity changes in serum and several tissues were investigated in female mice during pregnancy and the estrous cycle. In addition, the effects of steroid hormones on the CAP activity were examined in non-pregnant female and in male mice.

MATERIALS AND METHODS

Animals

Inbred SHN mice established as a high mammary tumor strain (Nagasawa *et al*., 1976) or ICR mice purchased from CLEA Japan Inc. (Tokyo, Japan) were used. The animals were housed in plastic cages, four to seven a cage, under controlled lighting (12 hr light-dark cycle: lights on at 06:00) and temperature (25 ± 0.5°C), and were provided with a commercial diet (CA-1 : CE-7 = 1 : 2, CLEA) and tap

water *ad libitum*. All procedures used on the mice were approved by the Animal Care and Use Committee of the Graduate School of Science, University of Tokyo. Mice were sacrificed at between 80 and 90 days of age in experiment 1 or between 50 and 70 days in experiment 2 and 3.

Experiment 1: Changes in CAP activity during pregnancy in SHN mice

To induce pregnancy, virgin female SHN mice were caged with males. The day when sperm was found in the vaginal smear was designated as day 1 of pregnancy. Mice were sacrificed by cervical dislocation at days 5, 10, 13, 17 and 19 of pregnancy or within 24 hr after parturition. All of these mice showed diestrous smears.

Immediately after sacrifice, blood samples were collected and allowed to clot at room temperature for 1 hr. After centrifugation at 2,000 g for 20 min, serum samples were stored at –70°C until CAP assay. The serum CAP activity in female mice at mid-pregnancy was 105.9 ± 25.3% of the activity in the raw blood (n = 3). Since there was little difference in the activities between serum and raw blood samples, the degradation of CAP activity during collection and storage of serum samples was considered to be negligible. From the mice at day 13 and 17 of pregnancy, placentas, uteri except for the placental tissue and livers were removed, washed with ice-cold 0.9% NaCl to remove excess blood and stored at –70°C for later assay.

In addition, five groups of mice were prepared as follows: normal mice on the 2nd day of diestrus, normal mice on the day of estrus, mice ovariectomized seven days before autopsy and given four daily subcutaneous injections of 1 mg progesterone (Sigma Chemical Co., St. Louis, MO, USA) dissolved in 0.1 ml sesame oil at 14:00 starting on the 3rd day after ovariectomy (OX + Prog group), mice ovariectomized seven days before autopsy and given four daily injections of the vehicle only (OX + Oil group), and normal male mice. All mice were sacrificed between 14:00 and 15:00. Immediately after sacrifice, the serum, liver and uteri, if any, were collected and stored for later assay as described above.

Experiment 2: Effects of steroid hormone treatments on serum CAP activity in female ICR mice

Female ICR mice kept intact or ovariectomized seven days earlier were sacrificed between 14:00 and 15:00, and the serum samples were collected and stored immediately as mentioned above.

First, normal cycling mice on the 2nd day of diestrus or on the day of estrus and ovariectomized mice were sacrificed without further treatment, and the serum was used for later assay (Diestrus, Estrus and OX groups for controls).

Next, female mice were divided into four groups. The first group of intact mice was given four daily injections of 1 mg progesterone at 14:00 (Intact+Prog group). The second group of ovariectomized mice received four daily injections of 1 mg progesterone starting on the 3rd day after ovariectomy (OX + Prog group). The third group of ovariectomized mice was given four daily injections of 5 μg estradiol-17β (Sigma) dissolved in 0.1 ml sesame oil at 14:00 (OX + Est group). The fourth group of ovariectomized mice was given four daily injections of 1 mg progesterone in combination with 5 μg estradiol-17β at 14:00 (OX + Prog + Est group). All these mice were sacrificed the day after the last injection.

Experiment 3: Effects of steroid hormone treatments on serum CAP activity in male ICR mice

Male ICR mice kept intact or castrated seven days before were sacrificed between 14:00 and 15:00, and the serum samples were collected and stored immediately as mentioned above.

First, intact and castrated male mice were sacrificed without further treatment, and the serum was used for later assay (Male and Cast groups for controls).

Then, castrated male mice were divided into three groups. The first group was given four daily injections of 1 mg progesterone at 14:00 starting on the 3rd day after castration (Cast + Prog group). The second group was given four daily injections of 5 μg estradiol-17β at 14:00 (Cast + Est group). The third group was given five daily injections of 100 μg testosterone propionate (Nacalai Tesque Inc., Kyoto, Japan) dissolved in 0.1 ml sesame oil at 14:00 starting on the 3rd day after castration (Cast + TP group). All mice were sacrificed the day after the last injection.

Preparation of organ extract

CAP was extracted from the fetal and maternal placentas, uteri and liver according to the method of Ikenaga *et al*. (1993). These tissues were minced in small pieces and homogenized with Teflon-glass homogenizers in 5 vol of 5 mM Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose. Homogenates were centrifuged at 600 g for 10 min to remove nuclei and undisrupted cells and the supernatants containing the membrane fraction were stored at –70°C for the CAP assay.

Determination of CAP activity

CAP activity was determined essentially by the method of Suzuki *et al*. (1981) using a fluorogenic substrate, S-benzyl-L-cysteine-4-methylcoumaryl-7-amide (S-benzyl-L-cysteine-MCA; BACHEM Feinchemikalien AG, Bubendorf, Switzerland). A 0.1 M sodium acetate buffer containing 0.1 M sodium monochloroacetate, pH 4.3, was used as the stopping reagent (Barrett, 1980).

A reaction mixture (500 μl for the assay of serum or 400 μl for the assay of tissue samples) containing 25 μM of S-benzyl-L-cysteine-MCA and 0.25 M sodium phosphate buffer, pH 7.4 as recommended by Durham (1976), was added to three tubes. Two of these tubes were used as duplicates and the third served as the zero control. This reaction mixture was prepared from 1 mM substrate in ethylene glycol dimethyl ether (Nacalai) and freshly prepared 0.5 M phosphate buffer. After preincubation for 3 min at 37°C, the reaction was initiated with the addition to the test tubes of 10 μl serum, 10 μl supernatant of uteri or liver, 5 μl supernatant of fetal placenta and 2.5 μl supernatant of maternal placenta, respectively. Incubation was carried out at 37°C for 5 min, and the reaction was terminated by an addition of 2.5 ml (for the assay of serum) or 2.8 ml (for the assay of tissue samples) of stopping reagent. The liberated 7-amino-4-methylcoumarin (AMC) was measured by spectrofluorophotometer (Model RF-540; Shimadzu, Kyoto, Japan) at an excitation wavelength of 370 nm and an emission wavelength of 460 nm using AMC (Peptide Institute Inc., Osaka, Japan) as the standard. In the zero control tube, the stopping reagent was added before the addition of assay samples, and then the mixture was treated in the same manner as the test tubes. The release of 1 nmol of AMC per minute from the substrate was estimated at 1 mU of CAP activity.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance followed by the Fisher PLSD test or by the unpaired t-test, using the StatView program (Abacus Concepts, Inc., Berkeley, CA, USA).

RESULTS

Experiment 1: Changes in CAP activity during pregnancy in SHN mice

The serum CAP activity in SHN mice was highest at day 13 of pregnancy and lowest at day 17, the activity at day 17 being significantly lower than those in the groups of day 5, day 10 and day 13 of pregnancy. The serum CAP level at day 17 was also significantly lower than those in intact diestrous (13.4 ± 0.5 mU/ml serum) and estrous (10.2 ± 0.7 mU/ml serum) mice ($p < 0.05$) (Fig. 1). While the activity at diestrus was significantly higher than that at estrus ($p < 0.01$), both the ac-

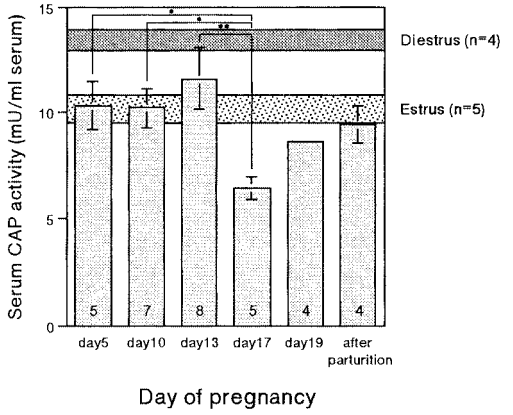

Fig. 1. Changes in serum cystine aminopeptidase (CAP) activity in SHN mice during pregnancy. The number of mice examined is indicated in each column. Vertical bars indicate the SEM. The CAP activities in normal cycling mice (Estrus and Diestrus) are presented as the range (mean ± SEM). $*p < 0.05$, $**p < 0.001$.

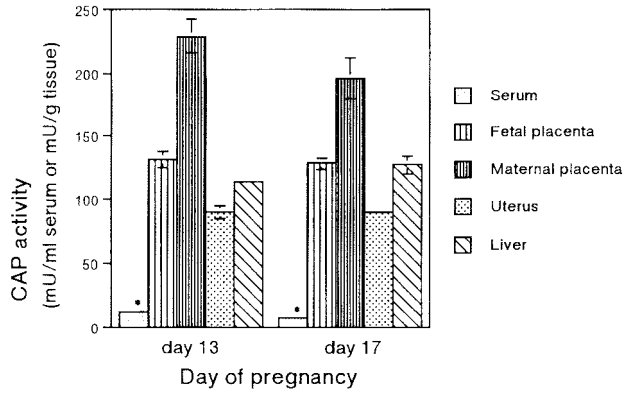

Fig. 2. Changes in serum and tissue cystine aminopeptidase (CAP) activities in pregnant SHN mice. The number of mice examined in each group is four, except for the serum samples of which the number is indicated in Fig. 1. Vertical bars indicate the SEM. $*p < 0.05$ by the unpaired t-test.

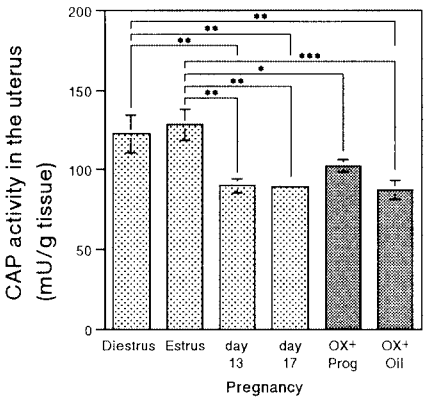

Fig. 3. Changes in uterine cystine aminopeptidase (CAP) activity in SHN mice. Four mice were examined in each group. Vertical bars indicate the SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

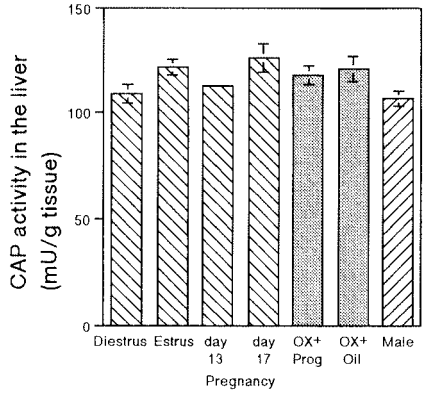

Fig. 4. Changes in liver cystine aminopeptidase (CAP) activity in SHN mice. Four mice were examined in each group. Vertical bars indicate the SEM.

tivities at diestrus and at estrus were significantly higher than that in male mice (5.4 ± 0.7 mU/ml serum; n = 6) at the 0.001 level. In ovariectomized mice, the serum CAP level was almost the same as that at estrus, and progesterone treatment induced no significant change in the activity (data not shown).

CAP activities in the fetal and maternal placentas, uterus and liver during pregnancy are shown in Fig. 2. Among the tissues examined, extremely high CAP activity was detected in the maternal placenta. However, no significant differences in CAP activities were found in any tissue samples between the groups of day 13 and day 17 of pregnancy (Fig. 2).

In the uterine tissue, CAP activity during pregnancy was significantly lower than in normal cycling mice and the activity after ovariectomy was also decreased compared with normal mice. The low activity in the ovariectomized mice was slightly increased after the progesterone treatment (Fig. 3).

No significant differences were detected in the liver CAP activity among the groups examined, though it was lowest in males (Fig. 4).

Experiment 2: Effects of steroid hormone treatments on serum CAP activity in female ICR mice

In ICR mice, no significant differences in the serum CAP activity were detected among three groups, diestrous mice (12.7 ± 1.0 mU/ml serum; n = 4), estrous mice (10.3 ± 1.1 mU/ml serum; n = 6) and ovariectomized mice (11.7 ± 0.6 mU/ml serum; n = 18). Progesterone injection into intact mice induced a significant decrease in the serum CAP activity compared with that at diestrus, and the decreased activity was also significantly lower than those in the OX and OX + Prog groups. In ovariectomized mice, the serum CAP activity was significantly lowered by the administration of estrogen either alone or when combined with progesterone compared with that in the OX group, though the administration of progesterone alone affected little the activity (Fig. 5).

Experiment 3: Effects of steroid hormone treatments on serum CAP activity in male ICR mice

The serum CAP activity in intact males (9.8 ± 0.5 mU/ml

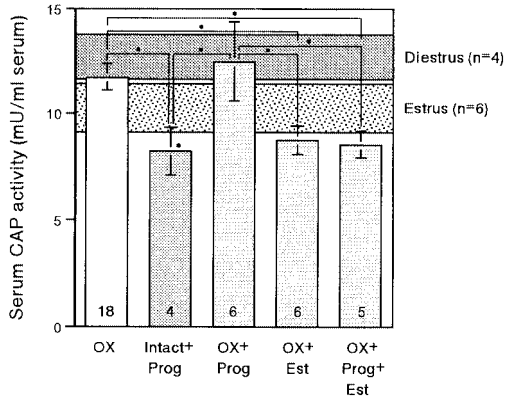

Fig. 5. Changes in serum cystine aminopeptidase (CAP) activity in female ICR mice treated with steroid hormones. The number of mice examined is indicated in each column. Vertical bars indicate the SEM. The CAP activities in normal cycling mice (Estrus and Diestrus) are presented as the range (mean ± SEM). OX = ovariectomy; Prog = progesterone; Est = estradiol-17β. $*p < 0.05$.

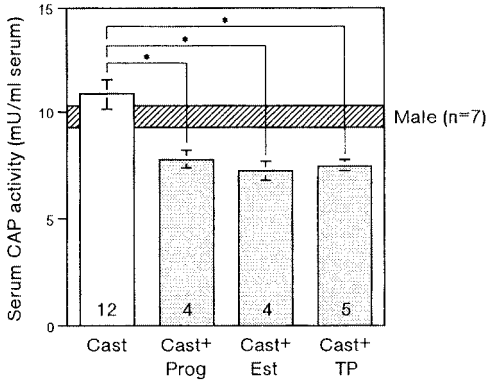

Fig. 6. Changes in serum cystine aminopeptidase (CAP) activity in male ICR mice treated with steroid hormones. The number of mice examined is indicated in each column. Vertical bars indicate the SEM. The CAP activities in intact male mice (Male) are presented as the range (mean ± SEM). Cast = castration; Prog = progesterone; Est = estradiol-17β; TP = testosterone propionate. $*p < 0.01$.

serum; n = 7) was significantly lower than that in females on the 2nd day of diestrus ($p < 0.05$).

Effects of progesterone, estrogen and androgen treatments on serum CAP activity in males are shown in Fig. 6. The serum CAP level in castrated mice was almost the same as that in intact males and was significantly decreased by the administration of progesterone, estrogen or androgen (Fig. 6).

DISCUSSION

Changes in serum CAP activity were investigated in pregnant and non-pregnant mice. Until now, CAP activity changes have been reported only in humans, and some of the findings differ from the present results. The activity of human CAP in the circulation, which is extremely low in men and non-pregnant women (less than 5 mU/ml serum in both), rises progressively after conception to reach a maximum at or near term (360 mU/ml serum) and decreases after parturition (Babuna and Yenen, 1966a,b; Durham, 1976; Roy and Karim, 1983; Mizutani and Tomoda, 1992). In contrast, of the SHN mice examined in the present study, a high activity of serum CAP was detected in the non-pregnant females on the 2nd day of diestrus, and the activity was low in the males. The result that the serum CAP level was higher at diestrus than at estrus implies a suppressive effect of estrogen on the activity. In mice, pregnancy lasts 20 days and the implantation occurs on the 4th day of fertilization. We observed that during pregnancy, the peak of the serum CAP activity appeared at day 13, and even the maximum level was not higher than that at diestrus. The enzyme activity was significantly lowered at day 17 of pregnancy, and then increased thereafter. On the other hand, CAP activities in the mouse placentas were not changed between day 13 and 17. The serum CAP level involving its release from tissues is regulated by some humoral factors, probably by the serum steroid hormone levels.

The present result that ovariectomy caused a significant decrease of the uterine CAP activity indicates ovarian steroids participate in the modulation of the CAP level. In contrast, no significant change was found in the liver CAP activity in any female group examined. The difference in the activity changes between the uterus and liver suggests that the expression of CAP is modulated in a tissue-specific manner. This may be due to different responsiveness of the tissues to steroid hormones. CAP activity was markedly high not only in the maternal and fetal placentas but also in the uterus and liver, as compared with the activity in serum. In humans, CAP activity is detected in liver, kidney, pancreas, mammary gland, uterus, ovary, pituitary and hypothalamus (Roy and Karim, 1983). It is suggested that these tissues produce CAP and release it into the blood.

Treatment with estrogen alone or in combination with progesterone led to a significant decrease of the serum CAP activity in ovariectomized ICR mice. This result suggests that estrogen suppresses the CAP synthesis in the tissues and/or the release into the blood. The administration of progesterone caused significant decrease of the serum CAP activity in intact females but no change in ovariectomized mice, compared with that in normal diestrous mice. Therefore, in females, progesterone can enhance a suppressive effect of estrogen on the serum CAP activity. In addition, the serum CAP activity in castrated males was slightly higher than that in intact males and was significantly lowered by estrogen, progesterone or testosterone treatment. It is possible that the higher circulating level of testosterone in males than in females leads to the low serum CAP activity in the former.

Human placenta is considered to be a main source of the circulating CAP (Page *et al*., 1961) and the enzyme is derived from the lysosomes in placental cells (Oya *et al*., 1974a,b), although the releasing mechanism of the enzyme into the blood remains unknown. The placental CAP activity was markedly high in mice, whereas the highest serum activity at day 13 of pregnancy was almost the same as that in intact diestrous mice. This result suggests the different releasing mechanism of the enzyme into the blood in pregnant mice from that in

pregnant women. In humans, it is reported that the serum CAP level during pregnancy rises progressively until parturition, associated with consistent increase of the serum progesterone level. In addition, the serum CAP level stops its increase just before the onset of labor in humans, associated with the increase of the serum estrogen level and the decrease of the serum progesterone level (Babuna and Yenen, 1966a,b; Boroditsky *et al*., 1978; Ottesen and Lebech, 1979; Mizutani *et al*., 1982; Roy and Karim, 1983; Mizutani and Tomoda, 1992). However, the enzyme activity in mice decreased significantly at day 17 of pregnancy. Soares and Talamantes (1984) and Kosaka *et al*. (1988) reported that the serum progesterone level in mice increased at day 2 of pregnancy, remained high until day 12, increased again from day 13 to day 14, was maintained at this level until day 17, and decreased thereafter. Thus, the lowered CAP activity at day 17 may be due to a suppressive effect of progesterone. As CAP inactivates oxytocin, a potent inducer of uterine contraction, the lowered serum CAP level can cause a decrease of the metabolic clearance of oxytocin in the blood. In addition, the result that CAP activity in the uterus was significantly low in pregnant mice compared to normal mice also implies an elevation of the uterine response to the oxytocin, the uterus being ready for the contraction after the increase of serum oxytocin level. Further studies on the different changes in the CAP activities between humans and mice are needed to clarify the mechanism of the onset of labor.

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REFERENCES

Babuna C, Yenen E (1966a) Further studies on serum oxytocinase in pathologic pregnancy. Am J Obstet Gynecol 94: 868–875

Babuna C, Yenen E (1966b) Enzymatic determination of placental function: a rapid method. Am J Obstet Gynecol 95: 925–934

Barrett AJ (1980) Fluorimetric assays for cathepsin B and cathepsin H with methylcoumarylamide substrates. Biochem J 187: 909–912

Boroditsky RS, Reyes FI, Winter JS, Faiman C (1978) Maternal serum estrogen and progesterone concentrations preceding normal labor. Obstet Gynecol 51: 686–691

Durham BH (1976) Spectrophotometric end-point method for assay of serum cystyl-aminopeptidase in pregnancy. Clin Chem 22: 79–82

Fekete K (1930) Beiträge zur physiologie der gravidität. Endokrinologie 7: 364–369

Ikenaga H, Mizuta Y, Ono K, Sawazaki T, Suzuki N, Tomoda I (1993) Placental and plasma cystine aminopeptidase in pregnant animals. J Vet Med Sci 55: 479–480

Keller SR, Scott HM, Mastick CC, Aebersold R, Lienhard GE (1995) Cloning and characterization of a novel insulin-regulated membrane aminopeptidase from Glut4 vesicles. J Biol Chem 270: 23612–23618

Kosaka T, Saito TR, Takahashi KW (1988) Changes in plasma progesterone levels during the estrous cycle and pregnancy in 4-day cyclic mice. Exp Anim 37: 351–353

Mizutani S, Sakura H, Akiyama H, Kobayashi H (1982) Simultaneous determinations of total cortisol and progesterone by radioimmunoassay during late pregnancy. Radioisotopes 31: 185–189

Mizutani S, Tomoda Y (1992) Oxytocinase: placental cystine aminopeptidase or placental leucine aminopeptidase (P-LAP). Semin Reprod Endocrinol 10: 146–153

Mizutani S, Goto K, Tsujimoto M, Nakazato H, Matsuzawa K, Furuhashi Y, Arii K, Tomoda Y (1996) Possible effects of placental leucine aminopeptidase on the regulation of brain-gut hormones in the fetoplacental unit. Biol Neonate 69: 307–317

Nagasawa H, Yanai R, Taniguchi H, Tokuzen R, Nakahara W (1976) Two-way selection of a stock of Swiss albino mice for mammary tumorigenesis: establishment of two new strains (SHN and SLN). J Natl Cancer Inst 57: 425–430

Ottesen B, Lebech PE (1979) Plasma progesterone, serum estriol and plasma HPL determinations during the last trimester to detect changes before spontaneous labor: comparison of progesterone assay using RIA and CPB. Acta Obstet Gynecol Scand 58:423–427

Oya M, Yoshino M, Asano M (1974a) Human placental aminopeptidase isozymes. Experientia 30: 985–986

Oya M, Yoshino M, Mizutani S, Wakabayashi T (1974b) The origin of human pregnancy serum oxytocinase. Gynecol Invest 5: 276–283

Page EW, Titus MA, Mohun G, Glendening MB (1961) The origin and distribution of oxytocinase. Am J Obstet Gynecol 82: 1090–1095

Rogi T, Tsujimoto M, Nakazato H, Mizutani S, Tomoda Y (1996) Human placental leucine aminopeptidase / oxytocinase: a new member of type II membrane-spanning zinc metallopeptidase family. J Biol Chem 271: 56–61

Roy AC, Karim SM (1983) Review: significance of the inhibition by prostaglandins and cyclic GMP of oxytocinase activity in human pregnancy and labour. Prostaglandins 25: 55–70

Roy AC, Viegas OA, Sen DK, Ratnam SS (1993) Decreased levels of amniotic fluid oxytocinase activity in preeclampsia. Gynecol Obstet Invest 35: 166–168

Ryden G (1966) Cystine aminopeptidase and oxytocinase activity in pregnancy: a comparative study in human and rat tissues. Acta Obstet Gynecol Scand 45 Suppl 3: 1–105

Sakura H, Lin TY, Doi M, Mizutani S, Kawashima Y (1981) Purification and properties of oxytocinase, a metalloenzyme. Biochem Int 2: 173–179

Soares MJ, Talamantes F (1984) Pre-parturitional changes in serum prolactin, placental lactogen, growth hormone, progesterone, and corticosterone in the C3H/HeN mouse. J Dev Physiol 6: 423–429

Suzuki M, Ueno T, Takahashi T, Kanaoka Y, Okuyama T, Furuya H, Sekine T (1981) A new fluorometric ultramicro determination of serum cystine aminopeptidase using an aminocoumarine derivative. Clin Chim Acta 115: 223–228

Tsujimoto M, Mizutani S, Adachi H, Kimura M, Nakazato H, Tomoda Y (1992) Identification of human placental leucine aminopeptidase as oxytocinase. Arch Biochem Biophys 292: 388–392

Tuppy H (1968) The influence of enzymes on neurohypophysial hormones and similar peptides. In “Handbuch der Experimentellen Pharmakologie Vol 23” Ed by B Berde, Springer-Verlag, Berlin, Heiderberg, New York, pp 67–129

Wood PL, Durham BH (1988) Change in plasma cystyl aminopeptidase (oxytocinase) between 30-34 weeks’ gestation as a predictor of pregnancy-induced hypertension. Obstet Gynecol 72: 850–852

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