

## Full Paper

Localized Expression of Histamine H<sub>1</sub> Receptors in Syncytiotrophoblast Cells of Human PlacentaKazuto Matsuyama<sup>1</sup>, Tatsuya Ichikawa<sup>1</sup>, Yuichi Nitta<sup>1</sup>, Yoshio Ikoma<sup>2</sup>, Kazutaka Ishimura<sup>3</sup>, Shuhei Horio<sup>1</sup>, and Hiroyuki Fukui<sup>1,\*</sup><sup>1</sup>Department of Molecular Pharmacology, and <sup>3</sup>Department of Anatomy and Cell Biology, Institute of Health and Biosciences, the University of Tokushima, 1-78-1 Shomachi, Tokushima 770-8505, Japan<sup>2</sup>Department of Industry-Academia Collaboration Research Planning, Intellectual Property Office, the University of Tokushima, 2-1 Minami-Jousanjima-cho, Tokushima 770-8506, Japan

Received August 1, 2006; Accepted October 3, 2006

**Abstract.** The previous Northern blot analysis and in situ hybridization studies showed that histamine H<sub>1</sub>-receptor (H<sub>1</sub>R) mRNA is expressed in human placenta and suggested that H<sub>1</sub>R plays some roles in the function of placenta in pregnancy. To investigate further, it is essential to show the precise location of H<sub>1</sub>R in the placenta. In the present study, we investigated H<sub>1</sub>R expression in human placenta by radioligand binding assay and immunohistochemical study using an antibody against human H<sub>1</sub>R. Placentas were obtained from normal uncomplicated deliveries. Membranes prepared from the tissue exhibited saturable [<sup>3</sup>H]mepyramine binding ( $K_d = 4.0 \pm 0.6$  nM and  $B_{max} = 91.4 \pm 4.9$  fmol/mg of protein). Stereoisomers of chlorpheniramine inhibited [<sup>3</sup>H]mepyramine binding; *d*-chlorpheniramine inhibited more potently than *l*-chlorpheniramine,  $K_i$  values being  $1.1 \pm 0.4$  and  $270 \pm 170$  nM, respectively. The placenta tissues were positively immunostained with anti-H<sub>1</sub>R antibody only in the region of the syncytiotrophoblast of chorionic villus. The tissues were double stained with anti-H<sub>1</sub>R antibody and an antibody against human chorionic gonadotropin (hCG) that is solely expressed in placental syncytiotrophoblast cells. The results showed that H<sub>1</sub>R and hCG were expressed on the same cells, that is, syncytiotrophoblast cells. These results indicate that H<sub>1</sub>Rs are specifically expressed in syncytiotrophoblast cells of human placenta organ.

**Keywords:** histamine H<sub>1</sub> receptor, mepyramine, placenta, syncytiotrophoblast, immunohistochemistry

## Introduction

Histamine is a biogenic amine formed from L-histidine by histidine decarboxylase (HDC), and plays an important role in both central and peripheral tissues (1). The role of histamine in pregnancy has been studied for a long period. Histamine is required during pregnancy in processes such as embryo development, implantation, and decidualization (2 – 9). On the other hand, accumulating data suggest that high levels of circulating maternal blood histamine is harmful to human pregnancy and is involved in a number of complications,

including preeclampsia, spontaneous abortion, preterm labor, and hyperemesis gravidarum (10). Thus histamine may have dual effects in pregnancy. Knowledge of the precise mechanism of histamine actions in pregnancy is essential for treating the complications in pregnancy, and it also provides important information about the influence of various drugs related to histamine actions on pregnancy.

The placenta is an organ essential for successful pregnancy. It mediates the physiological exchange between mother and fetus and secretes hormones, growth factors, cytokines, and other bioactive molecules and also protects the fetus from harmful agents getting into the fetal circulation. It has been known that the placenta is a primary source of histamine during pregnancy in humans (11 – 14). If histamine has functions in

\*Corresponding author. hfukui@ph.tokushima-u.ac.jp  
Published online in J-STAGE: November 10, 2006  
doi: 10.1254/jphs.FP0060862

the placenta, the expression of histamine receptors in this organ would be expected. However, there is only limited information on histamine receptors in human placenta. Histamine receptors are now classified into four subtypes, H<sub>1</sub>, H<sub>2</sub>, H<sub>3</sub>, and H<sub>4</sub> receptors (1, 15). Among these receptor types, H<sub>1</sub> receptor (H<sub>1</sub>R) has been suggested to exist in human placenta. Northern blot analysis revealed high levels of H<sub>1</sub>R mRNA in human placenta tissues (16), and in situ hybridization study also showed the expression of H<sub>1</sub>R mRNAs in cultured human placental tissues (17). Studies on the role of histamine receptors in human placenta are also very limited, but Liu et al. (8) suggested an involvement of H<sub>1</sub>R in trophoblast invasion that produce invasive extravillous trophoblasts, whose impairment results in pregnancy complications such as preeclampsia.

To investigate further the role of histamine in pregnancy, the knowledge of the expression and the precise location of histamine receptors in human placenta is essential. Therefore, in the present study we investigated the expression of H<sub>1</sub>R in the human placenta tissue to localize H<sub>1</sub>R expression and identify H<sub>1</sub>R-containing cells by immunohistochemical study using an antibody against human H<sub>1</sub>R. Some of these results have appeared in preliminary form (18).

## Materials and Methods

### Materials

[<sup>3</sup>H]Mepyramine ([pyridinyl-5-<sup>3</sup>H]pyrilamine, 0.74 TBq/mmol) was purchased from NEN Life Science Products (Boston, MA, USA). Cell culture reagents were from Life Technologies (Rockville, MD, USA). Antibody against c-myc was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), and antibodies against human histamine H<sub>1</sub> receptor and human chorionic gonadotropin (hCG) were from CHEMICON International, Inc. (Mississauga, Canada). Goat antibodies against mouse and rabbit IgGs were purchased from Santa Cruz Biotechnology, Inc. An antibody against rabbit IgG was purchased from Dako Cytomation (Kyoto). *d*-Chlorpheniramine was obtained from Wako Pure Chemicals (Osaka), and *l*-chlorpheniramine was a generous gift from Schering Corporation (Bloomfield, NJ, USA). All other reagents, unless otherwise stated, were of analytical grade and were from Wako Pure Chemicals or Sigma (St. Louis, MO, USA).

### Tissues

Placentas were obtained from normal uncomplicated deliveries at the University of Tokushima affiliated hospital and transported immediately to the laboratory on ice. Placentas from non-smoking and non-drinking

mothers who were not medicated were selected for the experiments. Their use was approved by the Ethics Committee of the University of Tokushima.

### [<sup>3</sup>H]Mepyramine binding assay

Membranes were prepared from human placenta according to the method of Jacobs and Cuatrecasas (19). Briefly, tissues were homogenized using a Polytron in 0.25 M sucrose containing 20 µg/ml PMSF. After centrifugation at 600 × *g* for 10 min, the supernatant was adjusted to 0.1 M NaCl and 0.2 mM MgCl<sub>2</sub> and then centrifuged at 40,000 × *g* for 50 min. The pellet was suspended in 50 mM sodium-potassium phosphate buffer (pH 7.4), re-centrifuged, and suspended in the same buffer. The [<sup>3</sup>H]mepyramine binding assay was performed as described previously (20). A suspension of cell membranes (500 µg of protein) was incubated at 25°C for 60 min with various concentrations of [<sup>3</sup>H]mepyramine ranging 0.5–8.0 nM in the absence (total binding) or presence (nonspecific binding) of 10 µM triprolidine in 50 mM sodium-potassium phosphate buffer (pH 7.4) containing 1 µM quinine in a final volume of 600 µl. The membrane-bound radioligands were separated from free radioligands by rapid filtration through a Whatman GF/B glass fiber filter (Whatman, Maidstone, UK). The filter was placed in 10 ml of Aquasol II (Packard Instrument Inc., Meriden, CT, USA), and the radioactivity on the filter was counted in a liquid scintillation counter. The specific binding was calculated by subtracting nonspecific binding from total binding. Inhibition study of [<sup>3</sup>H]mepyramine bindings (5 nM) was performed with increasing concentrations of either *d*-chlorpheniramine (10<sup>-11</sup>–10<sup>-5</sup> M) or *l*-chlorpheniramine (10<sup>-8</sup>–10<sup>-4</sup> M).

### Chinese hamster ovary (CHO) cells expressing c-myc-tagged human H<sub>1</sub>Rs (CHO-H<sub>1</sub>R cells)

Expression of H<sub>1</sub>Rs in CHO cells was described previously (21, 22). Briefly, the mammalian expression vector pdKCR-dhfr containing c-myc epitope-tagged human H<sub>1</sub>R gene was introduced into CHO cells that were deficient in dihydrofolate reductase [CHO(-) cells] using the calcium phosphate precipitation method. Transfected cells were cultured in  $\alpha$ -minimum essential medium without ribonucleosides and deoxyribonucleosides, and CHO cells stably expressing c-myc-tagged H<sub>1</sub>R (CHO-H<sub>1</sub>R cells) were cloned.

### Western blot analysis

Western blotting was performed on membrane preparations from CHO-H<sub>1</sub>R and CHO(-) cells. Cultured cells were harvested in ice-cold lysis buffer (548 mM NaCl, 8 mM EDTA, and 80 mM Tris-Cl, pH 7.4) and then

ultrasonically disrupted. The crude cell membrane preparations were collected by centrifugation and were suspended in SDS sample buffer. Aliquots of 50 µg protein were separated by 7% SDS-PAGE and then electroblotted to nitrocellulose membranes. After blocking the nonspecific binding sites with 5% nonfat dry milk in TS buffer (150 mM NaCl, 20 mM NaH<sub>2</sub>PO<sub>4</sub>, 0.05% Tween 20, pH 7.4), the blots were incubated with 1:100 dilution of monoclonal anti-c-myc antibody or 1:50 dilution of polyclonal anti-human H<sub>1</sub>R antibody at 4°C overnight. The blots were then washed with TS buffer and reincubated for 2 h at room temperature with 1:2000 dilution of horseradish peroxidase-labeled goat anti-mouse IgG (Santa Cruz Biotechnology, Inc.) or goat anti-rabbit IgG, respectively. The complexes were detected using the ECL Western blotting detection system (Amersham Biosciences, Buckinghamshire, UK).

#### *Immunohistochemistry*

For the detection of H<sub>1</sub>R expressed in CHO cells, cells were grown on coverslips and fixed with 4% formaldehyde for 2 h at room temperature. Then the cells were treated with 2% Triton X-100 in PBS for 2 h at room temperature, washed 3 times with PBS, and heated in a microwave oven for 10 min at 600 W in PBS containing 0.01 M citric acid. After the blocking procedure with 10% goat serum in PBS for 1 h at room temperature, the cells were incubated with 1:100 dilution of polyclonal anti-human H<sub>1</sub>R antibody at 4°C overnight. The cells were then washed with PBS buffer and incubated for 1 h at room temperature with peroxidase-conjugated anti-rabbit IgG (DAKO Envision<sup>+</sup>, DAKO). Peroxidase activity was visualized by incubating the cells at room temperature in the presence of 3,3'-diaminobenzidine tetrahydrochloride (DAB) and 3% hydrogen peroxide.

For immunohistochemical staining of placentas, the tissues were cut into small pieces on ice and fixed in 4% formaldehyde solution at 4°C for 12 h before dehydration in graded concentrations of ethanol, and embedded in paraffin. Then, the thin sections were treated essentially in the same way as described for immunohistochemical staining of the CHO cells. For staining by anti-H<sub>1</sub>R antibody, the sections were incubated with 1:100 dilution of polyclonal anti-human H<sub>1</sub>R antibody or normal rabbit IgG (as a control) at 4°C overnight, followed by incubation with peroxidase-conjugated anti-rabbit IgG for 1 h at room temperature. For staining by an antibody against hCG, the sections were incubated with 1:250 dilution of anti-hCG antibody at 37°C for 30 min. For double staining with anti-hCG antibody and anti-human H<sub>1</sub>R antibody, the sections were first incubated with 1:250 dilution of anti-hCG antibody at 37°C for 30 min, then with peroxidase-conjugated

anti-rabbit IgG, and reacted with DAB. Secondly, the sections were treated with 0.1 M glycine-HCl buffer (pH 2.2) for 2 h to remove antibodies, then incubated with 1:100 dilution of anti-human H<sub>1</sub>R antibody at 4°C overnight, followed by incubation with peroxidase-conjugated anti-rabbit IgG. Then, the sections were visualized by the treatment with 0.002% 4-chloro-1-naphthol and 0.005% hydrogen peroxide for 10 min.

## **Results**

#### *Radioligand binding to membranes from human placenta*

First, we examined whether H<sub>1</sub>Rs are expressed in the placenta by radioligand binding assay. Saturation studies performed with the selective H<sub>1</sub>R antagonist [<sup>3</sup>H]mepyramine revealed a single class of high affinity binding sites in membranes from human placenta. K<sub>d</sub> and B<sub>max</sub> values obtained from the Scatchard plot analysis were 4.0 ± 0.6 nM and 91.4 ± 4.9 fmol/mg protein (n = 3), respectively. To verify that the [<sup>3</sup>H]mepyramine binding was to H<sub>1</sub>Rs, we performed inhibition studies using stereoisomers of chlorpheniramine, another selective H<sub>1</sub>R antagonist (23). *d*-Chlorpheniramine inhibited [<sup>3</sup>H]mepyramine binding more potently than *l*-chlorpheniramine. K<sub>i</sub> values of *d*- and *l*-chlorpheniramine were 1.1 ± 0.4 and 270 ± 170 nM (n = 3), respectively. These values are closely comparable to the values obtained for human H<sub>1</sub>R in other tissues (24), and these data indicate that H<sub>1</sub>Rs are expressed in human placenta.

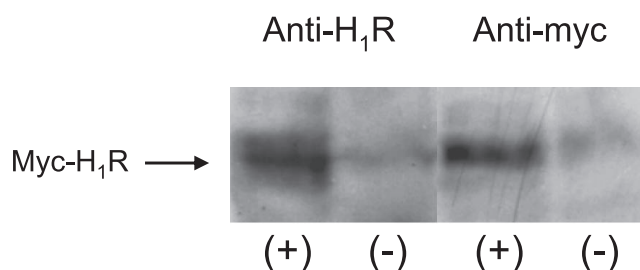
#### *Western blot analysis of c-myc-tagged H<sub>1</sub>Rs expressed in CHO-H<sub>1</sub>R cells*

To study the localization of H<sub>1</sub>R in human placenta, we performed immunohistochemistry by using an antibody against human H<sub>1</sub>R (CHEMICON International, Inc.). First, we examined the specificity of this antibody by Western blot analysis using CHO-H<sub>1</sub>R that expressed c-myc-tagged H<sub>1</sub>R. In this experiment, an antibody against c-myc was used to localize the migrated c-myc-tagged H<sub>1</sub>R, and it was detected as a band around the molecular weight of 57 kDa (Fig. 1). The antibody against H<sub>1</sub>R also bound to the band at the same position (Fig. 1). In contrast to this result, both antibodies failed to detect this band in CHO(-) cells that expressed no human H<sub>1</sub>R. These results indicate that this antibody against H<sub>1</sub>R can specifically detect human H<sub>1</sub>R.

#### *Immunohistochemistry of CHO-H<sub>1</sub>R cells*

To verify further the specificity of the antibody against H<sub>1</sub>R, we performed immunohistochemical staining of CHO-H<sub>1</sub>R cells. As shown in Fig. 2, CHO-H<sub>1</sub>R cells were positively stained, but control CHO(-) cells were

not stained at all by the antibody against H<sub>1</sub>R, indicating that this antibody can specifically detect human H<sub>1</sub>R in the cell.

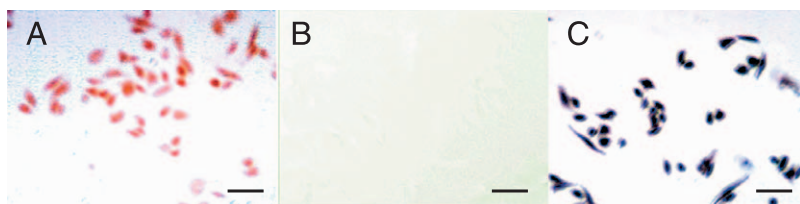


**Fig. 1.** Western blot analysis of CHO-H<sub>1</sub>R cells that expressed myc-tagged human H<sub>1</sub>R. The bands corresponding to myc-tagged H<sub>1</sub>R are indicated by an arrow. Immunodetection was performed with an antibody against human H<sub>1</sub>R (Anti-H<sub>1</sub>R) and an antibody against c-myc (Anti-myc) for CHO-H<sub>1</sub>R cells expressing myc-tagged H<sub>1</sub>R (+) and control CHO cells (-).

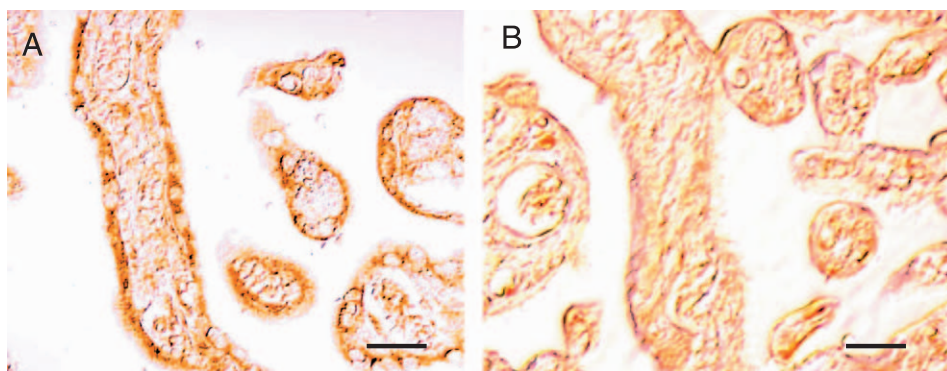
### Immunohistochemistry of human placenta

Human placentas from normal uncomplicated deliveries were immunostained with the antibody against human H<sub>1</sub>R. Positive immunostainings of H<sub>1</sub>R were observed only in the region of the syncytiotrophoblast of chorionic villus (Fig. 3A). No other cells including cytotrophoblasts, vessels, stroma cells, and intervillous space were positively stained. Normal rabbit IgG that was used as a control showed no positive staining (Fig. 3B).

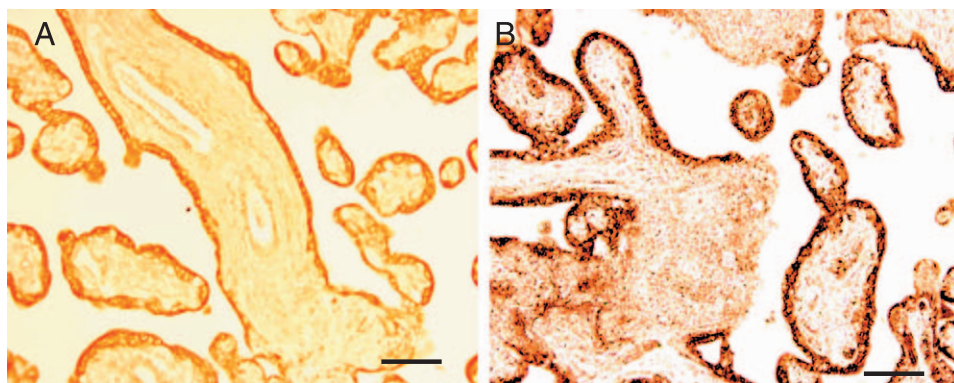
Then, the tissue was stained with an antibody against hCG that is specifically expressed in placental syncytiotrophoblast cells (25). As shown in Fig. 4A, positive stainings of hCG were found only in the syncytiotrophoblast region. Then, double staining with the anti-hCG antibody and the anti-human H<sub>1</sub>R antibody was performed. Antibodies against hCG were visualized with DAB, in which positive cells were stained in red, and



**Fig. 2.** Immunohistochemistry with an antibody against human H<sub>1</sub>R of CHO-H<sub>1</sub>R cells expressing myc-tagged human H<sub>1</sub>R (A) and control CHO cells (B). Control CHO cells were stained by hematoxylin-eosin to indicate their localization (C). Scale bars = 50  $\mu$ m.



**Fig. 3.** Immunohistochemistry of human placenta with an antibody against human H<sub>1</sub>R. A: Immunostaining with an antibody against human H<sub>1</sub>R. B: Immunostaining with normal rabbit IgG. Scale bars = 20  $\mu$ m.



**Fig. 4.** Immunohistochemistry of human placenta with antibodies against hCG and human H<sub>1</sub>R. A: Immunostaining with an antibody against hCG. B: Double immunostaining with an antibody against human H<sub>1</sub>R (visualized with 4-chloro-1-naphthol) and an antibody against hCG (visualized with DAB). Scale bars = 20  $\mu$ m.

antibodies against human H<sub>1</sub>R were visualized with 4-chloro-1-naphthol, in which positive cells were stained in dark blue. As shown in Fig. 4B, both of the stainings merged to reveal areas of co-localization in dark brown. These results indicate that H<sub>1</sub>Rs are specifically expressed in syncytiotrophoblast cells in human placenta.

## Discussion

The present study showed that H<sub>1</sub>R was specifically expressed in syncytiotrophoblast cells in human placenta organ. This was confirmed in this study by the following results: First, the ligand binding study with stereoisomers of the selective H<sub>1</sub>R antagonist chlorpheniramine clearly showed that H<sub>1</sub>Rs are expressed in human placenta tissue. Second, the anti-human H<sub>1</sub>R antibody was shown to be specific to H<sub>1</sub>R by Western blot analysis and immunohistochemical analysis of two types of CHO cells, one that expressed recombinant human H<sub>1</sub>R(CHO-H<sub>1</sub>R) and the other that did not (CHO(-)). Third, the anti-H<sub>1</sub>R antibody stained only the marginal regions of chorionic villus of human placenta, that is, syncytiotrophoblast regions (26). Forth, the double staining study using the antibody to H<sub>1</sub>R and that to hCG showed that H<sub>1</sub>R and hCG were expressed in the same cells. Because hCG is specifically expressed in placental syncytiotrophoblast cells (25), these results indicate that H<sub>1</sub>R is solely expressed in syncytiotrophoblast cells in this organ.

Histamine has been shown to be involved in several processes in pregnancy. Blockage of histamine action interrupts embryo development (4, 6), implantation (3, 5), and decidualization (2). Both H<sub>1</sub>R and H<sub>2</sub>R may be involved in implantation (7, 8). Histamine-deficient mice (HDC knockout mice) have some problems in pregnancy; their birth rate is low, their litter size is small, and their resorption rate is high compared to wild-type mice (9). Thus histamine is thought to participate in the fine tuning of the process of pregnancy.

The placenta is an organ essential for pregnancy and mediates the physiological exchange between mother and fetus and protects the fetus from harmful agents getting into the fetal circulation. The placental villi, which bathe in maternal blood, consists of the outer trophoblasts layer with multinuclear syncytium on the outside and mononuclear cytotrophoblasts on the inside followed by a connective tissue layer consisting of macrophages, fibroblasts, and fetal blood vessels (26, 27). The placenta has been known to contain large amount of histamine (11 – 14), suggesting that histamine plays a role in placenta during pregnancy. Although there has been only limited information on histamine

receptors in human placenta, the present study revealed that H<sub>1</sub>R is specifically expressed in the syncytium. The syncytium is a major transport, polarized, secretory epithelium that is essential for establishment and maintenance of pregnancy. Defects in this formation can be seen in several pregnancy complications (26 – 28).

How are histamine and H<sub>1</sub>R involved in syncytium function? One possibility is that H<sub>1</sub>R regulates the production of placental peptide hormones such as hCG and human placental lactogen (hPL). These hormones are thought to be involved in the establishment and continuation of pregnancy. For example, hCG that is mainly produced in syncytiotrophoblast cells has been shown to be involved in the differentiation of cytotrophoblasts to syncytium (29 – 31). Activation of H<sub>1</sub>R leads to induction of c-Fos (32 – 35) that forms activator protein-1 (AP1), which binds to promoter regions of various genes and regulates their expression. Thus, it is possible that H<sub>1</sub>R regulates the production of placental peptide hormones that play important roles in pregnancy.

Another possible role of H<sub>1</sub>R in the syncytium is to regulate the transport of nutrients such as amino acids and glucose from the maternal body to the fetus. It is now well known that amino acids and glucose are transported in placenta by several types of amino acid transporters (36, 37) and glucose transporters (38), respectively. Although little is known about the regulation of these transporters, several studies suggest that protein kinase C is involved in some types of amino acid transport (39 – 41). Since stimulation of H<sub>1</sub>R activates protein kinase C (24), it is possible that H<sub>1</sub>R regulates some types of amino acid transporters in syncytiotrophoblast cells, and thus regulates amino acids transport in the placenta.

In the human placenta, a subpopulation of stem cytotrophoblasts differentiates along two pathways that produce either syncytiotrophoblasts or invasive extravillous trophoblasts (42, 43). Cultured human cytotrophoblast cells (HTR-8/SVneo cells) express H<sub>1</sub>R and stimulation of this receptor by histamine enhances trophoblast invasion (8). Thus H<sub>1</sub>R may be involved in the production of invasive extravillous trophoblast that is required for early pregnancy. In this case, H<sub>1</sub>R is expressed in cytotrophoblast cells, and our study revealed H<sub>1</sub>R expression only in syncytiotrophoblast cells. This discrepancy may be due to the difference in the pregnancy stage of the preparations used in these studies; the cultured cytotrophoblast cell was derived from first-trimester pregnancy (8) and the placenta used in this study was obtained from normal uncomplicated delivery.

In conclusion, the present study showed that H<sub>1</sub>R is

expressed specifically in syncytiotrophoblast cells of human placenta, and this finding makes it possible to study the functional role of histamine and H<sub>1</sub>R in the placenta during pregnancy.

## Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science and by a fund from the Osaka Medical Research Foundation for Incurable Diseases.

## References

- Hill SJ. Distribution, properties, and functional characteristics of three classes of histamine receptor. *Pharmacol Rev.* 1990;42:45–83.
- Marcus GJ, Kracier PF, Shalesnyak MC. Studies on the mechanism of decidualization. II. The histamine-releasing action of pyrathiazine. *J Reprod Fert.* 1963;5:409–415.
- Dey SK, Johnson DC, Santos JG. Is histamine production by the blastocyst required for implantation in the rabbit? *Biol Reprod.* 1979;21:1169–1173.
- Dey SK, Johnson DC. Histamine formation by mouse preimplantation embryos. *J Reprod Fert.* 1980;60:457–460.
- Dey SK. Role of histamine in implantation: Inhibition of histidine decarboxylase induces delayed implantation in the rabbit. *Biol Reprod.* 1981;24:867–869.
- Hudgins L, Mukherjee S, Dey SK. Preimplantation embryo development in the mouse: role of histidine decarboxylase. *Gamete Res.* 1982;6:121–125.
- Zhao X, Ma W, Das SK, Dey SK, Paria BC. Blastocyst H(2) receptor is the target for uterine histamine in implantation in the mouse. *Development.* 2000;127:2643–2651.
- Liu Z, Kilburn BA, Leach RE, Romero R, Paria BC, Armant DR. Histamine enhances cytotrophoblast invasion by inducing intracellular calcium transients through the histamine type-1 receptor. *Mol Reprod Dev.* 2004;68:345–353.
- Pap E, Falus A, Mihalyi D, Borck H, Diel F, Pallinger E. Histamine regulates placental cytokine expression – in vivo study on HDC knockout mice. *Placenta.* 2006; in press.
- Brew O, Sullivan MH. The links between maternal histamine levels and complications of human pregnancy. *J Reprod Immunol.* 2006; in press.
- Wicksell F. Observations on histamine and histaminolysis in pregnancy. *Acta Physiol Scand.* 1949;17:395–414.
- Lindberg S, Lindell SE, Westling H. Formation and inactivation of histamine by human foetal tissues in vitro. *Acta Obstet Gynecol Scand.* 1963;42:49–58.
- Gunther RE, Glick D. Determination of histaminase activity in histologic samples and its quantitative distribution in intact human placenta and uterus. *J Histochem Cytochem.* 1967;15:431–435.
- Purcell WM, Hanahoe TH. A novel source of mast cells: the human placenta. *Agents Actions.* 1991;33:8–12.
- Nguyen T, Shapiro DA, George SR, Setola V, Lee DK, Cheng R, et al. Discovery of a novel member of the histamine receptor family. *Mol Pharmacol.* 2001;59:427–433.
- Fukui H, Fujimoto K, Mizuguchi H, Sakamoto K, Horio Y, Takai S, et al. Molecular cloning of the human histamine H1 receptor gene. *Biochem Biophys Res Commun.* 1994;201:894–901.
- Brew OB, Sullivan MH. Localisation of mRNAs for diamine oxidase and histamine receptors H1 and H2, at the feto-maternal interface of human pregnancy. *Inflamm Res.* 2001;50:449–452.
- Matsuyama K, Kawakami N, Ichikawa T, Nitta Y, Ishimura K, Horio S, et al. Expression of histamine H1 receptor in placenta. *Inflamm Res.* 2004;53 Suppl 1:S85–S86.
- Jacobs S, Cuatrecasas P. Purification of insulin receptor from human placenta. *Methods Enzymol.* 1985;109:399–405.
- Mizuguchi H, Fukui H, Yabumoto M, Wada H. Synaptic and extra-synaptic distribution of histamine H1-receptors in rat and guinea pig brains. *Biochem Biophys Res Commun.* 1991;174:1043–1047.
- Fujimoto K, Ohta K, Kangawa K, Kikkawa U, Ogino S, Fukui H. Identification of protein kinase C phosphorylation sites involved in phorbol ester-induced desensitization of the histamine H1 receptor. *Mol Pharmacol.* 1999;85:735–743.
- Miyoshi K, Kawakami N, Horio S, Fukui H. Homologous and heterologous phosphorylations of human histamine H<sub>1</sub> receptor in intact cells. *J Pharmacol Sci.* 2004;96:474–482.
- Hill SJ, Emson PC, Young JM. The binding of [<sup>3</sup>H]mepyramine to histamine H<sub>1</sub> receptors in guinea-pig brain. *J Neurochem.* 1978;31:997–1004.
- Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, et al. International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol Rev.* 1997;49:253–278.
- Hoshina M, Boothby M, Boime I. Cytological localization of chorionic gonadotropin alpha and placental lactogen mRNAs during development of the human placenta. *J Cell Biol.* 1982;93:190–198.
- Boyd JD, Hamilton WJ. The human placenta. London: MacMillan; 1970.
- Benirschke K, Kaufmann P. Pathology of the human placenta, 4th ed. New York: Springer; 2000.
- Frendo JL, Vidaud M, Guibourdenche J, Luton D, Muller F, Bellet D, et al. Defect of villous cytotrophoblast differentiation into syncytiotrophoblast in Down's syndrome. *J Clin Endocrinol Metab.* 2000;85:3700–3707.
- Shi QJ, Lei ZM, Rao CV, Lin J. Novel role of human chorionic gonadotropin in differentiation of human cytotrophoblasts. *Endocrinol.* 1993;132:1387–1395.
- Cronier L, Bastide B, Herve JC, Deleze J, Malassine A. Gap junctional communication during human trophoblast differentiation: influence of human chorionic gonadotropin. *Endocrinol.* 1994;135:402–408.
- Yang M, Lei ZM, Rao ChV. The central role of human chorionic gonadotropin in the formation of human placental syncytium. *Endocrinol.* 2003;144:1108–1120.
- Panettieri RA, Yadvish PA, Kelly AM, Rubinstein NA, Kotlikoff MI. Histamine stimulates proliferation of airway smooth muscle and induces c-fos expression. *Am J Physiol.* 1990;259:L365–L371.
- Satoh T, Sugama K, Matsuo A, Kato S, Ito S, Hatanaka M, et al. Histamine as an activator of cell growth and extracellular matrix reconstruction for human vascular smooth muscle cells. *Atherosclerosis.* 1994;110:53–61.
- Kjaer A, Larsen PJ, Knigge U, Moller M, Warberg J. Histamine

- stimulates c-fos expression in hypothalamic vasopressin-, oxytocin-, and corticotropin-releasing hormone-containing neurons. *Endocrinol.* 1994;134:482–491.
- 35 Megson AC, Walker EM, Hill SJ. Role of protein kinase C alpha in signaling from the histamine H(1) receptor to the nucleus. *Mol Pharmacol.* 2001;59:1012–1021.
- 36 Jansson T. Amino acid transporters in the human placenta. *Pediatr Res.* 2001;49:141–147.
- 37 Kudo Y, Boyd CA. Human placental amino acid transporter genes: expression and function. *Reproduction.* 2002;124:593–600.
- 38 Baumann MU, Deborde S, Illsley NP. Placental glucose transfer and fetal growth. *Endocrine.* 2002;19:13–22.
- 39 Kulanthaivel P, Cool DR, Ramamoorthy S, Mahesh VB, Leibach FH, Ganapathy V. Transport of taurine and its regulation by protein kinase C in the JAR human placental choriocarcinoma cell line. *Biochem J.* 1991;277:53–58.
- 40 Karl PI. Insulin-like growth factor-1 stimulates amino acid uptake by the cultured human placental trophoblast. *J Cell Physiol.* 1995;165:83–88.
- 41 Roos S, Powell TL, Jansson T. Human placental taurine transporter in uncomplicated and IUGR pregnancies: cellular localization, protein expression, and regulation. *Am J Physiol Regul Integr Comp Physiol.* 2004;287:R886–R893.
- 42 Fisher SJ, Damsky CH. Human cytotrophoblast invasion. *Semin Cell Biol.* 1993;4:183–188.
- 43 Cross JC, Werb Z, Fisher SJ. Implantation and the placenta: key pieces of the development puzzle. *Science.* 1994;266:1508–1518.