Histamine uptake by human endometrial cells expressing the organic cation transporter EMT and the vesicular monoamine transporter-2

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Cellular reuptake of monoamines, which is mediated by cell membrane transporters, is followed by accumulation in vesicles by vesicular monoamine transporters (VMAT). The aim of this study was to demonstrate the presence of functional monoamine transporters with high affinity for histamine in human endometrial tissue, since histamine has been implicated as a paracrine signal during endometrial decidualization and embryo implantation. In situ hybridization with 35S-labelled cRNA probes was used for detection of the organic cationic transporter-2 (OCT-2), the extraneuronal monoamine transporter (EMT), and VMAT-2 in cryosections of normal human endometrial tissue. To identify functional transporters for histamine in endometrial cells, we incubated primary cultures of stromal cells and cultures of attached glands with 3H-labelled histamine. Cultures were pretreated with either corticosterone, a specific inhibitor of EMT, or reserpine, a specific inhibitor of VMAT-2. EMT mRNA was localized in the stroma during the proliferative phase and in the epithelium during the secretory phase. Thus, EMT and VMAT-2, which both have high affinity for histamine, are strongly expressed in endometrial cells. Both the cycle. VMAT-2 mRNA was localized in the stroma during the proliferative phase and in the epithelium during the secretory phase. This indicates the presence of functional EMT and VMAT-2 transporter proteins throughout the cycle, even though their periods of maximal mRNA expression were limited. The results of uptake experiments with glandular epithelial cells confirmed not only the presence of functional VMAT-2 transporter protein in the secretory phase but also the absence of a histamine-specific plasma membrane transporter throughout the cycle. Thus, endometrial tissue contains both plasma membrane and vesicular monoamine monoamine transporters with high affinity for histamine. They can potentially influence the reproductive process by the uptake of extracellular histamine and subsequent release on demand.

Key words: decidualization/epithelial cell/histamine release/inflammatory mediator/stromal cell

Introduction

Monoamines such as histamine, serotonin, dopamine, norepinephrine and epinephrine are potent signalling substances, which, in addition to being neurotransmitters, also have important functions as endocrine and paracrine signals (Axelrod, 1971). For example, norepinephrine, the principal neurotransmitter of the sympathetic nervous system, is also a potent stress hormone released together with epinephrine by the adrenal medulla (Kandel and Schwartz, 1985). Histamine also has a dual role being a neurotransmitter in the central nervous system (Steinbusch and Mulder, 1984) and a potent peripheral vasoregulator, e.g. when released by mast cells during allergic reactions (White, 1990).

Unlike most transmitter substances, monoamines are recycled rather than enzymatically inactivated. Thus, monoaminergic signalling is terminated by reuptake of the transmitter substance. This uptake requires active transport across the cell membrane, and specific membrane-bound transporter proteins mediate such uptake (Amara and Kuhar, 1993; Brownstein and Hoffman, 1994). Reuptake of monoamines was first discovered in neural cells, and these transporters are therefore referred to as neuronal transporters. Genes coding for the neuronal monoamine membrane transporters, including that for serotonin (SERT) (Blakely et al., 1991; Hoffman, 1994; Chang et al., 1996), norepinephrine (NET) (Ramamoorthy et al., 1993) and dopamine (DAT) (Kilty et al., 1991; Usdin et al., 1991), have been cloned and characterized. It was subsequently discovered that these transporter proteins were not restricted to the nervous system, but occurred in other cell types like DAT in stomach, pancreas and kidneys (Mezey et al., 1996; Eisenhofer, 2001), NET in adrenal medulla, lung and placenta (Eisenhofer, 2001; Torres et al., 2003) and SERT in platelets, intestine, adrenal glands and the skin (Talvenheimo and Rudnick, 1980; Wade et al., 1996; Schroeter et al., 1997; Hansson et al., 1998). However, to date, no specific membrane transporter for histamine has been found.

Three members of another family—so-called non-neuronal membrane transporters—were cloned more recently, i.e. the organic cation transporters (OCT) (Gorboulev et al., 1997; Koehler et al., 1997; Zhang et al., 1997; Grundemann et al., 1998). This group consists of OCT-1, OCT-2 and extraneuronal monoamine transporter (EMT).
Unlike neuronal transporters, OCTs play a key role in the clearance of monoamines from the blood stream (Eisenhofer, 2001). These transporters are expressed in humans by the liver (OCT-1 and EMT), intestine (OCT-1), kidney and brain (OCT-2, EMT). EMT has a broad tissue distribution and is also found in the heart, blood vessels, placenta and retina (Eisenhofer, 2001).

In contrast to most other transmitter substances, the intracellular fate of monoamines is influenced by their lower affinity for inactivating enzymes than for vesicular monoamine transporters (VMATs), which are located on the membranes of intracellular vesicles (Erickson and Eiden, 1993; Peter et al., 1995). Thus, monoamines are preferentially transferred from the cytoplasm to storage vesicles, from which they can be subsequently released on demand. Cell membrane-bound monoamine transporters therefore function not only as part of a metabolizing system, but also as part of a recycling system operating together with the VMATs to replenish the transmitter stores.

We recently reported on the distribution and cyclic variation of mRNA for neuronal and VMATs in the human endometrium using in situ hybridization and real-time polymerase chain reaction (PCR) (Bottalico et al., 2003). The endometrial stroma did not express mRNA for any neuronal transporter, whereas the vesicular transporter VMAT-2 mRNA was present in stroma during the proliferative phase. Endometrial epithelial cells expressed mRNA for only one plasma membrane transporter, i.e. NET mRNA. The signal was weak and only present in the proliferative phase. The vesicular transporter VMAT-2 mRNA was strongly expressed in secretory phase epithelium but was absent during the proliferative phase. In this study, we examined the expression of non-neuronal transporters and found that the expression of EMT mRNA was strong in the secretory endometrium, have high affinity for histamine (Erickson et al., 1992; Peter et al., 1994; Merckel and Edwards, 1995). Histamine has been suggested to modulate transformation of endometrial tissue into decidua as well as to regulate local blood flow (Barkai and Kraicer, 1996). Histamine has particularly been shown to play a key role in blastocyst implantation and placentation (Dey, 1981; Hatanaka et al., 1982; Mitchell et al., 1983; Liu et al., 2004). In mice, the rate-limiting enzyme in histamine synthesis, histidine decarboxylase (HDC), has been demonstrated in uterine epithelial cells with peak expression at the time for implantation (Paria et al., 1998). The blastocyst expresses histamine type 2 receptor (H2), which is the target for histamine activation (Zhao et al., 2000). After implantation, the placenta is developed by invasion of trophoblasts into the endometrium and maternal vasculature. Histamine enhances the invasion by activation of H1 receptor on the cytotrophoblasts (Liu et al., 2004).

To elucidate functional aspects of EMT and VMAT-2 in endometrial cells, we examined the uptake of "H-labelled histamine by stromal cells in primary cultures and by epithelial cells in cultures of attached glands. Uptake was also assayed in the presence of corticosterone, a specific inhibitor of EMT, and reserpine, a specific inhibitor of VMAT-2.

**Materials and methods**

**Tissue sampling**

Endometrial tissue was collected at the Department of Obstetrics and Gynecology from healthy women, 45 years or younger, without hormonal treatment or intrauterine device having hysterectomy or endometrial curettage for reasons unrelated to endometrial dysfunction, e.g. cervical dysplasia, myoma or uterine prolapse. Patients with endometriosis and other genital pathology were excluded. Sampling with informed patient consent was approved by the review board for studies in human subjects at the Lund University Hospital. Each sample was evaluated by a histopathologist for exclusion of endometrial pathology and for identification of the cyclic phase. Samples were classified as belonging

to the early, mid or late part of the proliferative phase or to the early, mid or late part of the secretory phase in an ideal 28-day reproductive cycle (Noyes et al., 1950; Hendrickson and Kempson, 1980).

**Tissue culture**

Endometrial tissue from 23 patients was obtained under sterile conditions, transported in saline to the laboratory and rinsed in Hanks balanced salt solution (HBSS) to remove blood. It was subsequently cut into 1 mm pieces and incubated in 10 ml dissociation solution (collagenase 2.5 g/l, DNase-I 0.25 g/l, tosyl-l-lysine-chloromethyl-ketone 0.2 μmol/l in HBSS) for 45 min at 37°C on a shaking rotor (BioSan ES-20). Gentle pipetting up and down assisted dissociation. Following addition of 10 ml culture medium supplemented with fetal bovine serum (FBS) 10%, cells were fractionated over sterile sieves. A nylon sieve (350 μm pore size) removed undigested pieces of tissue, and another sieve (35 μm pore size) retained the glands, while stromal cells were collected in the flow-through. The glands were back-washed from the sieve with HBSS, and both cell types were collected by centrifugation at 300 g, plated and maintained in 37°C humidified atmosphere of 5% CO2 in air.

Stromal cells were grown in 75 cm2 culture flasks with M199 medium (Sigma, St Louis, MO, USA) supplemented with FBS 10%, penicillin 100 U/ml, streptomycin 100 μg/ml and glutamine 2 mmol/l. Incubation overnight allowed glands to attach to the plastic. Uncoated plastic was used to prevent interference with the subsequent protein assay. The advantage of performing the uptake assay with adherent glands, rather than epithelial cells growing on the plastic, is that these cells preserve their polarized morphology and hence their functional integrity.

**Cellular uptake of "H-labelled histamine**

Cellular uptake of "H-histamine was assayed by incubating subconfluent cultures of endometrial stromal cells or attached glands for 15 min at 37°C with "H-histamine 0.1 μmol/l (Amersham, TRK 631) in uptake buffer (KCl 4.8 mmol/l, KH2PO4 1.2 mmol/l, MgSO4 1.2 mmol/l, glucose 5.6 mmol/l, NaCl 125 mmol/l, CaCl2 1.3 mmol/l, ascorbic acid 1 mmol/l, pargyline 10 μmol/l and Hepes 25 mmol/l, pH = 7.4). Subsequently, the cultures were washed twice with the buffer and lysed with 0.5 mol/l NaOH, and the cell lysate was assayed for radioactivity in a liquid scintillator. To determine background non-specific uptake/binding of "H-histamine, identical experiments were performed in the presence of 100-fold unlabelled histamine, 10 μmol/l. Non-specific binding/uptake was measured for each experiment and subtracted from the total counts to yield the specific binding/uptake. We also estimated cellular binding as compared with active uptake by incubating the cultures with "H-labelled histamine 0.1 μmol/l at 0°C. Uptake was also measured after pretreatment with either an inhibitor of the organic cation transporter EMT, i.e. corticosterone for 10 min, or an inhibitor of VMAT-2, i.e. reserpine for 45 min. The cytosols were assayed for protein content using bovine serum albumin as a standard. Finally, results were expressed as uptake of "H-histamine/ mg cellular protein. Each inhibitor concentration was assayed in quadruplicate for each patient.

**Preparation of RNA probes**

For the human EMT (OCT3) mRNA, a probe was used corresponding to 440 NT (1300–1740), Genbank accession NM_021977 (Grundemann et al., 1998).

For the human VMAT-2 mRNA, a probe was used corresponding to 486 NT (1300–1786, exons 12–14), Genbank accession NM_003054 (Erickson et al., 1993).

DNA templates were generated by PCR from cDNAs, as described previously (Bottalico et al., 2003, 2004). Complementary RNA (cRNA) probes were transcribed from 25 ng of gel-purified DNA template using "S-UTP (Dupont NEN, 1300 Ci/mmol) and either T7 or T3 RNA polymerase according

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to the manufacturer’s instructions (Ambion MAXIscript) to generate sense and antisense probes, respectively.

**In situ hybridization**

Tissue samples, 3 × 3 × 3 mm, from 31 patients were snap frozen in liquid nitrogen and stored at -80°C. Cryostat sections (12 µm) were thaw mounted on sialinized slides and stored at -80°C until hybridized. Fresh frozen tissue rather than fixative-treated tissue was used to maximize mRNA detection. Thawing of tissue did not occur before mounting to ensure best possible tissue integrity. Tissue sections were fixed, dehydrated, delipidated and hybridized as previously described (Bradley and Towle, 1992; Bottalico et al., 2003, 2004).

**Statistical methods**

All data are presented as mean and SEM, since distribution of data within each group appeared normal. Differences between groups were evaluated with Wilcoxon signed rank test or Mann–Whitney U-test. Concentration-dependent reduction of uptake by inhibitors was evaluated with chi-square test for trend (Altman, 1991). All tests were two-sided, and the 5% level of significance was used.

**Results**

*In situ* hybridization demonstrated EMT mRNA in the endometrial stroma with high expression in the secretory phase but present also in the proliferative phase (Fig. 1E,F). No EMT mRNA was detected in epithelial cells. Also OCT-2 mRNA was present in the stroma, but the expression was found in few stromal cells without cyclic variation (not shown). It was absent in the epithelium. VMAT-2 mRNA was located in stromal cells in the proliferative phase (Fig. 1A,B) and in epithelial cells in the secretory phase (Fig. 1C,D).

For the functional experiments, we wanted to establish the optimal concentration of 3H-labelled histamine to be used, and hence primary stromal cell cultures from five different endometrial samples were incubated with increasing concentrations of 3H-histamine, i.e. 0, 0.1, 0.5, 1, 5, 10, 50 and 100 nmol/l. The dose–response curve for cell-related radioactivity showed a gradual increase up to 100 nmol/l without reaching a plateau (not shown). We therefore decided to use 100 nmol/l. Also, another study of histamine uptake in other cell types used the same concentration (Grundemann et al., 1999).

Our aim was to quantify specifically the uptake of 3H-histamine. To allow distinction between cellular uptake and binding of 3H-histamine, parallel experiments were performed at 37°C and on ice. Furthermore, to distinguish between specific and non-specific uptake/binding, experiments were done in the presence and absence of 100-fold unlabelled histamine. Stromal cell cultures from two different patients were used for these experiments, and each treatment was performed in quadruplicate. By combining the results from these experiments, we found that the mean specific uptake was 44%, specific binding 12%, non-specific uptake 13% and non-specific binding 31% (not shown). Subsequent experiments always included wells with 100-fold unlabelled histamine, and non-specific uptake/binding was subtracted from total uptake/binding. This allowed all results to be presented as specific uptake.

Specific uptake in the control groups of stromal cell cultures was not different between the proliferative phase (seven patients), 126 fmol/mg protein (102–247) (median and ranges), and the secretory phase (six patients), 124 fmol/mg protein (62–207).

To demonstrate that endometrial stromal cells have functional membrane transporter protein with affinity for histamine, we studied the uptake of 3H-histamine in the absence and presence of corticosterone, a highly efficient inhibitor of EMT-mediated uptake (Hayer-Zillgen et al., 2002). Stromal cell cultures, established from proliferative (seven patients) as well as secretory (six patients) phase endometria, were pretreated with corticosterone 0, 10, 20, 30, 40, 50 or 60 µmol/l for 10 min before the uptake of 3H-histamine was measured. Corticosterone 10 µmol/l inhibited specific uptake of 3H-histamine in both the proliferative (*P = 0.01) and the secretory (**P < 0.0001) phase (Fig. 2). However, further increasing concentrations of corticosterone resulted
in further decreasing histamine uptake \((P = 0.0004)\) in proliferative phase cultures only.

Functional VMAT-2 transporter was assayed using reserpine, a selective inhibitor of VMAT-2-mediated monoamine transport (Erickson et al., 1992; Merickel et al., 1995). Pretreatment of stromal cells for 45 min with reserpine 10 \(\mu\)mol/l inhibited subsequent histamine uptake in proliferative (seven patients) \((P < 0.0001)\) but not in secretory (six patients) phase cultures (Fig. 3). Increasing concentrations of reserpine resulted in dose-dependent reduction of uptake in proliferative \((P = 0.0004)\) as well as in secretory \((P = 0.005)\) phase cultures.

When the experimental design used for stromal cells was applied to the gland cultures, no specific uptake was detected either in the proliferative (three patients) or in the secretory (three patients) phase cultures. When non-specific binding/uptake was subtracted from total uptake, the remaining specific uptake was close to zero in all groups. Also, corticosterone and reserpine had no inhibitory effect on the total uptake of histamine in epithelial cells in this experimental design (not shown). However, since endometrial epithelial cells in the secretory phase express VMAT-2 mRNA but lack plasma membrane transporters, \(^3H\)-histamine is unlikely to be available to the vesicular transporter during short-term experiments. Therefore, in the next set of experiments, we increased the incubation period up to 3 h to allow diffusion of \(^3H\)-histamine into the cells, thereby making it available to vesicular membrane transporters. Glands obtained in the secretory phase (three patients) accumulated significantly more \(^3H\)-histamine over time than glands from the proliferative phase (two patients) (Fig. 4), suggesting the presence of an active transport mechanism. Furthermore, pretreatment of the glands with reserpine 10 \(\mu\)mol/l under these conditions resulted in the reduction of accumulated \(^3H\)-histamine after 2 and 3 h in secretory phase but not in proliferative phase glands.

**Discussion**

Based on our previous and present findings with *in situ* hybridization, EMT and VMAT-2 are the only two monoamine transporter genes with strong expression in human endometrial tissue (Bottalico et al., 2003). Since both these transporters have high affinity for histamine, we chose radiolabelled histamine for the functional experiments, and we used corticosterone and reserpine as specific inhibitors of EMT and VMAT-2 respectively. Our results show that primary cultures of endometrial cells are capable of specific uptake of histamine, thus indicating the presence of functional transporter proteins. Susceptibility of the uptake to corticosterone and reserpine suggests the presence of functional EMT and VMAT-2.

Stromal cell uptake of radiolabelled histamine, expressed as fmol/mg cellular protein, was not different between the proliferative and secretory phase. This was surprising, taking into account that EMT mRNA is mainly expressed in the secretory phase. Weak expression of both EMT and OCT-2 genes may add to the uptake in proliferative phase stromal cells, since both have high affinity for histamine, and both are sensitive to the inhibitory effect of corticosterone (Grundemann et al., 1999). However, OCT-2 transports histamine with only two-third efficiency of that of EMT (Grundemann et al., 1999) and corticosterone is about 100-fold more potent as inhibitor of human EMT than of human OCT-2 (Grundemann et al., 1998; Hayer-Zillgen et al., 2002). In addition, the specific uptake measured in our experiments reflects accumulation of intracellular histamine as an effect not only of plasma membrane transport but also of vesicular membrane transport under conditions when a plasma membrane transporter is present. The VMAT-2 mRNA is present only in the proliferative phase; thus VMAT-2 protein is likely to be more abundant in the proliferative phase. The combined effects of these transporter mechanisms offer an explanation for the lack of difference in basal uptake between the cyclic phases.

The uptake of radiolabelled histamine in stromal cells was inhibited by corticosterone in both the proliferative and the secretory phase cells. We noted that full inhibitory effect of corticosterone was seen already at 10 \(\mu\)mol/l in secretory phase cells, whereas it required 60 \(\mu\)mol/l in proliferative phase cells. Possible contribution of OCT-2 to uptake in the proliferative phase may explain the difference since its sensitivity to corticosterone is 100-fold less compared to that of EMT (Grundemann et al., 1998; Hayer-Zillgen et al., 2002).

The concentration-dependent inhibition of histamine uptake by reserpine shows different patterns in stromal cell cultures taken in the proliferative and secretory phases. In the proliferative phase, when
VMAT-2 mRNA is present, the inhibition of uptake by increasing concentrations of reserpine comes to a plateau, whereas in the secretory phase, when VMAT-2 mRNA was not detected, the inhibition did not reach a plateau. There is an apparent similarity between the inhibition patterns for EMT and VMAT-2, i.e. inhibition reaches a plateau in the cyclic phase where the transporter mRNA is strongly expressed and the transporter protein is likely to be abundant. Differences between the cyclic phases may suggest that higher concentration of the inhibitor is required at a lower number of transporter molecules. In fact, Merckel and Edwards (1995) showed full inhibition of histamine uptake by reserpine at 1 μmol/l in transfected COS cells having strong VMAT-2 expression. Alternatively, the observation suggests variation in sensitivity of transporter proteins to inhibitors or transporter activity between the cyclic phases, which results in different inhibition patterns in the proliferative and secretory phases. The ovarian steroids, estrogen and progesterone, apparently regulate the expression of monoamine transporters in endometrial cells over the menstrual cycle, directly or indirectly, but they have not been shown to modify transporter sensitivity to inhibitors like corticosterone or estradiol and progesterone, apparently regulate the expression of monoamine transporters in endometrial cells over the menstrual cycle, directly or indirectly, but they have not been shown to modify transporter sensitivity to inhibitors like corticosterone or progesterone.

In situ hybridization demonstrated stromal expression of VMAT-2 mRNA in the proliferative phase only, whereas results from uptake experiments indicate that functional VMAT-2 protein is present also in the secretory phase. This suggests that VMAT-2 protein synthesized in the proliferative phase persists into the secretory phase. Thus, human endometrial stromal cells seem to have functional cell membrane as well as vesicular membrane transporter proteins in both the proliferative and the secretory phases, even though periods of maximal mRNA expression do not overlap. Alternatively, as yet unidentified transporter protein(s) with similar sensitivity to inhibitors contribute to our results. In fact, PMAT, a new monoamine transporter, which shares high sequence and functional similarities to the OCT transporters, has recently been cloned (Engel et al., 2004). Despite low affinity for histamine, PMAT transports histamine at high rate (Engel and Wang, 2005).

Apart from NET mRNA expression, which is mainly found in the late proliferative phase (Bottalico et al., 2003), we were not able to demonstrate mRNA for any other known plasma membrane monoamine transporters in endometrial epithelial cells. NET has higher affinity for dopamine than for norepinephrine, but nothing has been reported on its ability to transport histamine. According to our uptake data, human endometrial epithelial cells appear to have no plasma membrane transporter with affinity for histamine. In accordance with the apparent lack of plasma membrane transporter for histamine in epithelial cells, we found that 3H-histamine was not available to the intracellular vesicular transporter during short-term (15 min) incubations. Prolongation of the incubation period allowed 3H-histamine to accumulate in the cells by diffusion. Under these conditions, pretreatment of secretory, but not proliferative, epithelial cells with reserpine resulted in substantial reduction of accumulated 3H-histamine, thus suggesting the presence of functional VMAT-2 protein.

From the early secretory phase on, no VMAT-2 mRNA-expressing cells were found in the stroma, whereas such cells gradually accumulate in the epithelium (Bottalico et al., 2003). One alternative explanation for this differential location might be that the observed expression of VMAT-2 mRNA in fact relates to migratory cells, which transiently pass the stroma in the proliferative phase migrating to the epithelium. Such cells could be mast cells or macrophages. However, our finding that histamine uptake was inhibited by reserpine in subcultured stromal cells suggests that the stationary cells, not migratory blood derived cells, are the origin of VMAT-2.

Similarities between the decidualization process in the endometrium and major effects of histamine during the inflammatory response have initiated many studies. Decidualization, which is initiated by presence of the conceptus after appropriate priming with estradiol and progesterone, involves oedema and hyperaemia, two classical effects of histamine (Hoffman et al., 1990; Paria et al., 2000; Rockwell et al., 2002). In addition, increased transudation of fluid due to increased capillary permeability at the implantation site in rodents was shown to be dependent on histamine released by the blastocyst (Dey et al., 1979; Dey and Johnson, 1980; White, 1990; Barkai and Kraicer, 1996; Hart, 2001). Thus, it is likely that inflammatory mediators, among them histamine, which are normally released during tissue repair and remodelling, are important mediators of decidualization and implantation. In fact, Barash et al. reported that local injury to the endometrium, caused by taking a biopsy, increased the incidence of implantation in IVF patients (Barash et al., 2003). Also, implantation in rats was induced by histamine when combined with suboptimal doses of estrogen (Johnson and Dey, 1980), and intrauterine application of inhibitors or antagonists to histamine receptors inhibits decidua formation (Shlesnyak, 1952, 1957; Hatanaka et al., 1982).

HDC was detected in endometrial tissue of mice but not rabbits (Dey et al., 1979; Dey, 1981; Paria et al., 1998; Zhao et al., 2000). Nevertheless, intra-luminal injection of α-α-methylhistidine, an inhibitor of HDC, was reported to delay implantation in rabbits (Dey et al., 1979; Dey, 1981). Expression of HDC has not been reported in the human endometrium. However, it should be noted that even minimal synthesis of histamine by mast cells will allow gradual storage in intracellular vesicles in stromal and epithelial cells, making histamine available when release is triggered.

The cellular origin of endometrial histamine is still controversial. Migratory mast cells are a well-known source of histamine and are present in human endometrium and uterine fluid (Casslen et al., 1982). However, some observations suggest that mast cells may not be the origin of endometrial histamine. Implantation progresses normally in genetically mast cell-deficient mice, as well as in rodents treated with a mast cell stabilizer (Hatanaka et al., 1982; Salamonsen et al., 1996). Uptake of histamine from the environment by EMT and VMAT-2 offers an alternative source of endometrial histamine. Even very low concentrations of histamine in blood and extracellular fluids may serve as substrate for the high-affinity transporters in the stationary endometrial cells.

Histamine-releasing factor (HRF) constitutes a heterogeneous group of compounds with identical function but with different modes of action (Budde and Aalberse, 2003). Human embryos were found to produce HRF (Cocchiara et al., 1986), and a recent report detected both mRNA and protein of HRF in non-pregnant human endometrium, mainly in epithelial cells (Oikawa et al., 2003). That study also found that overexpression of HRF accelerates invasive growth of endometrial tissue implants in a nude mouse model of endometriosis. The observation may be taken to indicate that expression of HRF is associated with invasive properties, which may be relevant also for the blastocyst. Embryonic HRF most likely targets endometrial epithelial cells.

If histamine is accumulated in stromal cell vesicles during the proliferative phase, it may be released to the extracellular fluid and subsequently enter epithelial cells in the secretory phase for storage in vesicles. Since VMAT-2 mRNA is expressed in epithelial cells during the secretory phase, and reserpine inhibited uptake in this phase, we propose that histamine is taken up in epithelial vesicles by VMAT-2 and may be released in response to embryonic HRF to stimulate decidua formation and support implantation. Johnson and Dey showed that estrogen-induced implantation was inhibited in mice treated with dexamethasone (Dey, 1981), possibly through inhibition.
of organic cation transporters. Knowledge of the regulation of histamine metabolism in the endometrium will increase the understanding of infertility problems and may offer new pharmacological approaches to optimize assisted reproduction.

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