Monoamine transporters in human endometrium and decidua

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Background:
Monoamines play important roles in decidualization, implantation, immune modulation and inflammation. Furthermore, monoamines are potent vasoactive mediators that regulate blood flow and capillary permeability. Regulation of the uterine blood flow is important both during menstruation and pregnancy. Adequate monoamine concentrations are essential for a proper implantation and physiological development of pregnancy. Unlike most transmitter substances, monoamines are recycled by monoamine transporters rather than enzymatically inactivated. Their intracellular fate is influenced by their lower affinity for inactivating enzymes than for vesicular transporters located in intracellular vesicles. Thus, cells are capable not only of recapturing and degrading monoamines, but also of storing and releasing them in a controlled fashion.

Methods:
The general objective of the present review is to summarize the role of the monoamine transporters in the female human reproduction. Since the transporter proteins critically regulate extracellular monoamine concentrations, knowledge of their distribution and cyclic variation is of great importance for a deeper understanding of the contribution of monoaminergic mechanisms in the reproductive process. MEDLINE was searched for relevant publications from 1950 to 2007.

Results:
Two families of monoamine transporters, neuronal and extraneuronal monoamine transporters, are present in the human endometrium and deciduas.

Conclusions:
New knowledge about monoamine metabolism in the endometrium during menstruation and pregnancy will increase understanding of infertility problems and may offer new pharmacological approaches to optimize assisted reproduction.

Key words: Keywordsdecidua / endometrium / histamine / monoamines

Introduction

Monoamines

Monoamines are neurotransmitters and neuromodulators that contain one amino group connected to an aromatic ring by means of a two-carbon chain (–CH2–CH2–). All, except histamine (H), derive from aromatic amino acids.

Monoamines include:

(i) catecholamines such as epinephrine (E), norepinephrine (NE) and dopamine (DA);
(ii) serotonin or 5-hydroxytryptamine (5-HT), an indolamine;
(iii) histamine.

NE is the principal neurotransmitter in the sympathetic nervous system and together with E is a potent stress hormone released into the bloodstream by the adrenal medulla (Kandel and Schwartz, 1985). H also has a dual role, being a neurotransmitter in the central nervous system (Brownstein et al., 1974; Steinbusch and Mulder, 1984) and a potent peripheral vasoregulator, e.g. when released by mast cells during allergic reactions (White, 1990). DA is a neurotransmitter and in addition a neurohormone released by the hypothalamus. As a hormone, its main function is to inhibit the release of prolactin from the anterior lobe of the pituitary.
As neurotransmitters, monoamines take part in a wide variety of processes such as modulation of motor functions, arousal, attention, mood and anxiety. Neuroendocrine cells in the gastrointestinal and respiratory tracts also contain monoamines, which play important paracrine roles in these organs (Axelrod, 1971).

Monoamines are among the earliest neurotransmitters to be detected during human fetal brain development (Olson et al., 1973). Since monoamines and their synthesizing enzymes are present before the nerve terminal areas are developed, their early role differs from their role in the synopsis.

5-HT is the first monoamine to appear in the fetal rat brain and has been detected at embryonic Day 12, followed by DA on Day 13 and NE on Day 14 (Olson and Seiger, 1972; Olson et al., 1973).

During development, monoamines regulate morphogenesis through cell differentiation and migration; some have also been shown to release trophic factors and induce trophic effects (Hansson et al., 1998). In particular, 5-HT plays a role in early gastrulation, induction of neurogenesis and neural differentiation, formation of the neural tube and migration of cranial neural crest cells. At a later stage, it inhibits synaptogenesis and causes release of neuronal growth factors (Buznikov, 1984; Marshak, 1990; Whitaker-Azmitia et al., 1990; Fox, 1995; Moiseiwitsch and Lauder, 1995; Whitaker-Azmitia et al., 1996).

Synthesis of catecholamines is mediated by two enzymes: tyrosine hydroxylase, which is a rate-limiting enzyme and converts tyrosine to DA, and DA beta hydroxylase that converts DA to NE. NE is then converted to E by phenylethanolamine-N-methyltransferase. 5-HT is synthesized from tryptophan by tryptophan hydroxylase. Histidine decarboxylase (HDC) is the rate-limiting enzyme for H biosynthesis.

Neuronal monoamine degradation is mediated by monoamine oxidase (MAO), and the extraneuronal catabolism of 5-HT, NE and DA is mediated by MAO-A, and of NE, E and DA by catechol-O-methyltransferase (COMT) (Eisenhofer, 2001). In addition, both MAO-A and MAO-B are found in various peripheral organs and therefore contribute to extraneuronal monoamine catabolism. H is degraded by histamine-N-methyltransferase and diamine oxidase.

Unlike most transmitter substances, monoamines are recycled rather than enzymatically inactivated, and over 70% of recaptured catecholamines are recycled into storage vesicles rather than being deaminated (Eisenhofer et al., 2004). Their intracellular fate is influenced by their lower affinity for inactivating enzymes than for vesicular transporters located in the membranes of intracellular vesicles (Bremens and Eiden, 1993; Nirenberg et al., 1995). Thus, such cells are capable not only of capturing and degrading monoamines, but also of reuptake, storage and release.

**Monoamines in human reproduction**

**Uterus**

The uterus has an extensive innervation of sympathetic neurons and the adrenergic nerve fibers play a role in uterine contractility (Amenta et al., 1992). Selective vascular constriction has been shown for 5-HT in pregnant uterine arteries (Lang et al., 1993), and it induces collagenase production in isolated uterine smooth muscle cells, which may be a mechanism involved in the massive collagen degradation in the uterus post-partum (Jeffrey et al., 1991; Wilcox et al., 1992).

**The endometrium**

Enzymes involved in monoamine-synthesis have been demonstrated in normal endometrium as well as in early pregnancy deciduas (Mangona et al., 1998). Local synthesis of monoamines may play an important physiological role.

Adequate monoamine concentrations are essential for a proper implantation and physiological development of pregnancy. Many factors are involved in this regulation. For example, progesterone stimulates COMT and MAO activities in endometrium and decidua, which increase degradation of catecholamines (Hobble et al., 1981).

Prostaglandins are of crucial importance for implantation and survival of the blastocyst. Their synthesis can be modulated by NE, DA and H (Schrey et al., 1995; Yanagawa et al., 1997; Skarzynski et al., 1999).

Monoamines are potent vasoactive mediators that regulate blood flow and, in the case of H, capillary permeability (Kraicer, 1996). Regulation of the uterine blood flow is important both during menstruation and pregnancy.

5-HT and H play a role in decidualization, implantation and, in the case of H, in immunomodulation (Dey, 1981; Hatanaka et al., 1982; Mitchel et al., 1983; Cocchiara et al., 1986; Maekawa and Yamanouchi, 1996, 1223). 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, 1303). Thus, it is likely that inflammatory mediators, including H (Beer et al., 1984), which normally released during tissue repair and remodelling function as mediators of decidualization and implantation. Implantation in rats was also induced by H when combined with suboptimal doses of estrogen (Johnson and Dey, 1980), while intrauterine application of inhibitors or antagonists to H receptors inhibits decidua formation (Shelesnyak, 1952, 1957; Hatanaka et al., 1982).

In mice, the rate-limiting enzyme in H synthesis, HDC, has been observed in uterine epithelial cells with peak expression at the time of implantation (Paria et al., 1998).

**Monoamine transporters**

Since monoamines are potent mediators of physiological and pathophysiological events throughout the body, their extracellular concentrations are tightly regulated. Unlike other transmitter substances, monoamines are recycled rather than enzymatically inactivated. Specific membrane-bound transporter proteins mediate reuptake of monoamines from the synaptic cleft or extracellular fluids (Amara and Kuhar, 1993; Brownstein and Hoffman, 1994) and they accumulate intracellularly.

Monoamine transporters can be grouped into three main families, two in the cell membranes and one in vesicular membranes

(i) neuronal monoamine transporters;
(ii) non-neuronal monoamine transporters;
(iii) vesicular monoamine transporters (VMATs).

**Neuronal monoamine transporters**

The presence of an active transport system for NE uptake at sympathetic nerve endings was first suggested in early 1960 (Whitby et al., 1961; Axelrod, 1971). Chemical signalling by neural cells is terminated
by the reuptake of the monoamine from the synaptic cleft by the pre-
synaptic neuron and at extrajunctional sites. In the last decade, several
genes coding for transporter proteins have been cloned and character-
ized including the transporters for 5-HT (SERT), NE (NET) and DA
(DAT) (Blakely et al., 1991; Kilty et al., 1991; Usdin et al., 1991;
Amara and Kuhar, 1993; Erickson and Eiden, 1993; Hoffman, 1994;
Borowsky and Hoffman, 1995; Chang et al., 1996) (Table I). An E
transporter has been cloned in the bullfrog (Apparsundaram et al.,
1997), but no such transporter has been described in mammals in
general or humans in particular.

To date, no specific membrane transporter for H has been found.
It was subsequently discovered that these transporter proteins are
not restricted to the nervous system, but occur in other cell types
such as DAT in the 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).

Both SERT (Balkovetz et al., 1989) and NET (Ramamoorthy et al.,
1993b) are expressed in the placenta (Table II).

### Non-neuronal monoamine transporters

#### Organic cation transporters

It has been known for many years that another family of plasma membran
transporters, the organic cation transporters (OCT), is responsible for the so-called type 2 uptake of monoamines (Iversen, 1965). OCTs, also known as non-neuronal membrane transporters, were only recently cloned (Gorboulev et al., 1997; Koehler et al., 1997; Zhang et al., 1997; Grundemann et al., 1998). Unlike neuronal monoamine transporters, which mainly regulate synaptic levels of monoamines, OCTs play a key role in the clearance of monoamines from the bloodstream. Also in contrast to the neuronal transporters, OCTs have a broad affinity for all biogenic amines as well as exogenous drugs, xenobiotics and organic anions and cations (Grundemann et al., 1999; Wieland et al., 2000; Eisenhofer, 2001; Hayer-Zillgen et al., 2002; Koepsell, 2004). Compared to neuronal monoamine transporters, OCTs have 12 putative transmembrane domains (TMDs), a lower affinity for catecholamines (i.e. higher $K_{m}$), favour E over NE, and exhibit a higher maximum rate of catecholamine uptake (i.e. higher $V_{max}$). In addition, uptake is not an Na$^{+}$- and Cl$^{-}$- dependent process (Eisenhofer, 2001) (Table I).

<table>
<thead>
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<th>Table I  Substrate specificity and inhibitors of monoamine transporters</th>
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<td><strong>Principal substrates and specificity</strong></td>
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<td>Neuronal transporters</td>
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<td>NET</td>
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<td>DAT</td>
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<td>SERT</td>
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<td>Non-neuronal transporters</td>
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<td>OCT1</td>
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<td>OCT2</td>
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<td>EMT (OCT3)</td>
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<td>PMAT</td>
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MPP$^+$, 1-methyl-4-phenylpyridinium; E$_2$, 17β-estradiol; P, progesterone 17β-estradiol; NMN, normetanephrine; MN, metanephrine; Parg., pargyline, a monoamine oxidase inhibitor; SSRI, selective serotonin reuptake inhibitor.

<table>
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<th>Table II Regional expression of monoamine transporters (Eisenhofer, 2001, modified)</th>
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<tr>
<td><strong>Neuronal transporter</strong></td>
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<tr>
<td>NET</td>
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<td>DAT</td>
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<td>SERT</td>
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<td>VMAT1</td>
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<td>VMAT2</td>
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OCTs include OCT1, OCT2 and OCT3. OCT 3 is identical to the ‘extra-neuronal monoamine transporter’ (EMT) since their functional properties match those of the corticosterone-sensitive catecholamine transport system (Eisenhofer, 2001). In humans, non-neuronal monoamine transporters are mainly expressed by the liver (OCT1, EMT), intestine (OCT1), kidney and brain (OCT2, EMT). EMT has a broad tissue distribution and is also found in the heart, blood vessels, placenta and retina (Eisenhofer, 2001) (Table II).

Their pharmacological properties have recently been analysed in stably transfected cell lines (Hayer-Zillgen et al., 2002). OCTs 1 and 2 as well as EMT are greatly inhibited by corticosterone, progesterone and 17β-estradiol (Hayer-Zillgen et al., 2002) (Table I).

**Plasma membrane monoamine transporter**

Yet another non-neuronal monoamine transporter, the plasma membrane monoamine transporter (PMAT), which has functional similarities to the OCT transporters, has recently been cloned and characterized (Engel et al., 2004; Engel and Wang, 2005). PMAT is a protein characterized by 11 TMDs and in common with OCTs, its uptake is Na+-dependent (Engel et al., 2004). A peculiarity of this transporter is its ability to function bidirectionally (Engel and Wang, 2005). Despite the fact that PMAT belongs to the equilibrative nucleoside transporter (ENT) family, it reportedly functions as a polyspecific OCT (Engel and Wang, 2005). Unlike ENT1–3, which exclusively transport nucleosides, PMAT (or ENT4) has a low-affinity and high-capacity for monoamines (Table I). In particular, H is transported with a low affinity (i.e. high Km), but efficiently (high Vmax). The best substrate is 1-methyl-4-phenylpyridinium (MPP+), which is also the case for OCTs (Km 1/433 mM). In humans, PMAT mRNA is strongly expressed in the brain and skeletal muscle, but transcripts are also found in the liver, kidney and heart (Engel et al., 2004, 1454) (Table II). Nicotine is a strong inhibitor of PMAT. In general, most inhibitors exhibited similar or close inhibitory potencies towards PMAT and OCTs, with the exception of corticosterone, which inhibits low-affinity PMATs (Table I).

**Vesicular monoamine transporters**

In the cytoplasm, monoamines are further concentrated in vesicles by another set of transporter proteins, i.e. the VMATs (Peter et al., 1995a,b). Two VMAT forms have been cloned, VMAT1 and VMAT2 (Peter et al., 1995b). They exhibit different anatomical distribution; VMAT2 is mainly expressed in monoaminergic cell groups of the CNS (Hoffman et al., 1991; Usdin et al., 1991) and adrenal medulla (Erickson et al., 1992, 1996), whereas VMAT1 is mainly found in the adrenal medulla and neuroendocrine cells of the gastrointestinal tract (Weilhe et al., 1994).

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.

**Monoamine transporters, selective serotonin reuptake inhibitor and drug addiction**

Neuronal monoamine transporters have been of particular interest because of their central role in modern treatment of depression and anxiety by means of selective serotonin reuptake inhibitor (SSRI) (Pacholczyk et al., 1991; Tatsumi et al., 1997).

Antidepressants and psychoactives affect monoamine transporters enhancing serotoninergic, noradrenergic or dopaminergic neurotransmission by binding to the corresponding transporters SERT, NET and DAT, thereby inhibiting neurotransmitter reuptake and raising active levels in the synapse. Examples include fluoxetine, an SSRI; reboxetine, an NE reuptake inhibitor and bupropion, which inhibits both NET and DAT transporter.

Monoamine transporters are also involved in mechanisms associated with drug addiction (Schuldiner et al., 1993). Cocaine and amphetamine block the uptake of monoamines in a similar way to that of SSRI, leading to increased levels of extracellular monoamines.

The euphoric and addictive properties of amphetamine, methamphetamine and cocaine are considered to derive from their potent DAT-blocking activity.

**Normal human endometrium**

The stroma is characterized by a dynamic leukocyte population. Endometrial leukocytes include T and B cells, mast cells, macrophages, neutrophils and the uterine natural killer (uNK) cells. These cells vary across the menstrual cycle. The uNK cells are mainly present in the late secretory phase and early pregnancy; macrophages are always present but increase in number in the mid-late secretory phase and in decidua; mast cell distribution does not vary during the cycle, although the cells are activated in the late secretory phase, prior to menstruation (Poropatich et al., 1987). Mast cells are a well-known source of H and could contribute to the H pool present in the endometrium.

**Decidualization, implantation and placentation**

Monoamines in general and H in particular have been shown to play a role in the process of decidualization of endometrial tissue in the secretory phase (Dey et al., 1979; Dey, 1981; Barkai and Kraicer, 1996). However, in mast cell-deficient mice, deciduomata formation still occurred, suggesting an H source other than mast cells (Hatanaka et al., 1982). In mice HDC was localized to epithelial cells preceding implantation and decreased after initiation of implantation, suggesting an important role for locally synthesized H during implantation (Paria et al., 1998). An embryonic H-releasing factor has been described (Cocchiara et al., 1986). Local release of stored H, triggered by a factor released by the embryo, could be a mechanism to prevent maternal immuno-rejection at the implantation site and/or increase the blood flow locally (Barkai and Kraicer, 1996). A cross-talk between the decidua and the embryo occurs. The blastocyst expresses an H type 2 receptor (H2), which is the target for H (Zhao et al., 2000). After implantation, the placenta develops by trophoblastic cells invading the endometrium and the maternal vasculature. H enhances the invasion by activating the H type 1 (H1) receptor on the cytotrophoblasts (Liu et al., 2004).

The general objective of the present review was to summarize our knowledge of monoamine transporters in the female reproductive tract. Since these transporter proteins critically regulate extracellular concentrations of very potent signalling molecules, knowledge of their distribution and cyclic variation is of great importance for...
Materials and Methods

Search methods
This review includes medical papers published in the English language on monoamines and their transporters. Search for publications have been made using MEDLINE, combining medical subject heading terms: monoamines, serotonin, E, H and DA, transporters, endometrium, decidua and placenta. All pertinent articles were retrieved and selected through systematic review. Relevant references to December 2007 and dating back to 1950 have been included.

Experimental methods

Patients and tissue sampling
Tissue was collected at the Department of Obstetrics and Gynaecology, Lund University Hospital, Lund after informed consent. The study was approved by the Research Ethical Committee Review Board for studies in human subjects at Lund University.

Endometrial tissue was collected from healthy women, under 45 years of age, who were undergoing hysterectomy or diagnostic curettage for benign reasons unrelated to endometrial dysfunction. Each sample was evaluated by a histopathologist in order to exclude endometrial pathology and identify the cyclic phase (Noyes et al., 1950; Hendrickson and Kempson, 1980). Decidual tissue was collected from first trimester elective abortions.

Placental tissue was collected from pre-eclamptic and normal pregnancies. The controls were matched for maternal age and parity. Patients with essential hypertension and renal or other systemic diseases were excluded.

Tissue samples were taken from the placenta immediately after delivery and, in the case of Caesarean section (29% in the control and 50% in the pre-eclamptic groups), from the uterine wall. Placenta bed biopsies were collected from the uterine wall during Caesarean section in order to study gene expression in the modified spiral arteries.

In situ hybridization histochemistry
RNA probes of ~400–560 nucleotides were used for the human SERT, NET, DAT, VMAT1, VMAT2, OCT1, OCT2, EMT, PMAT mRNAs (Pacholczyk et al., 1991; Erickson and Eiden, 1993; Ramamoorthy et al., 1993a; Uhl and Kitayama, 1993; Erickson et al., 1996; Gorboulev et al., 1997; Koehler et al., 1997; Zhang et al., 1997; Grundemann et al., 1998; Engel et al., 2004). Hybridization was done as previously described (Bradley et al., 1992; Bottalico et al., 2003, 2007).

Real-time PCR amplification
Primers and probes were designed using Assays on-Design/Demand™ (Applied Biosystems). Analysis were done as previously described (Bottalico et al., 2003, 2007).

Tissue culture
Endometrial tissue was obtained under sterile conditions, disintegrated, and cell fractions prepared as previously described (Noskova et al., 2006). Overnight incubation allowed glands to attach to the plastic, preserving cell polarized morphology and hence the functional integrity of the glands.

Results and discussion
Human endometrial tissue expresses mRNA for several monoamine transporters, mainly the neuronal cell membrane transporter NET in late proliferative epithelium (Fig. 1), the extraneuronal cell membrane...
transporters EMT in secretory stroma (Fig. 2), PMAT in proliferative stroma (Fig. 3) and the vesicular membrane transporter VMAT2 in proliferative stroma and secretory epithelium (Fig. 4) (Bottalico et al., 2003, 2007). The DAT, SERT, OCT1 and OCT2 mRNA species were detected either in very low concentrations or only in sporadic cells. VMAT1 mRNA was not detected at all. Furthermore, primary cultures of endometrial stromal cells showed specific uptake of H, indicating the presence of functional transporter proteins (Noskova et al., 2006). The highest affinity for H has been ascribed to EMT and VMAT2 (Erickson et al., 1992; Merickel and Edwards 1995; Grundemann et al., 1999). Susceptibility of the uptake to corticosterone suggests the presence of functional PMAT and/or EMT, whereas susceptibility to reserpine is indicative of functional VMAT2 protein. Differences in expression pattern and localization for the various transporters imply different roles in endometrial biology as well as the reproductive process.

Figure 2 Bright (A) and darkfield (B) images of in situ hybridization for EMT mRNA in secretory endometrial tissue. EMT mRNA localized to the stroma, and not in the epithelium. Intensity of the expression increased during the proliferative phase reached a peak in the early secretory phase, and then decreased gradually during the secretory phase. There was no expression in the menstrual phase. This pattern was confirmed by real-time PCR. Expression of EMT mRNA was low in decidual tissue. Immuno-histochemistry showed stromal distribution of EMT protein that matched the distribution of mRNA, and also a weak staining in epithelial cells (not shown). EP, early proliferative; MP, mid-proliferative; LP, late proliferative; ES, early secretory; MS, mid-secretory; LS, late secretory; M, menstrual; DE, decidua. Adapted from Bottalico et al. with permission (Bottalico et al., 2007).

Figure 3 Bright (A) and darkfield (B) images of in situ hybridization for PMAT mRNA in proliferative endometrial tissue. PMAT mRNA localized in the stroma. No signal was seen in epithelial cells. Intensity was high in the proliferative phase and low in the secretory and menstrual phases. Real-time PCR confirmed this pattern, including a significant difference between the proliferative and secretory phases. It was not detected in early pregnancy decidua. EP, early proliferative; MP, mid-proliferative; LP, late proliferative; ES, early secretory; MS, mid-secretory; LS, late secretory; M, menstrual; DE, decidua. Adapted from Bottalico et al. with permission (Bottalico et al. 2007).

Proliferative stroma
The proliferative phase is characterized by endometrial growth, and such growth involves paracrine signalling between the various cell types. Our knowledge of monoamines in endometrial tissue during the proliferative phase is scanty, but differential expression of several monoamine transporters in the stroma suggests that cellular uptake and/or release of monoamines is important. None of the neuronal monoamine transporters had detectable amounts of mRNA in the stroma during this phase. Presence of the extraneuronal cell membrane transporter OCT2 mRNA in isolated scattered cells suggests expression related to some migratory cell type. Owing to very few cells, this expression did not show in the real-time PCR results. The situation with OCT1 was the opposite. Low levels of mRNA, which were detected by real-time PCR throughout the cycle, may represent weak diffuse gene expression, which, however, could not be detected with in situ hybridization.

In contrast, the proliferative stroma has significant expression of the other extraneuronal cell membrane transporters EMT and PMAT, as well as the VMAT2. PMAT has the highest expression in the proliferative phase, whereas EMT peaks in the secretory phase. Despite lower affinity for H, PMAT transports H at a high rate (Engel and Wang, 2005). Our functional study showed that uptake of radiolabelled H in
endometrial stromal cells obtained in the proliferative phase was inhibited by corticosterone in a gradual dose-dependent way (Noskova et al., 2006). This is consistent with reports that corticosterone is a low-affinity inhibitor of PMAT (Grundemann et al., 1998; Hayer-Zillgen et al., 2002). Corticosterone is also a low-affinity inhibitor of OCT2, but in case this transporter is associated with migratory cells within the proliferative stroma, neither the cells nor the OCT2 transporter is likely to be present in the stromal cell cultures, since most migratory cells do not attach and are washed away. The in vitro experiments with proliferative stromal cells showed inhibition of H uptake by reserpine, an inhibitor of VMAT2-mediated monoamine uptake, thus verifying our in situ hybridization results.

In situ hybridization as well as real-time PCR demonstrated mRNA for both PMAT and VMAT2 in the proliferative stroma, and uptake experiment confirms the existence of functional transporter proteins. Thus, endometrial stromal cells are capable of uptake, storage and regulated release of monoamines. The stromal content of mRNA for both PMAT and VMAT2 peaks in the late proliferative phase. Such timing usually suggests a function at midcycle. An additional fact to consider is that PMAT has a bidirectional function (Engel and Wang, 2005), which allows for a regulated release of monoamines from cells. Thus, VMAT2, which can release monoamines from the vesicles, and PMAT, which can release monoamines from the cell, may cooperate to deliver the vesicular content of monoamines to the pericellular environment. It is tempting to speculate that at midcycle some monoamines, maybe H, could be released from stromal cells through this mechanism, and diffuse to the uterine cavity with the potential to influence sperm migration. Such transport through the epithelium has, however, not been demonstrated for monoamines, but occurs with other compounds, e.g. a protease inhibitor TIMP-4 (Pilka et al., 2006).

**Proliferative epithelium**

In situ hybridization revealed that strong expression of NET mRNA was exclusively found in epithelial cells, and specifically in the late proliferative phase. A weaker expression was detected also in other parts of the cycle with real-time PCR. This pattern of NET expression suggests a role in the epithelium at midcycle, i.e. at the time of sperm migration. It is possible that NET mediates uptake of NE or DA from the uterine fluid. In vitro uptake of NE by human endometrial tissue has in fact been demonstrated (Pedroza-Garcia et al., 1975). Uptake by NET is enhanced by estradiol and modulated by cocaine, and animal experiments have shown glandular epithelial cell uptake of NE to have these characteristics (Alm et al., 1975; Declercq de Perez Bedes and Garcia Bienere, 1975; Kennedy and de la Lande, 1986). The fact that DAT mRNA was not detected in the human endometrium does not rule out DA as a transmitter, since NET actually has a higher affinity for DA than for NE (Pacholczyk et al., 1991; Eisenhofer, 2001). Proliferative epithelial cells had no expression of any plasma membrane transporter with affinity for H, and did not show any uptake of radiolabelled H in standard short-term experiments (Noskova et al., 2006). Also, there was no expression of any vesicular transporter, which was also born out by our functional study.

**Secretory stroma**

EMT mRNA is mainly expressed in the secretory phase, whereas PMAT mRNA is more abundant in the proliferative phase. Even though EMT has higher affinity for H than PMAT, basal uptake of radiolabelled H occurred in both secretory and proliferative stromal cells (Noskova et al., 2006). This is probably related to the fact that PMAT transports H at high rate, despite the lower affinity for H (Engel and Wang, 2005).

The functional study showed that the uptake of radiolabelled H was inhibited by corticosterone in stromal cells obtained in the secretory as well as in the proliferative phase (Noskova et al., 2006). However, the pattern was different, i.e. full inhibition was achieved in secretory phase cells already with the lowest concentration of corticosterone, 10 µmol/L. This difference between proliferative and secretory stromal cells is likely to relate to the different sensitivities of PMAT and EMT to corticosterone. Since corticosterone is a high-affinity inhibitor of EMT, full inhibition is achieved by the lowest concentration in secretory phase stromal cells, whereas increasing concentrations of corticosterone resulted in gradually increasing inhibition of uptake in proliferative phase when PMAT had highest expression. In addition, OCTs including EMT as well as PMAT are sensitive to 17β-estradiol and progesterone (Hayer-Zillgen et al., 2002; Engel and Wang, 2005), and the cyclic variation of these
hormones may modulate EMT or PMAT-mediated uptake differently in the proliferative and secretory phases.

H uptake was inhibited by reserpine in secretory stromal cells to a similar extent as in proliferative stromal cells. However, according to the in situ hybridization results, stromal cells expressed VMAT2 mRNA only in the proliferative and not in secretory phase. This observation suggests either that VMAT2 protein synthesized in the proliferative phase persists into the secretory phase or that a yet unidentified vesicular transporter protein with similar sensitivity to reserpine is expressed in this phase. We noted in functional experiments that the uptake of H was less sensitive to inhibition by reserpine in the secretory than in the proliferative phase (Noskova et al., 2006). This could be due either to a lower concentration of VMAT2 protein in the secretory phase or to a variation in sensitivity of the transporter proteins to inhibitors.

In conclusion, endometrial stromal cells have functioning transporters both in the cell membrane and in the vesicular membranes throughout the menstrual cycle.

**Secretory epithelium**

Interestingly, secretory phase epithelium showed no expression of any plasma membrane transporter, which would accompany the strong expression of VMAT2. Confirming this, epithelial cells did not show any uptake of radiolabelled H in standard short-term experiments (Noskova et al., 2006). An increase of VMAT2 mRNA throughout the secretory phase suggests that a gradual accumulation of monoamines may take place in the epithelial cells. If H is accumulated in secretory stromal cells, it may be released to the extracellular fluid and subsequently enter epithelial cells in early secretory phase for storage in vesicles. H can enter the epithelial cells by diffusion and subsequently be accumulated in vesicles, since we found that prolonged incubation of secretory epithelial cells with \(^{3}H\)-histamine, resulted in cellular uptake, which was inhibited by reserpine (Noskova et al., 2006). Hypothetically, H stored in epithelial vesicles by VMAT2 could be released in response to embryonic stimuli, e.g. H-releasing factor (Cocchiara et al., 1986), in order to stimulate decidua formation and thus support implantation.

**Menstrual endometrium**

Virtually, no species of transporter mRNA were expressed in endometrial samples from the menstrual phase. Apparently, the need for further production of transporter proteins is no longer present in this degrading tissue. However, since regulation of uterine blood flow is important during menstruation, and monoamines in general are potent vasoactive agents, monoamines, which have been accumulated in endometrial vesicles by VMAT2 during the secretory phase, can be released during tissue disintegration and play a role for vasoconstriction of the uterine spiral arteries, as a measure to control bleeding.

**Early pregnancy decidua**

Occasionally, in situ hybridization identified mRNA for DAT and SERT in single cells in early pregnancy decidua. This appearance suggests localization in migratory cell types within the tissue. Also, the sporadic pattern makes the physiological significance of these two transporters doubtful in early pregnancy decidua. Doubtful significance is also the case for OCT1, whose mRNA was not detected by in situ hybridization, but was demonstrated in very low levels with real-time PCR in early pregnancy decidua. The content of EMT mRNA was on similar level in decidual tissue as in early proliferative phase tissue. Thus, EMT is the only cell membrane transporter, which seems to be expressed at a significant level in early pregnancy decidua.

Neither of the mRNA species for vesicular transporters VMAT1 and VMAT2 were detected in decidual tissue. Apparently, the need for reuptake of monoamines in decidual tissue is limited, at least when the pregnancy is 6–10 weeks old.

**Decidualization and implantation**

Numerous studies suggest that monoamines, H in particular, play a role during decidualization of endometrial tissue in the mid-late secretory phase of fertile cycles, although exact mechanisms are not known (Dey et al., 1979; Dey, 1981; Barkai and Kraicer, 1996). In fact, early reports claim a role for H not only in decidualization, but also in implantation, placenta formation, immune modulation and blood flow regulation (Dey, 1981; Hatanaka et al., 1982; Cocchiara et al., 1986; Barkai and Kraicer, 1996). Decidualization, which is initiated by the presence of a conceptus after appropriate priming with estradiol and progesterone, involves edema and hyperaemia, two classical effects of H (Hoffman et al., 1990; Paria et al., 2000; Rockwell et al., 2002). Furthermore, estrogen-induced implantation in mice was inhibited by dexamethasone (Dey, 1981), an effect that may involve inhibition of H uptake by steroid-sensitive monoamine transporters.

In mice, the rate-limiting enzyme in H synthesis, HDC, has been demonstrated in endometrial epithelial cells with peak expression at the time of implantation (Paria et al., 1998), and the blastocyst expresses a target receptor, i.e. H2 (Zhao et al., 2000). The cellular origin of human endometrial H is still controversial. HDC, the rate-limiting enzyme that would indicate H synthesis, has not been reported in human endometrium. Migratory mast cells are a well-known source of H, and they occur in human endometrium and uterine fluid (Casslen et al., 1982). Uptake of H from the environment by EMT, PMAT and VMAT2 offers an alternative source of endometrial H in humans. Even very low concentrations of H in blood and extracellular fluids may serve as substrate for the high affinity transporters in stationary endometrial cells. Following implantation, the placenta begins to develop by trophoblastic cell invasion of the endometrium and the uterine spiral arteries. H can potentially modulate invasion by activating H1 receptors on the cytotrophoblastic cells (Liu et al., 2004). Being a potent vasoactive agent, H can also modulate local blood flow and capillary permeability (Barkai and Kraicer, 1996).

**Detoxification**

The uterine cavity ideally represents the environment which is optimal for implantation and survival of the blastocyst. However, environmental toxins have been shown to affect both preimplantation embryos and embryonic growth (Godfrey, 2002). EMT and PMAT may represent a mechanism whereby the uterine cavity can ensure the optimal and toxin-free milieu, which is a prerequisite for normal programming of the conceptus. Both EMT and PMAT contribute strongly to the removal of catecholamines from extracellular fluids (Eisenhofer, 2001; Engel et al., 2004; Engel and Wang, 2005). In addition, they mediate...
cellular uptake of a wide variety of noxious substances, in particular neurotoxins. They have a broad tissue distribution, especially in toxin-eliminating organs such as liver, kidney and placenta (Hayer-Zillgen et al., 2002; Engel et al., 2004). Impaired detoxification of xenobiotics has actually been associated with both endometriosis and malignant transformation (Baxter et al., 2001). Thus, EMT and/or PMAT may have a protective function in the non-pregnant endometrium, at implantation, as well as during pregnancy.

High levels of stress hormones, such as corticosterone, also have a negative impact on reproduction. Since corticosterone is an inhibitor of EMT (Hayer-Zillgen et al., 2002) and PMAT (Engel and Wang, 2005), our results suggest a possible cellular mechanism, which could explain stress-related impairment of implantation due to excessive levels of H. Nicotine is a strong inhibitor of PMAT, a fact that could be relevant to the higher rate of miscarriages as well as the lower birthweight in smokers compared with non-smokers (Shiverick et al, 1999).

SSRIs are widely used in modern treatment of depression and anxiety (Pacholczyk et al., 1991; Tatsumi et al., 1997). Lately, new indications for SSRI are introduced into clinical practice, as aid to quit smoking (Foley et al., 2006), neuropathic pain (Matsuzawa-Yanagida et al., 2008) and treatment of premenstrual symptoms (Shah et al., 2008) are some examples. The use of SSRI in first trimester pregnancy increases the risk of miscarriage, but without complications of major malformations (Pastuszak et al., 1993). Since many women, in fertile age, are using SSRI, the effects on the endometrium ought to be further studied.

Cocaine and amphetamine block the uptake of monoamines in a similar way to that of SSRI (Schuldiner et al., 1993). Cocaine abuse during pregnancy has been well studied, both from a neurodevelopmental perspective (Meyer et al., 1993; Leslie et al., 1994; Battaglia et al., 1995) and regarding placenta function (Pacholczyk et al., 1991; Ramamoorthy et al., 1993b; Prasad et al., 1994). Cocaine also has negative effects on the pregnant uterus, increased miscarriage rate as well as premature delivery have been described for this drug but the underlying mechanisms are not fully understood (Woods, 1998).

### Blood flow regulation during pregnancy

Regulation of uterine blood flow is critical during pregnancy when blood flow increases remarkably. Since monoamine transporters regulate extracellular concentrations of monoamines, they are likely to play a role in maintaining homeostasis in the fetoplacental circulation, which is crucial for fetal development (Ganapathy et al., 1993; Prasad et al., 1994; Ganapathy and Leibach, 1995). Monoamine transporters may represent a protective mechanism against fluctuating levels of vasoactive substances by uptake from both the maternal and the fetal circulation, thereby controlling vascular tonus and placental blood supply. On the basis of the anatomical distribution of monoamine transporters in the uterine wall and placenta, we suggest that the transporters form three lines of defence against inappropriate monoamine concentrations (Bottalico et al., 2004).

The first line of defence consists of trophoblastic cells lining the spiral arteries closest to the myometrium. These cells express mRNA for both NET and VMAT2, indicating that they are capable of both uptake and regulated release. Controlling monoamine concentrations at this levels offers an approach to regional control of blood flow.

The second line of defence is represented by numerous trophoblastic cells, which surround the entrance of the spiral arteries of the decidua. These cells express high levels of NET mRNA, and can represent a mechanism to clear the maternal blood of excessive amounts of NE. Laporte and DeRoth demonstrated changes in the contractile response of the porcine uterine artery by inhibiting NE uptake in early pregnancy (Laporte and DeRoth, 1997). Therefore, NET may have a protective role in preventing sympathetic hyperactivity from reducing uterine blood flow. In fact, we have, in an animal model, recently shown increased fetal blood pressure after pharmacological inhibition of NET (Hansson et al., 2001). The third line of defence is made up of OCT, EMT in particular, which are located in elongated cells in intralobular septa and in the extravascular stroma of larger vessels. Their location suggests that they may be myofibroblasts. The ability of these cells to contract villi is important for placental auto-regulation of blood flow (Demir et al., 1997).

### Conclusions

This review highlights new knowledge about monoamine metabolism in the endometrium during the menstruation cycle and early pregnancy. Monoamines in general, and H in particular, appear to be important players in reproduction. The knowledge about monoamine transporters may increase understanding of infertility problems and may offer new pharmacological approaches to optimize assisted reproduction.

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