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Serotonin localization and its functional significance during mouse preimplantation embryo development

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Summary

Serotonin is a neurotransmitter functioning also as a hormone and growth factor. To further investigate the biological role of serotonin during embryo development, we analysed serotonin localization as well as the expression of specific serotonin 5-HT1D receptor mRNA in mouse oocytes and preimplantation embryos. The functional significance of serotonin during the preimplantation period was examined by studying the effects of serotonin on mouse embryo development. Embryo exposure to serotonin (1 μM) highly significantly reduced the mean cell number, whereas lower concentrations of serotonin (0.1 μM and 0.01 μM) had no significant effects on embryo cell numbers. In all serotonin-treated groups a significant increase in the number of embryos with apoptotic and secondary necrotic nuclei was observed. Expression of serotonin 5-HT1D receptor mRNA in mouse oocytes and preimplantation embryos was confirmed by *in situ* hybridization showing a clearly distinct punctate signal. Immunocytochemistry results revealed the localization of serotonin in oocytes and embryos to the blastocyst stage as diffuse punctate cytoplasmic labelling. It appears that endogenous and/or exogenous serotonin in preimplantation embryos could be involved in complex autocrine/paracrine regulations of embryo development and embryo-maternal interactions.

Keywords: Embryo, 5-HT1D receptor, Oocyte, Preimplantation development, Serotonin

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter which also functions as a hormone and growth factor and is implicated in a large array of physiological and pathological pathways. Evolutionarily, serotonin existed in plants even before the appearance of animals. Serotonin may be tied to the evolution of life itself, particularly through the role of tryptophan, its precursor molecule. In animal cells serotonin alters the cytoskeleton of cells and influences the formation of contacts. In addition, serotonin regulates cell proliferation, migration and maturation in a variety of cell types (Azmitia, 2001).

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The majority of serotonin cellular activities are mediated through receptors which belong to the group of G-protein coupled receptors, except for the 5-HT3 receptor, which is a ligand-gated ion channel. Serotonin receptors are classified into seven different families depending on their signalling pathways. The family including 5-HT1 and 5-HT5 receptors interacts negatively with adenylyl cyclase; the 5-HT2 receptor family is coupled to the activation of phospholipase C; and the family including 5-HT4, 5-HT6 and 5-HT7 receptors activates adenylyl cyclase (Hoyer *et al*., 2002).

Serotonin has been shown to play a role in cell proliferation. Recent testing for functional activity of serotonin (10^{-14} to 10^{-10} M) has shown variable effects depending on cell type and culture conditions. Serotonin stimulated proliferation of melanocytes in a medium deprived of growth factors, while inhibiting cell growth in the presence of growth factors (Slominski *et al*., 2003). Serotonin caused a dose-dependent increase in DNA synthesis in primary cultures of rat hepatocytes in the presence of epidermal

growth factor (EGF) and insulin. The serotonin antagonists, ketanserin (1 μM) and spiperone (1 μM), blocked stimulation of DNA synthesis by serotonin (Balasubramanian & Paulose, 1998).

Serotonin and its receptors have been found both in the central and peripheral nervous system, as well as in various non-neural tissues: in the gut, cardiovascular system, blood and reproductive tract, including human ovaries and placenta (Itoh *et al*., 1999; Huang *et al*., 1998). High levels of serotonin were detected in human follicular fluid, the concentrations changing in association with ovulatory cycle (Bodis *et al*., 1993). Serotonin is locally released by mast cells within the ovary, oviduct and uterus, or is transported to the reproductive organs through the nerve terminals and blood leukocytes (Sirotkin & Schaeffer, 1997).

The effects of serotonin or its antagonists on postimplantation embryo development are well documented. Lauder & Sadler (1988) observed defects in embryos at the headfold stage grown in a medium containing serotonin. The embryotoxic effect of serotonin during early pregnancy was also reported by Chebotar *et al*. (1995). Rats were treated by intra-amniotic (direct action) and subcutaneous (indirect action) injections of serotonin. A dose of 15 mg/kg of serotonin caused embryo death, whereas a dose of 5.0 mg/kg had no lethal effect on development. Decreased levels of endogenous serotonin during early postimplantation stages led to abnormalities in development (Khozhai *et al*., 1995). Lauder *et al*. (2000) used immunocytochemistry to analyse the expression of specific serotonin (5HT-2) receptors during mouse embryogenesis. The functional significance of these receptors was demonstrated by the spectrum of malformations induced by receptor antagonists in the whole embryo culture; the most potent was ritanserin (1 μM), which caused 100% malformation of embryos.

Recently we have analysed the expression of three types of serotonin receptors – 5-HT1B, 5-HT2C and 5-HT1D – by reverse transcription-polymerase chain reaction (RT-PCR) in mouse preimplantation embryos (Veselá *et al*., 2003). 5-HT1D receptor mRNA was detected in unfertilized oocytes, zygotes, 2-cell embryos, compacted morulae and expanded blastocysts derived *in vivo*. Expression of 5-HT1D receptor mRNA was also detected in blastocysts cultured *in vitro*. Transcripts for 5-HT1B and 5-HT2C receptors were not detected in any stages of preimplantation embryo development *in vivo*. These data suggest the possible involvement of 5-HT1D receptors in early embryonic development.

To further investigate the biological implication and significance of serotonin, we analysed the developmental pattern of serotonin localization as well as expression of serotonin receptor (5-HT1D) mRNA in mouse oocytes and preimplantation embryos. The functional significance of serotonin during the preimplantation period was demonstrated by the effects of serotonin on mouse embryo development.

Materials and methods

Embryo recovery

Female mice (ICR strain, Velaz, Prague, Czech Republic; 4–5 weeks old) underwent superovulation treatment by intraperitoneal injection of 5 IU of pregnant mare's serum gonadotropin (PMSG, Bioveta, Ivanovice na Hane, Czech Republic), followed 46 h later by administration of 5 IU of human chorionic gonadotropin (hCG, Organon, Oss, The Netherlands). Females were mated with males of the same strain overnight. Mating was confirmed by identification of a vaginal plug. Females were killed by cervical dislocation (55–57 h after hCG treatment) and embryos were recovered at the 4-cell stage, by flushing the oviduct using a flushing-holding medium (FHM; Lawits & Biggers, 1993).

Embryo culture

The 4-cell embryos were washed three times in KSOM culture medium (Lawits & Biggers, 1993) containing serotonin or appropriate solvent, transferred to 30 μl drops of KSOM media (with serotonin or solvent) and cultured in a humidified atmosphere with 5.0% CO_2 at 37 °C to blastocyst stage (day 5). Serotonin (Sigma-Aldrich, Steinheim, Germany) at concentrations of 1 μM to 0.01 μM was dissolved in dimethylsulfoxide (DMSO; Sigma-Aldrich). The control groups of embryos were cultured in the presence of equivalent amounts of solvent (DMSO) added into KSOM. The embryo cell number and the detection of apoptosis incidence were analysed by morphological triple staining (Fabian *et al*., 2004).

Morphological triple staining

For cell death assessment, nuclei were stained with the cell-permeant dye Hoechst 33342 (HO, 20 μg/ml; Sigma-Aldrich; stains all cells) and the cell-impermeant dye propidium iodide (PI, 20 μg/ml; Sigma-Aldrich; stains dead cells only), and cytoplasm was stained with Calcein AM (5 μM; BioChemika; stains live cells only) for 40 min at 37 °C. The embryos were then washed, sealed with coverslips and observed under fluorescence microscopy (BX 51 Olympus, Japan).

The number of nuclei and corresponding morphological profile were assessed in all embryos: healthy nuclei, oval, with uniform Hoechst staining, or with visible chromosomes; condensed nuclei with dense Hoechst staining, smaller than normal nuclei; fragmented nuclei, in the process of karyorhexis

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or disintegrated into apoptotic bodies; disseminated fragments; and polar bodies. Healthy nuclei were counted as Hoechst normal nuclei, and condensed and fragmented nuclei as Hoechst damaged nuclei. Additional PI and Calcein staining was used for their subsequent exact categorization.

Hoechst normal (stained but healthy), PI- and Calcein+ nuclei were classified as normal nuclei; Hoechst damaged, PI- and Calcein+ nuclei were classified as apoptotic nuclei in early stages of apoptosis; and Hoechst damaged/normal, PI+ and Calcein+ nuclei were classified as apoptotic nuclei in later stages of apoptosis; Hoechst damaged/normal, PI+/- and Calcein- nuclei were counted as secondary necrotic nuclei (Fabian *et al*., 2004).

For the analysis of embryo distribution each embryo was assigned to one of four groups depending on its developmental stage. The first group contained embryos with cell numbers less than 16, the second group comprised embryos with cell numbers from 16 to 32, the third group had 33 to 64 cells per embryo and the fourth group comprised embryos with cell numbers of 65 and more.

The following numbers of embryos were examined by morphological triple staining on day 5: serotonin 1 μM, $n = 181$; DMSO, $n = 143$; serotonin 0.1 μM, $n = 117$; DMSO, $n = 62$; serotonin 0.01 μM, $n = 103$; DMSO $n = 76$.

Immunostaining

Oocytes and preimplantation embryos were isolated and the zona pellucida was removed with 0.25% pronase in KSOM at 37 °C. Zona-free oocytes and embryos were washed three times in KSOM and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4 °C for 30 min. Free aldehyde groups were blocked with 0.1 M glycine (Merck, Darmstadt, Germany) in PBS/BSA (PBS containing bovine serum albumin; Sigma-Aldrich, Germany) twice for 10 min, followed by short washes in PBS/BSA and PBS/BSA/SAP (PBS/BSA containing 0.05% saponin; Sigma-Aldrich, Germany). Non-specific immunoreactions were blocked with 5% normal goat serum (Amersham, UK) and 1% saponin (Sigma-Aldrich, Germany) in PBS for 45 min at room temperature. Oocytes and embryos were incubated with primary serotonin antibody (ab8882, Abcam, Cambridge, UK) diluted 1:2000 in PBS/BSA/SAP at 4 °C overnight. Control groups of oocytes and embryos were incubated without primary serotonin antibody. After extensive washing in PBS/BSA, affinity-purified specific secondary antibodies (20 μl/ml; anti-rabbit IgG, Jackson Immunoresearch, West Grove, USA) coupled with fluorescein (FITC) were used to visualize primary antibody reactions (30 min at room temperature). Nuclei of oocytes and embryos were stained with Hoechst 33342 in PBS/BSA/SAP (20 μl/ml; Sigma-Aldrich, Germany). Afterwards, they were washed, mounted in Mowiol on glass slides, sealed with coverslips and observed under fluorescence microscopy (BX 51 Olympus, Japan). Oocytes and embryos in each experimental group were evaluated by comparison with control groups of oocytes and embryos.

Fluorescence *in situ* hybridization (FISH)

Probe

The rat 5-HT1D cDNA cloned into pcDNA3 plasmid was kindly provided by Thierry Wurch (Wurch *et al*., 1997). A 775 bp DNA fragment from the rat 5-HT1D coding region was obtained by digestion of the plasmid with *Pst*I and used for probe preparation (the 775 bp DNA fragment shows 95% nucleotide identity with mouse 5-HT1D coding sequence). It was labelled with DIG-11-dUTP by random priming (DIG-High Prime reagent, Roche Diagnostics, Mannheim, Germany) and the DIG-labelled DNA was quantified by DIG Teststrips (Roche Diagnostics, Mannheim, Germany). To distinguish 5-HT1D signal from non-specific signals, a lambda bacteriophage DNA (Lambda *Bst*EII digest, New England BioLabs, Beverly, USA) was similarly labelled and used as a negative control probe.

Oocyte and preimplantation embryo collection

Oocytes and preimplantation embryos were isolated and the zona pellucida was removed with 0.25% pronase in KSOM at 37 °C. Zona-free oocytes and embryos were washed three times in KSOM and fixed in 3% paraformaldehyde in PBS at 4 °C for 30 min, twice washed in 0.75% glycine in PBS/BSA twice for 10 min, followed by short washing in PBS/BSA and PBS/BSA/SAP.

Prehybridization and hybridization treatment

Prehybridization mixture contains all the components of hybridization mixture except for the probe: 2× SSC, 50% deionized formamide, 25 mM NaH_2PO_4, 10 mM EDTA, 1× Denhart solution, 5% dextran sulfate, 250 μg/ml DNA carrier–salmon sperm DNA. After fixation and permeabilization, oocytes and preimplantation embryos were incubated in prehybridization mixture for 20 min at room temperature (RT). Afterwards they were incubated in hybridization mixture containing DIG-labelled 5-HT1D DNA probe or lambda phage DNA probe 1 μg/ml overnight at 39 °C. Post-hybridization consisted of successive four washes in 2 × SSC, 60% formamide, 0.2% BSA at RT, once for 5 min at 37 °C, incubation in PBS containing 0.2% BSA for 5 min at RT before transfer into blocking solution for immunofluorescence detection.

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Table 1 Analysis of development and apoptosis incidence in mouse embryos cultured in KSOM containing serotonin or corresponding solvent (DMSO) on day 5, evaluated by triple staining (Hoechst 33342, PI, Calcein-AM)

	Serotonin	DMSO	Serotonin	DMSO	Serotonin	DMSO
	1 μM		0.1 μM		0.01 μM	
Cultured embryos	181	143	117	62	103	76
Mean cell number	$57.7 \pm 25.9^{***}$	72.1 ± 21.7	71.8 ± 17.5^{NS}	70.3 ± 17.6	68 ± 20.6^{NS}	72.1 ± 21.7
% <16 cells	7.7	2.1			1.9	1.3
% 16–32 cells	11.6	3.5	3.4	4.8	4.9	
% 33–64 cells	37	22.4	22.2	22.6	25.2	32.9
% >64 cells	43.7^{***}	72	73.4^{NS}	72.6	68^{NS}	65.8
Normal nuclei%	98.4	98.7	98.8	99.2	98.5	99.3
Apoptotic and secondary necrotic nuclei%	1.6^{*}	1.3	1.2^{*}	0.8	1.5^{**}	0.7

Values are means ± SD.
Normal nuclei are defined as Hoechst normal, PI−, Calcein+; apoptotic nuclei are defined as Hoechst damaged, PI+/−, Calcein+; secondary necrotic nuclei are defined as Hoechst normal/damaged, PI+/−, Calcein−.
$^{NS}p > 0.05$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Student's *t*-test was used for the mean cell number, chi-square test for the distribution of embryo cell number and for the profile of cell death incidence.

Immunofluorescence detection

After a wash with blocking solution (100 mM Tris-HCl, 150 mM NaCl, 0.5% (w/v) blocking reagent; Roche Diagnostics, Mannheim, Germany), oocytes and preimplantation embryos were incubated in the same blocking solution for 20 min at RT. For the detection of 5-HT1D mRNA, oocytes and embryos were incubated in solution containing anti-digoxigenin-fluorescein for 45 min at 37 °C (Roche Diagnostics, Mannheim, Germany). Following this, they were washed three times at RT in post-detection washing solution: 100 mM Tris-HCl, 150 mM NaCl, 0.05% (v/v) Tween-20, 0.2% BSA. Nuclei of oocytes and embryos were stained with PI in PBS/BSA/SAP (1 μl/ml; Sigma-Aldrich, Germany). Afterwards, they were washed, mounted in Mowiol on glass slides, sealed with coverslips and observed under fluorescence microscopy (BX 51 Olympus, Japan).

Statistical analysis

The results are expressed as mean values ± SD. The chi-square test was used to detect differences in the profile of cell death incidence and preimplantation embryo distribution. Statistical analyses of mean cell numbers in embryos were done using Student's *t*-test. Values of $p < 0.05$ were considered as significant.

Results

Effect of serotonin on embryo development

The analysis of blastocyst development of embryos cultured in KSOM with serotonin and corresponding controls (solvent DMSO) on day 5 is shown in Table 1.

Exposure to serotonin at concentration of 1 μM highly significantly ($p < 0.001$) reduced mean cell number (57.7 ± 25.9) compared with the control group of embryos (72.1 ± 21.7), whereas lower concentrations of serotonin (0.1 μM and 0.01 μM) had no significant ($p > 0.05$) effects on embryo cell numbers. Serotonin at 1 μM concentration in the culture medium had a highly significant ($p < 0.001$) negative influence on preimplantation development, as the proportion of embryos in the most developed groups is evidently lower compared with controls. No significant ($p > 0.05$) differences in embryo distribution were found between embryos exposed to lower concentrations of serotonin (0.1 μM and 0.01 μM) and the respective control embryos. The analysis of cell death incidence showed a small albeit significant increase ($p < 0.05$) in embryos with apoptotic and secondary necrotic nuclei in all serotonin-treated groups compared with control groups.

Hybridization *in situ* of 5-HT1D receptor mRNA

The results of expression of 5-HT1D receptor mRNA in oocytes and preimplantation embryos using hybridization *in situ* are shown in Fig. 1.

Distinct fluorescent punctate signals were detected in oocytes and embryos hybridized with 5-HT1D probe at the morula and blastocyst stages. Similar results were also observed in 1-cell embryos (data not shown). No signal was detected in oocytes and embryos hybridized with the negative control probe (lambda bacteriophage DNA).

Serotonin localization

We analysed the localization of serotonin in oocytes and preimplantation embryos by immunocytochemistry

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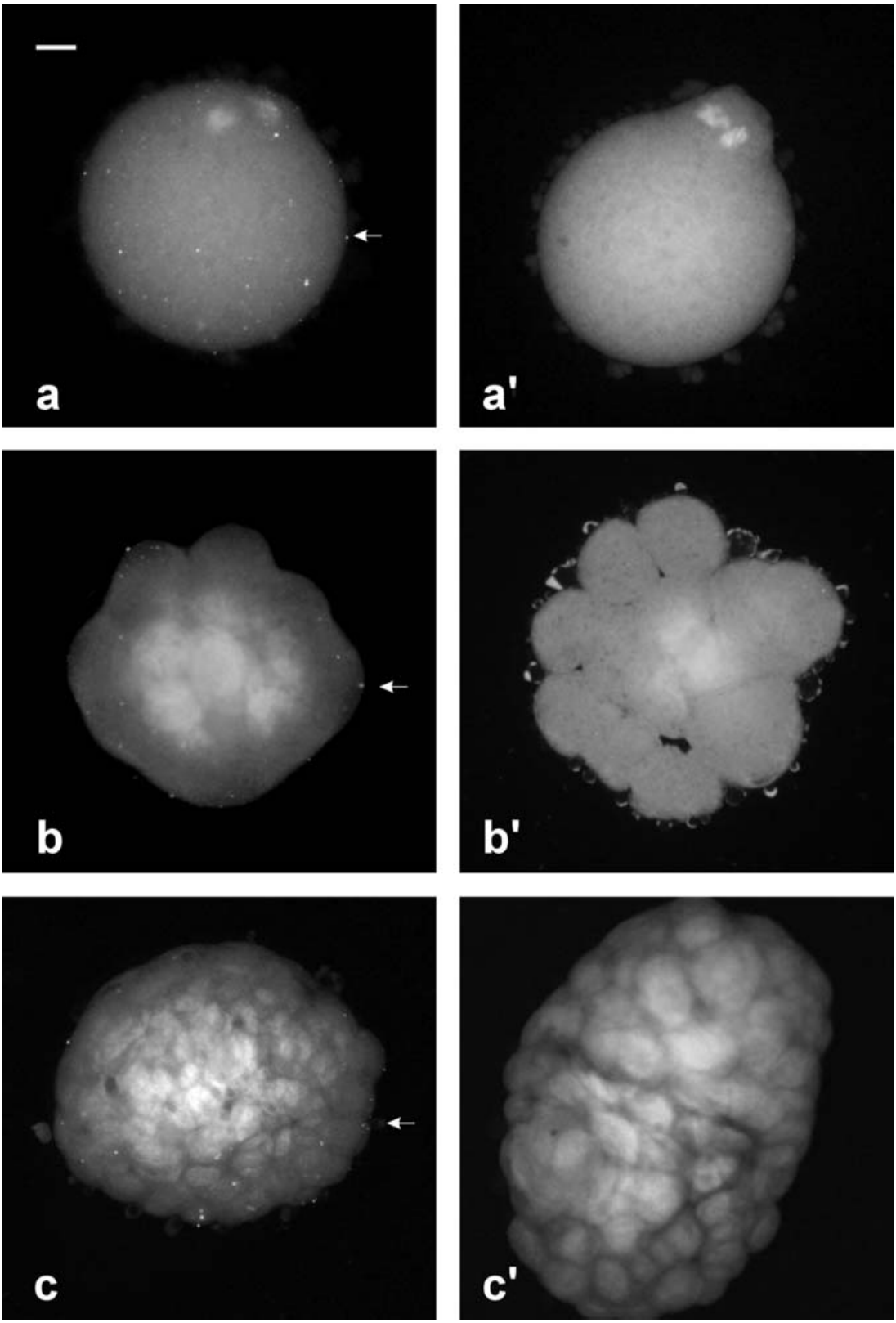

Figure 1 Fluorescence *in situ* hybridization of 5-HT1D receptor mRNA from the mouse oocyte to blastocyst. (*a*) Unfertilized oocyte (24 h after hCG); (*a'*) unfertilized oocyte (control with a lambda bacteriophage DNA), (*b*) 8- to 16-cell embryo (72 h after hCG); (*b'*) 8- to 16-cell embryo (control with a lambda bacteriophage DNA); (*c*) early blastocyst (104 h after hCG); (*c'*) early blastocyst (control with a lambda bacteriophage DNA). Distinct fluorescent punctate signals (arrows) were detected in oocytes and embryos hybridized with 5-HT1D probe. Scale bar represents 10 μm.

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using specific polyclonal rabbit antibody (ab8882, Abcam). We identified faint serotonin labelling in oocytes and zygotes. Stronger and more distinct immunolabelling was observed in 2- to 4-cell and 8-cell stage embryos. Less distinct but still specific labelling was also found in blastocysts, predominantly in trophoblast cells (Fig. 2). Serotonin immunostaining in all developmental stages revealed diffuse punctate cytoplasmic labelling.

Discussion

Serotonin plays important roles in different developmental processes, including cell proliferation, differentiation, cleavage division and morphogenesis. The literature data about the importance of serotonin for mammalian preimplantation embryo development are scarce. The effect of serotonin and its antagonists on 2-cell embryos developed to the blastocyst stage was firstly described by Leonov *et al*. (1969). In their experiment 1 mg/ml of serotonin (2.6 mM) inhibited the development of cultured embryos. Lower concentrations of serotonin (0.01 and 0.1 mg/ml) slightly decreased embryo development, whereas 0.0001 mg/ml of serotonin had a positive influence on development. Similar concentration dependence (but at much lower concentrations) was also confirmed in our experiment, where serotonin at 1 μM concentration significantly impaired embryo development. The addition of 0.1 μM and 0.01 μM serotonin did not significantly influence embryo development. However, serotonin at all concentrations (0.01–1 μM) significantly increased the incidence of apoptosis. The observed discrepancy in effective serotonin concentrations (at least 26-fold) inhibiting embryo development between our experiments and those of Leonov *et al*. could be explained by the different experimental design and the unknown quality of serotonin in Leonov *et al*.'s experiment. More recently Sakharova *et al*. (1997) demonstrated enhanced viability of preimplantation mouse embryos after cryopreservation at the stage of four blastomeres after pretreatment with serotonin (5 μM). Although serotonin concentrations in the oviductal fluid are not described in the literature, the serotonin concentrations used in our experiment were well within the range of values detected in human follicular fluid and sera (Bodis *et al*., 1992; Zarate *et al*., 2002). Also in our recent experiment (Veselá *et al*., 2003) it was shown that the specific serotonin 5-HT1D agonist sumatriptan at 1 μM concentration significantly inhibited *in vitro* development of mouse embryos.

In parallel with the present experiment we have also tested the effect of the 5-HT2A receptor antagonist ketanserin, which is also able to block the human 5-HT1D receptor with some preference over the 5-HT1B receptor (Pauwels *et al*., 1996). Ketanserin at 1 μM concentration significantly increased mean cell number (data not shown) in embryos, presumably due to the suppression of endogenous serotonin effects. It is interesting that similar results were also reported using a different model – embryonic chick skin *in vitro* – where it was shown that serotonin inhibited proliferation at 10 μg/ml concentration (approx. 26 μM) and ketanserin at concentrations from 0.05 to 5 μg/ml (0.12 to 12 μM) stimulated proliferation (Beele *et al*., 1989). On the other hand, Markova *et al*. (1990) showed the adverse effect of serotonin antagonist (cyproheptadine) on *in vitro* development of mouse embryos. Serotonin antagonist at a concentration of 60 μM inhibited the development of embryos, whereas 80 μM concentration arrested development and no significant effect was seen at a concentration of 37 μM.

Bolanos-Jimenez *et al*. (1997) first described the expression of 5-HT1D receptors mRNA and specific 5-HT1D binding in mouse embryos at 8 days of prenatal development using RT-PCR and the selective binding of radioactive ligand. Very recently we demonstrated the expression of serotonin 5-HT1D receptor mRNA in mouse oocytes and preimplantation embryos using RT-PCR (Veselá *et al*., 2003). These observations are confirmed by our experiment, as *in situ* hybridization of 5-HT1D receptor mRNA shows a distinct punctate signal in oocytes and preimplantation embryos.

5-HT synthesis seems to be important in the first steps of mammalian embryonic development. This can be deduced from the pharmacological actions of *p*-chlorophenylalanine (PCPA), a specific inhibitor of tryptophan hydroxylase (TPH; EC 1.14.16.4), the rate-limiting enzyme in the biosynthesis of the neurotransmitter 5-HT. Administration of PCPA to female mice during pregnancy has been shown to cause absence of cytokinesis at the zygote stage, or arrest of cleavage in preimplantation embryos (Khozhai *et al*., 1995). Walther & Bader (1999) used immunohistochemical analysis to reveal specific staining of embryonic stem (ES) cell clones using antibodies against TPH with no staining of the feeder-cell monolayer on which the ES cells were grown. ES cells abundantly synthesize 5-HT using TPH similarly to serotonergic neurons of the central nervous system, mast cells and enterochromaffin cells. Very interestingly immunohistochemistry showed also that no TPH protein is present in oocytes and that its expression is induced immediately after fertilization, as is visible in pronuclear-stage zygotes. This observation confirms our immunocytochemistry results revealing the localization of serotonin in embryos from 1-cell to blastocyst stage as diffuse punctate cytoplasmic labelling. Very recently serotonin (or a serotonin-like substance) was detected immunochemically using laser scanning microscopy in early embryos of the

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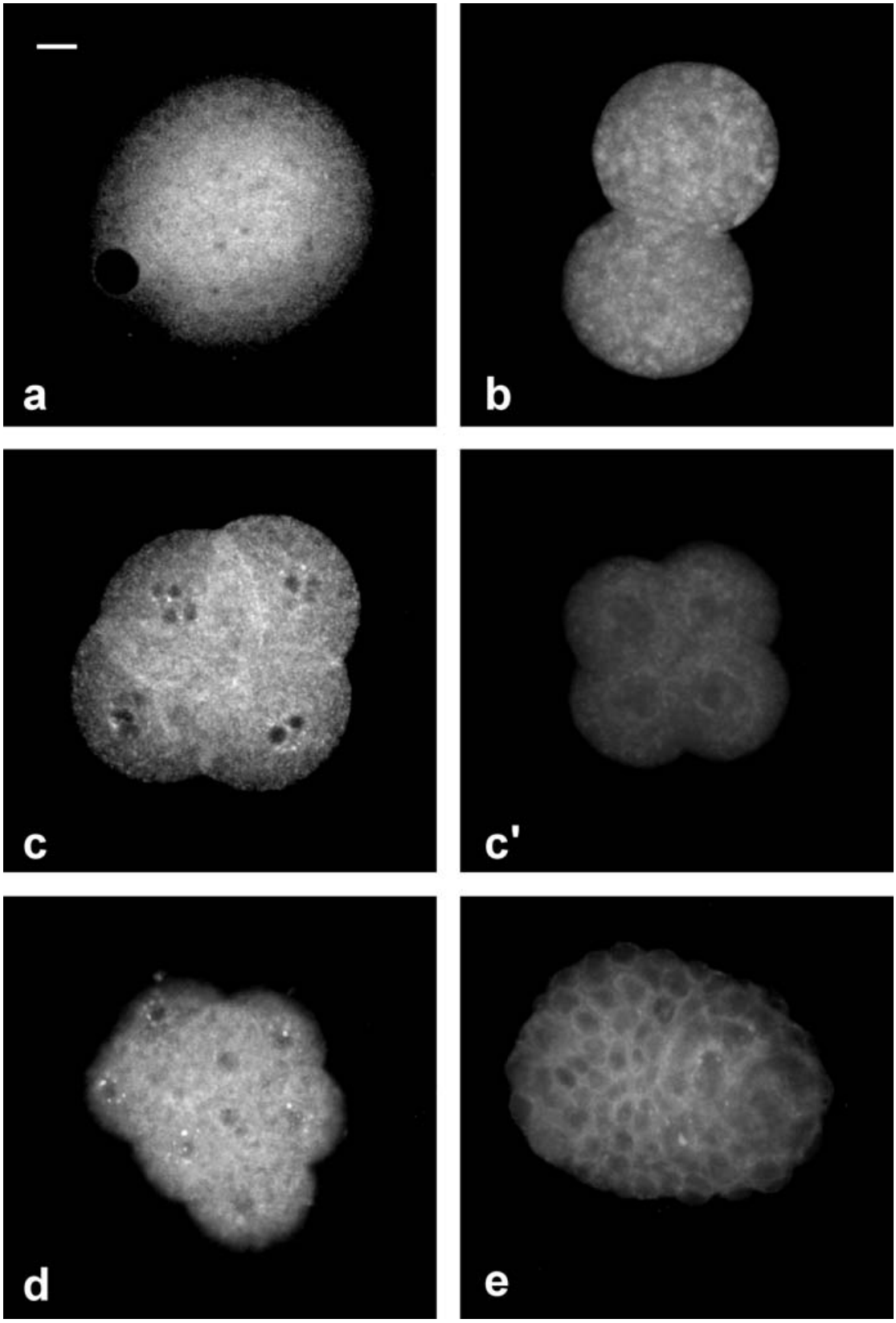

Figure 2 Immunofluorescence staining of serotonin from the mouse oocyte to blastocyst. (*a*) Immature oocyte (46 h after PMSG); (*b*) 2-cell embryo (54 h after hCG); (*c*) 4-cell embryo (54 h after hCG); (*c′*) control 4-cell embryo (without serotonin antibody); (*d*) 8-cell embryo (72 h after hCG); (*e*) hatching blastocyst (120 h after hCG). Serotonin immunostaining in all developmental stages revealed diffuse punctate cytoplasmic labelling. Scale bar represents 10 μm.

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mollusc *Tritonia diomedea*. At the 1- to 8-cell stages, immunolabelling suggested the presence of serotonin in the cytoplasm close to the animal pole. At the morula and blastula stages, a group of micromeres at the animal pole showed immunoreactivity. The serotonin antagonists ritanserin and cyproheptadine blocked cleavage divisions or distorted their normal pattern (Buznikov *et al*., 2003).

Our results show that serotonin and its specific 5-HT1D receptor are present in preimplantation embryos and that the addition of serotonin could influence embryo development. These observations support the concept of pre-nervous functions of neurotransmitters (Buznikov *et al*., 2001), because serotonin was detected during zygotic cleavage divisions, gastrulation and neurulation in embryos of sea urchins, frogs and chickens. The presence of serotonin and its receptors early in embryogenesis and the ability of serotonin-specific pharmacological agents to interfere with embryonic development have led to the suggestion that early embryos use serotonin before the onset of neurogenesis in the regulation of cell proliferation and/or morphogenetic movements (Buznikov *et al*., 1996).

It is known that embryo development is regulated at least partially by autocrine and paracrine mechanisms, as several growth factors are synthesized by the preimplantation embryo itself. Growth factors may act directly by regulating proliferation and/or differentiation of competent target cells or as indirect signals by inducing the expression of a cascade of other genes, which influence specific developmental events. Lee *et al*. (2002) hypothesize that fetal-maternal communication exists in the preimplantation period, allowing optimal development of embryos. It seems probable that not only do embryotrophic factors from the oviduct affect the development of embryos, but that embryos could even affect their own transport in the oviduct, by enhancing a specific set of mRNAs in the oviduct.

In conclusion, our results indicate that serotonin is synthesized in early preimplantation embryos and its specific receptor (5-HT1D) mRNA is already expressed in oocytes as well as in preimplantation embryos. It appears from our results that endogenous and/or exogenous serotonin in preimplantation embryos could be involved in complex autocrine/paracrine regulations of embryo development and embryo-maternal interactions.

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