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EXPERIMENTAL STUDY

Antagonistic effect of low Deprenyl dose on the preimplantation embryo development in rat

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Abstract: To investigate the role of potent MAO-B inhibitor deprenyl in fertilized females, we have evaluated the effect of chronic treatment with deprenyl at a low dosage on preimplantation embryo development in Wistar rats. We have found that the number of isolated embryos per rat did not differ between experimental and control groups. But morphological analysis of embryos isolated from deprenyl-treated animals had revealed improved rates in the distribution pattern compared with controls. On the other hand, harmful impact of deprenyl administration on the mean cell number in blastocysts and on their cell proliferation has been recorded.To our knowledge this is the first paper describing antagonistic effect of deprenyl administration on the preimplantation embryo development in mammals. Potential mechanisms mediating biphasic deprenyl-induced impact on embryonic development are proposed *(Tab. 3, Ref. 17)*. Full Text (Free, PDF) www.bmj.sk.
Key words: MAO-B inhibitor, selegiline.

Monoamine oxidases (MAO) are enzymes that degrade biogenic monoamines. They are strongly bound to the outer mitochondrial membrane. MAO-A has a higher affinity for the substrates serotonin and noradrenaline and MAO-B has a higher affinity for ß-phenylethylamine. Dopamine is a common substrate of both monoamine oxidases. MAO are involved in many behavioral processes and their inhibition has marked effect on brain function, blood pressure regulation and the detoxification of potentially harmful exogenous amines (Singer and Ramsay, 1995).

In recent years, increasing numbers of claims have been made about the therapeutic effects of MAO inhibitors in psychiatric and neurological disorders, including depression, bulimia, schizophrenia, Parkinson's disease, neurodegenerative diseases in general, Alzheimer's disease etc. Although the molecular bases of these diseases are often complex, the fact that most of them have been linked with abnormal MAO activity provides a biochemical rationale for further pursuit of the therapeutic potential of MAO inhibitors. Synthesis of new MAO inhibitors could dramatically increase the effectivness of the treatment and decrease the risk of undesirable side effects (Keung, 2002).

The total lack of information as to whether long-term MAO inhibitor administration can influence the course of pregnancy during the preimplantation period of embryo development prompted us to start an investigation into this area. We have decided to begin our work employing the potent MAO-B inhibitor deprenyl (selegiline), which is known for its neuroprotective effects (Semkova et al, 1996), antiapoptic effect in both cultured neurons (Le et al, 1997) and animal models (Simon et al, 2001) and exerting a cardiac neuroprotective effect in congestive heart failure (Hare, 2001).

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Materials and methods

Animals

All procedures performed with animals adhered to the permission of the Committee for Ethical Control of Animal Experiments at Šafárik University and the permission of the Slovak State Veterinary and Alimentary Administration (permission no.# 7881/04–220/3). All efforts were made to minimize both the number of animals and their suffering.

Young, virgin female Wistar rats (200–240 g, 85–90 days old) were obtained from the animal facility of the University. The animals were given free access to standard diet and water and were maintained in a 12 h light/12 h dark cycle. Rats were injected i.p. daily for 30 days, either with saline (control animals) or with the dose (0.25 mg/kg/day) of deprenyl (Sigma, M003) dissolved in saline (experimental animals). After the last drug administration, females were mated during the following week from 07:00–09:00 a.m. with males of the same strain. The first day on which a vaginal plug was present was designated day 1 of pregnancy.

Embryo collection

Pregnant rats were killed by lethal dose of thiopental (40 mg/kg; ICN Czech Pharma, Prague, Czech Republic) 97–99 hours after fertilization at 08:00 on day 5 of pregnancy. Oviducts and uter-

Tab. 1. Number of embryos collected from oviducts and uteri at day 5 of pregnancy from deprenyl treated (0.25 mg/kg) and control rats.

	Deprenyl		Control
Number of rats	29		29
Total number of embryos (%)	282 (100%)		290 (100%)
embryos/rat±S.D.	9.72±2.48		10±3.01
paired t-test		p>0.05	
No of embryos (%):			
flushed from oviducts	2 (0.71%)		3 (1.03%)
flushed from uteri	280 (99.29%)		287 (98.97%)

Tab. 2. Developmental stages of embryos and distribution pattern of degenerated embryos collected at day 5 of pregnancy from deprenyl treated (0.25 mg/kg) and control rats.

	Deprenyl		Control
No of blastocysts (%)	87 (30.85%)		96 (33.10%)
No of morulae (%)	162 (57.45%)		140 (48.28%)
No of degenerated embryos (%)	33 (11.70 %)		54 (18.62%)
χ^2 test		p<0.01	
No of oocytes and zygotes (%)	13 (39.39%)		9 (16.67%)
No of 2–7 cells embryos (%)	15 (45.45%)		33 (61.11%)
No of fragmented embryos (%)	5 (15.15%)		12 (22.22%)
χ^2 test		p<0.001	

ine horns were immediately removed and embryos were gently flushed from them with prewarmed PBS + PVP (3 mg/ml). Embryos were counted and classified according to developmental stage as follows: (a) degenerated – oocytes, often with cumular cells, 2–7 cells embryos or damaged embryos with cytoplasmic fragmentation, (b) morulae, (c) blastocysts.

Cell staining

The total cell number of individual morulae and blastocysts was counted as was described before (Mihalik et al, 2000). Briefly, the zona pellucida was removed using short incubation in acid Tyrode solution (pH 2.3–2.4). Embryos were transferred onto a glass slide into small drops of hypotonic solution of 0.88 % sodium citrate and kept at room temperature for 3–5 minutes. Drops of fixative solution (methanol:acetic acid; 3:1) were applied on top of the embryos just at the moment before cells dried out. Embryos were embedded between a slide and coverslip using one drop of fluorescent mounting medium VECTASHIELDR with DAPI (VECTOR, VC-H-1200-L010). Cell number counting was performed using UV light epifluorescence (Jenalumar a/d contrast, Carl Zeiss Jena, Germany).

Statistical analysis

Mean numbers of embryos, the cell number in morulae and blastocysts were analyzed by two-tailed unpaired Student's t-test. Results are given as means S. D. Differences in the distribution of preimplantation embryos and the cell number distribution in blastocysts were compared by the chi-square (χ^2) test. $p<0.05$ was considered as significant. Data presented here are pooled from three independent replications of the same experiment.

Results

Embryo collection. Neither the mean number of embryos recovered per female nor the number of embryos flushed from oviducts and uteri did not differed significantly between the experimental and control groups ($p>0.05$) (Tab. 1). But the morphological analysis embryos isolated from deprenyl-treated females revealed significantly improved development ($p<0.01$) (Tab. 2)

Tab. 3. Cell number in morulae and blastocysts and distribution of cell number in blastocysts (DAPI staining) isolated from deprenyl treated (0.25 mg/kg) and control rats.

	Deprenyl		Control
No of stained morulae	111		94
cell number±S.D.	13.62±2.26		13.70±2.24
unpaired t-test		p>0.05	
No of stained blastocysts	62		68
cell number±S.D.	19.21±3.13		21.31±6.95
unpaired t-test		p<0.05	
No of stained blastocysts	62		68
% 16–24 cells	91.94%		77.94%
% 25–32 cells	8.06%		20.59%
% >32 cells	0.00%		1.47%
χ^2 test		p<0.05	

because of the higher incidence of morulae and the lower incidence of degenerated embryos. Moreover, comparing degenerated embryos we have found that significantly less 2–7 cell embryos and those with cytoplasmic fragmentation were isolated from experimental animals comparing to controls ($p<0.001$) (Tab. 2).

Cell number. No significant effect of deprenyl administration on the mean cell number in morulae was found ($p>0.05$) (Tab. 3). On the other hand, significant decline of the mean cell number and decreased cell proliferation in blastocysts derived from experimental females in comparison with the control group has been recorded ($p<0.05$) (Tab. 3).

Discussion

Our results demonstrated that the long-term intraperitoneal administration of low deprenyl dose to rat females significantly improved the developmental rates of preimplantation embryos. In contrast to this, deprenyl had detrimental effect on the mean cell number in healthy blastocysts, when analysis of cell proliferation revealed a higher percentage of blastocysts with a lower cell number in the experimental group.

The hypothalamus regulates the sexual behavior of rats and works in the neural control of ovulation. The MAO value in the

hypothalamus is the highest of all areas of the brain. This suggests the presence of high levels of biogenic amines in this area (Kono et al, 1994). Substantial activity of monoamine oxidase B, using a coupled peroxidatic technique, was identified in several hypothalamic regions, such as the periventricular and paraventricular nuclei (Willoughby et al, 1988). MAO activity is also detected in various reproductive organs. MAO-B type was found in the ovarian blood vessels, where it has shown characteristic changes of activity during the oestrous cycle. These histochemical results suggest that MAO-B activity might possibly be involved in ovulation, presumably in association with humoral information (Yoshimoto et al, 1986). But we were not able to detect any detrimental effect of deprenyl, a potent MAO-B inhibitor, administrated at dose 0.25 mg/kg/30 days on the mean number of isolated embryos from experimental rats. That means this dose can not impair ovulation rates in rat on the hypothalamic or even ovarian levels.

On the contrary, significantly more morulae and less degenerated embryos were recovered from experimental females, when deprenyl proved to be a potent enhancer of embryonic quality. As was reported by Knoll (1998), maintenance of rats on (–) deprenyl during the postdevelopmental phase of their life slows the age-related decline of sexual and learning performances and prolongs life significantly. As was described previously in research of neuronal cells (Ebadi et al, 2002), selegiline reduces the production of oxidative radicals, delays apoptosis, and blocks apoptosis-related fall in the mitochondrial membrane potential. (–)-Deprenyl in much lower concentrations needed to induce MAO-B inhibition (10^{-9} to 10^{-13} M) potently inhibits apoptosis in tissue cultures of neuroectodermal origin, maintaining Caspase 3 activity on control level. The (+)-enantiomer of deprenyl lacks of this property. The anti-apoptotic activity of (–)-deprenyl can be prevented by inhibiting the metabolism of the drug, which suggests that some of the presently unknown metabolites could be responsible for the anti-apoptotic activity (Magyar and Szende, 2004, Szende et al, 2001).

On the other hand, compared with embryos collected from control rats, exposure to long-term treatment with deprenyl significantly decreased mean cell number of blastocysts on day 5 of gestation and delayed their cell proliferation. But the mean cell number in blastocysts collected from control animals was close to the values reported by Pampfer et al (1990) in healthy rats. Significant differences were not observed between the two groups of morulae in our work suggesting that the susceptibility on deprenyl was expressed after blastocyst formation. The mean number of cells was in keeping with the values of 16.10 reported by Pampfer et. al (1990) in rat morulae collected 90 hours after fertilization. Depending on the dose can deprenyl express both anti-apoptic and apoptic action. Higher dose (10^{-13} M) of (–)-deprenyl induced apoptosis and very high Caspase 3 activity in non-serum-deprived A-2058 cell culture (Szende et al, 2001). It seems that dose 0.25 mg/kg of deprenyl for long time is still very high to improve and protect rat embryonic development during whole preimplantation period and more experiments are needed to achieve its correct levels of anti-apoptic and enhanced properties. But as far as we know, this is the first paper describing positive impact of chronic deprenyl administration in mammals on the distribution pattern of preimplantation embryos.

Since Ilkova et al (2004) have proven the expression of serotonin 5-HT1D receptor mRNA from mouse oocytes and preimplantation embryos to the blastocyst stage it appears that endogenous and/or exogenous serotonin in preimplantation embryos could be involved in the regulation of embryo development and embryo-maternal interactions. Deprenyl is well known for its capability to inhibit not only MAO-B but also MAO-A, an enzyme which preferentially oxidises serotonin (Lakhshmana et al, 1996). To date there has been no relevant published work describing the influence of deprenyl administration on serotonin and MAO-A levels in the reproductive system. Due to this, we should not eliminate the possibility that the impaired blastocyst development described in our paper was achieved through the disturbed MAO-A level in the oviduct/uterus, because mice embryo exposure to serotonin in vitro had a detrimental effect (Iľková et al, 2004).

Despite the fact that deprenyl administration enhanced number of morulae and reduced number of degenerated embryos at dose 0.25 mg/kg over 30 days in rats, its negative impact on the process of blastocyst development has been recorded. These findings might be relevant to human clinical practice and strongly underline the importance of medicine control in women with neuropsychiatric disorders who want to achieve pregnancy. Our data presented here will be used as the basis for further experiments in the research into the influence of MAO inhibitors on the reproductive system in rats and humans.

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