NEGATIVE IMPACT OF FENOTEROL, A MYOMETRIAL RELAXANT ON THE HISTO-ARCHITECTURE OF THE MICE OVARY

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KEYWORDS

- β2-adrenoceptor agonists
- Ovarian hormones
- Ovulation
- Tocolysis


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ABSTRACT

Myometrial relaxants are β₂-adrenoceptor agonists, a group of drugs that are usually used to treat asthma. Fenoterol has long been used in tocolysis with the aim to reduce premature labour and fetal mortality. In the present study, the effects of fenoterol on ovary under short term treatment were examined. Virgin female mice were treated with single oral dose of fenoterol (2.5mg/kg of body weight) and sacrificed at 2, 4, 10 and 20h. Our study was mainly focused on the ovarian histology that revealed detailed changes after fenoterol administration. Little change was exhibited at 2h as compared with the control. However, after 4h onwards, ovarian stromal cells were undergoing degenerative changes following fenoterol administration. Follicular cells began to develop necrosis and the granulosa cells were no longer present in ovarian follicles and all of the cells have been sloughed off. Role of antioxidant defense system was also investigated. Fenoterol caused marked increase in SOD activity at 4h stage, with levels reaching almost 24.28% higher than corresponding vehicle control, while catalase activity exhibited slight increase (16.60%). The present study showed that fenoterol has dramatic effects on ovary and disrupted process of ovulation which may result in significant structural changes in ovarian follicles and stroma. Such fenoterol induced alterations in ovary can affect reproductive functions of exposed animals. The results of this research suggested negative influence of fenoterol on the reproductive health of the animals which might affect the fertility in general.

INTRODUCTION

Increased uterine activity associated with threatened miscarriage and abnormal uterine contractility is linked to dysmenorrhea, a condition associated with painful uterine cramping. Uterine cramps are the result of strong myometrial contractions that occur before and during reproductive cycle (Bulletti et al., 2001). Many women can alleviate painful uterine cramps by taking drugs, such as aspirin and uterorelaxant (fenoterol, isoxsuprine) with the belief that they would reduce contractions and prolong pregnancy. Perhaps due to the infrequent use of uterine relaxing agents in the management of miscarriages and other uterine discomforts, there has no recent trials conducted to study adverse effects of these drugs. There is thus dearth of trials conducted to study the effects of uterine relaxing agents in female reproductive system. Fenoterol is a selective β₂-adrenoceptor agonist. Tocolytic therapy with beta adrenergic receptor agonists is a standard regimen to prevent preterm birth, however, agonist stimulation of beta 2-adrenergic receptors causes receptor desensitization in many other cells and tissues (Gleiter, 1999).

During reproductive life when the female is not pregnant, ovaries exhibit cyclic changes in both structure and functions such as follicular growth and maturation, ovulation, formation and degeneration of corpus luteum which are controlled primarily by ovarian hormones. In absence of these hormones, the ovaries remain inactive. It is suggested that ovarian beta 2-adrenergic receptors have a regulatory role in ovarian hormone secretion. In humans, these changes are called menstrual cycles and in rodents these changes are called estrus cycle. The ovarian changes that occur during the sexual cycle depend completely on the gonadotropic hormones, FSH (follicle stimulating hormone) and LH (luteinizing hormone) secreted by anterior pituitary gland (Sullivan et al., 1999). In normal reproductive cycle, after the ovulation of the mature follicle LH stimulate granulosa cells to secrete estrogen and large amount of progesterone. The presence of these hormones will stop additional ovulation. It is suggested that the fenoterol administration locally stimulated ovarian hormones both estrogen and progesterone (Zsolnai et al., 1982). However, it is not known how fenoterol affects estrogen and progesterone-dependent processes in the ovary.

Reactive oxygen species (ROS) and antioxidants have also been implicated in the regulation of reproductive processes such as follicular development, ovulation etc. Imbalance between ROS production and antioxidant systems induces oxidative stress that negatively impacts reproductive processes (Kais et al., 2010). Oxidative stress (OS) may have an important role in spontaneous abortion, preterm labor (Jenkins et al., 2000), infertility in men and women (Rayman 2000; Xu, 2001), follicular growth and development (Murray et al., 2001). A role has been demonstrated for ovarian steroids, primarily estrogen, in the modulation of infiltration and function of mononuclear phagocytes, which produce superoxide radicals/hydrogen peroxide and cause apoptosis and damage to cells (Mc Master et al., 1992; Sugino et al., 2002). There is thus increased interest in investigating role of OS in the female reproduction. This was investigated by examining whether fenoterol disrupted ovarian cycle and follicular development through some feed-
back influence on the ovary. Thus, effect of fenoterol on histology and biochemical parameters of ovary was studied at different time intervals (2-20h).

MATERIALS AND METHODS

Animals: Female virgin Balb/c mice weighing 20-25g were procured from Central Research Institute, Kasauli (H.P.), India. They were maintained in polypropylene cages in the animal house of Department of Biosciences, H.P. University, under hygienic conditions of temperature and light (24 ± 2°C, 12:12 hrs light dark cycle). Mice were fed upon Hindustan lever pellet diet and water ad libitum. All the methodologies opted for the whole investigation had prior approval of Institutional Animals Ethics Committee of the University.

Experimental design: Normal healthy animals showing no sign of morbidity were randomly divided into two groups: First group (n = 5) served as control and received an equal volume of saline. Mice of second group designated as treated received single oral dose of fenoterol for different experimental stages.

Drug administration and tissue harvesting: Stock solution of fenoterol (2.5mg/mL) was prepared in physiological saline solution (0.9%). Each mouse received a single oral dose of fenoterol (2.5mg/kg of body wt.). The animals from each group were sacrificed at 2h, 4h, 10h and 20h after drug treatment respectively. The abdomen was cut open and reproductive tracts were exposed. The excised ovary was employed for histological and biochemical studies.

Histological study: Ovary was fixed in 10% neutral buffered formalin. After fixation, paraffin embedding was done and 4-5μm sections were prepared and stained with heamatoxylin and eosin for histological examinations.

Antioxidant enzyme assay: A tissue homogenate (10%) was homogenized in 0.15 M Tris HCl buffer (pH 7.2). The homogenate was centrifuged at 3500 x g for 10 min at 4°C. Then supernatant was collected and used for assay. Protein concentration from the supernatant was calculated according to the method of Lowry et al. (1951). Superoxide dismutase (SOD) activity was determined by the method of Mishra and Fridovich (1972) whereas catalase assay was done as per the method of Aebi (1984).

RESULTS AND DISCUSSION

Histopathology

Normal ovary has numerous ovarian follicles in the stroma at various stages of development. Connective tissue of the ovary is highly cellular and is referred to as the ovarian stroma (OS). Multilaminar primary follicles (MPF) are very similar to unilaminar primary follicles (UPF) and growing follicle (GF) with the major difference being their large size. Moreover the stratification of the follicular cells (FC) has increased. MPF’s display primary oocyte (PO) surrounded by several layers of granulosa cells (GC) and a single layer of radially arranged corona radiata cells (CR) that are attached to noncellular glycoprotein layer the zona pellucida (ZP). The stroma was being reorganized around the follicle to form the theca interna (TI). The presence of basal membrane (BM) between the follicular cells and the theca interna was also noticed (Fig. 1).

It was observed that the fenoterol did not evoke any acute changes at 2h. The theca interna and the granulosa cells were intact; however, some of the corona radiata cells were beginning to slough off. Also, zona pellucida has been disrupted (Fig. 2). However, after 4h of fenoterol administration, numerous ovarian follicles undergo degenerative changes (atresia) before reaching maturity. Atretic follicle (AF) was seen in moderate regression. Also degenerating ovary connective tissue filled with blood vessels was observed (Fig. 3 to 4). At later stages further shrinkage of granulosa cells, pyknosis of their nuclei (PN) was indicated. Connective tissue invades the regressing granulosa cells and replaces them as they degenerate. However, serial changes of degeneration can be recognized by noting follicles in different stages of atresia. At 10h, a follicle in later atresia was also illustrated and developing follicles involuted. The theca interna is still visible. The granulosa cells were no longer present; most of the cells have sloughed off and been resorbed (Fig. 5). Loose connective tissue filled with small blood vessels was clearly seen after 20h of drug administration. Entire follicle was replaced by connective tissue. Degenerating follicle (DF) with its ruptured follicular wall was also observed. Follicles rupture and some surrounding cells were slowly extruded from the ovary (Fig. 6).

Histological observations clearly revealed that after fenoterol administration, follicles do not attain maturity and undergo degeneration (a process called atresia) at various stages of growth, thus becoming atretic follicles. These follicles were gradually replaced by stroma. The cause of the atresia was unknown, but it has been postulated to be because of the large amounts of estrogen from the most rapidly growing follicles which act on the hypothalamus to depress further enhancement of secretion of FSH by the anterior pituitary gland, in this way blocking further growth of less well developed follicles. The present study showed that fenoterol has dramatic effects on ovary which resulted in significant structural changes in ovarian follicles and stroma. This might disrupt ovarian cycle. Early development of the follicle is dependent on local factors while later development depends on FSH and LH. During the follicular phase of ovarian cycle, FSH influences the growth and maturation of ovarian follicles and stimulates the granulosa and theca cells of the maturing follicles to produce estrogen. LH induces the development of the corpus luteum from the theca interna and membrane granulosa and this LH surge results in ovulation (Sullivan et al., 1999). As it seems that there is no further development of follicles, means a detectable corpus luteum did not form during reproductive cycle thus, indicating inhibition of corpus luteum (CL) development also. The failure of CL development would in itself cause the death of any embryos.

Prostaglandins may cause localized contractions in the smooth muscles in the ovary (Koos and Clark, 1982). Excessive prostaglandins secretion resulted in painful cramps. Many women can alleviate painful cramps by taking uterorelaxing substances that inhibit prostaglandin biosynthesis. If ovarian prostaglandin synthesis is inhibited, ovulation does not take place because the physical expulsion of the mature oocyte from the follicle appears to be due to an LH induced increase in prostaglandin within the follicle (Lemaire et al., 1973).
Traditionally, relaxation of smooth muscle by beta 2-adrenoceptor agonists has been thought to be via stimulation of adenylyl cyclase and the formation of adenosine 3, 5-cyclic monophosphate (cAMP) (Scheid et al., 1979). Some unusual degenerative changes were observed in follicular cells after fenoterol treatment. The follicle cells appear to be an important source of oocyte cAMP, and changes of cAMP concentration in the follicle cells were reflected in oocyte cAMP levels (Bornslaeger and Schultz, 1985; Racowsky, 1985). In response to this elevation, the follicle cells synthesize hyaluronic acid, which causes a physical disruption of the contact between the follicle cell processes and the oocyte (Eppig, 1979; Larsen et al., 1986). Because the elevation of cAMP levels inhibits oocyte maturation (Cho et al., 1974), it has been proposed that the fenoterol might increase cAMP concentrations through the gap junctions from the follicular granulosa cells to the oocyte. Infertility can be caused by failure to ovulate a mature oocyte (Mc Veigh and Barlow, 2002).

Circulating level of sex steroids, estrogen and progesterone produced by the ovaries fluctuate as a result of the reproductive cycle. These steroid sex hormones cause immediate changes in ovaries (Johnson et al., 1997; Geffrey et al., 2007). Progesterone inhibits the production of FSH, thereby preventing the maturation of any more follicles and ova. For oocyte maturation to occur, the follicle needs to be at a certain stage of development when the waves of gonadotropin arise. Fenoterol administration locally stimulated secretion of sex steroid ovarian hormones. The increased estrogen and progesterone have a negative feedback on gonadotropins released from the hypothalamus which prevents the large increase in LH and FSH secretion that triggers ovulation so that normal hormonal secretion and normal reproductive cycle cannot occur. For such reason, a combination of estrogen and progesterone are used in oral contraceptives which effectively suppress fertility in females. It was hypothesized from our results that fenoterol affects the follicular growth which might have altered ovarian histology. Although the exact mode of action of fenoterol was not known, it was assumed that probably this action might be due to fluctuations in ovarian hormones. Similar results were observed by Camargo et al. (2009) where female rats treated with steroidal agents presented estral acyclicity and impair fertility in animals.

Also, gradual increase in SOD and catalase activity was noticed at 2, 4, 10h stage after fenoterol treatment. Elevation of superoxide dismutase (SOD), catalase specific activity (expressed as units/mg protein) on mice ovary and percent increase in activity of enzymes as compared to control during 2-20 h period.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Post-administration time (Hours)</th>
<th>SOD activity (units/mg protein)</th>
<th>Catalase activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.19±0.72</td>
<td>10.30±0.2</td>
<td></td>
</tr>
<tr>
<td>2h</td>
<td>14.06±0.23*</td>
<td>15.34</td>
<td>11.08±0.3* 7.57</td>
</tr>
<tr>
<td>4h</td>
<td>15.15±1.14*</td>
<td>24.28</td>
<td>12.01±0.23* 16.60</td>
</tr>
<tr>
<td>10h</td>
<td>14.82±1.14*</td>
<td>21.57</td>
<td>11.76±0.23* 14.17</td>
</tr>
<tr>
<td>20h</td>
<td>12.92±0.41</td>
<td>5.98</td>
<td>10.76±0.3 4.46</td>
</tr>
</tbody>
</table>

Values have been expressed as mean ± SEM (n=5); *p < 0.05 significantly different from the control group.

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Table 1: Effect of fenoterol (2.5mg/kg body wt. Single dose) on superoxide dismutase (SOD), catalase specific activity (expressed as units/mg protein) on mice ovary and percent increase in activity of enzymes as compared to control during 2-20 h period.

Figure 1: T.S. of normal mice ovary showing ovarian follicles in different stages of development. Note growing follicle (GF), unilaminar primary follicle (UPF) and multilaminar primary follicle (MPF) with its primary oocyte (PO) surrounded by granulosa cells (GC). H and E. 400x

Figure 2-6: Representative morphological changes in ovary at different time points (2-20h) after fenoterol administration. H and E. 400x; (2) The ovary showing oocyte surrounded by zona pellucida (ZP) which has been disrupted after 2h; (3 and 4) At 4h, atretic follicle (AF) and degenerating ovary connective tissue (CT) filled with small blood vessels (→) is observed; (5) At 10h, ovary showing further degenerative changes, pyknotic nuclei (PN) and atretic follicle is noticed; (6) Further process of atresia (→) and replacement of the theca interna cells of follicle is visualized at 20th stage.
of these enzymes suggests an increased need for protection against free radicals. Fenoterol administration to the mice enhanced the SOD activity by 15.34% initially and maximum increase i.e. 24.28% was noticed during four hour stage. Similar increase in levels of catalase, initially 7.57% with levels reaching almost 16.60% higher than corresponding vehicle control was observed (Table 1). A role for superoxide in infertility and/ or miscarriage associated with various reproductive diseases has been suggested (Ota et al., 1999). Our results were in accordance with the findings of other researchers where beta agonist induces elevation in the antioxidant enzyme levels (Sasikumar and Shyamala Devi, 2000). It is assumed that fenoterol administration resulted in altered cellular toxicity due to increased superoxide radicals responsible for oxidative stress in ovary which demonstrated negative impact of fenoterol on development and maturation of ovarian follicles.

From our study, it appears that definite pathological changes occurred in the ovary after fenoterol administration with in hours. Even though these changes were not significant enough to be classified as cause of infertility, but they were abnormal and should be noted. Since an ovulatory cycle may occur periodically even in fertile women, it may be said that the action of fenoterol on ovarian hormones can result in disturbances of ovarian cycle. Further work is to be done to determine whether these abnormal changes are associated with use of tocolytic drugs. In conclusion, it was possible that treatment with uterorelaxing substances, particularly β-adrenoceptor agonists, may alleviate the uterine discomfort. However, side effects encountered with high dose and oral administration limit their utility. Thus, further attention is needed to enhance their efficacy and reducing side effects.

REFERENCES


