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Pethidine induced changes in ovarian follicular kinetics and biochemical parameters in albino rats

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SUMMARY

Pethidine at the dose level of 0.5 mg and 0.75 mg/100 g body weight administered for 20 days to the cycling albino rats caused decrease in the ovarian weight and its protein content. The ovarian folliculogenesis in treated rats is hampered; as a result the follicles which are at the different stages of growth underwent regression. Therefore, the number of healthy follicles is reduced and atretic follicles increased. The elevated levels of ovarian cholesterol and decreased level of glycogen in the pethidine treated rats indicates the inhibition brought in steroidogenesis, which is dependent on pituitary gonadotrophins.

Key words: Pethidine; Rats; Healthy follicles; Atretic follicles; Ovarian steroids

INTRODUCTION

Pethidine (meperidine, Demerol, or phenylpiperidine) is a synthetic analgesic drug introduced by Eisleb and Schaumann in 1939. Like other opiods pethidine binds to opiod receptors and exerts its chief pharmacological actions on the CNS. Opiods act on the hypothalamus and inhibit the release of gonadotrophin releasing hormone (GnRH) and corticotropin releasing factor (CRF), thus decreasing the circulating concentrations of luteinising hormone (LH), follicle stimulating hormone (FSH), adreno-corticotropic hormone (ACTH) and β-endorphin (Jaffe and Martin, 1985). Secretions of pituitary gonadotrophins are regulated by brain and neurons situated in the anterior parts of the hypothalamus that synthesize the GnRH (Krieger *et al.*, 1982; Teresawa and Davis, 1983). According to several investigators CNS influencing drugs inhibit the release of FSH and LH from the pituitary acting through hypothalamus, blocking the neural stimulus to the gonadotrophin releasing hormone (Blake *et al.*, 1972; Blake, 1974, 1978; Anderson *et al.*, 1982).

Morphine, an opiod administered during parturition inhibits the uterine contractibility, disturbs the normal maternal behaviour and reduces the survival of pups. It also affects the established maternal behaviour in lactating rats (Russell and Spears, 1984; Russell *et al.*, 1989). Pethidine is an analgesic used much more commonly than morphine in obstetric practice. It also has opiate actions, probably mediated by μ-opiod receptors thereby having similar effects like that of morphine on inhibition of oxytocin secretion and parturition in rats (Russell *et al.*, 1991). However morphine an opiod has a well established tonic inhibitory influence on the

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hypothalamo-hypophyseal axis that disturbs the normal reproductive activities (Bruni *et al.*, 1977; Cicero *et al.*, 1977; Johnsten *et al.*, 1982; Ching, 1983; Cicero *et al.*, 1985). Reports on the effects of pethidine on reproduction are scanty. Therefore the present study is aimed to understand the effect of pethidine on ovarian activities which are dependent on hypophyseal gonadotrophins in albino rats.

MATERIALS AND METHODS

Sexually matured, healthy, colony bred virgin male and female rats of Wistar strain, aged 3 months and weighing 150 - 200 g were used for the experiments. The rats were housed in polypropylene cages measuring $12'' \times 10'' \times 8''$, under well ventilated animal house conditions (temperature: 28 - 31°C, photoperiod: 12 h natural light and 12 h dark; humidity: 50 - 55%). The rats were fed with balanced diet as per CFTRI formula and water *ad libitum*. The rats were divided into three groups of six animals each.

Group I : Received 0.2 ml saline/100 g body weight for 30 days.

Group II : Received 0.5 mg pethidine in 0.2 ml saline/100 g body weight for 30 days.

Group III : Received 0.75 mg pethidine in 0.2 ml saline/100 g body weight for 30 days

The treatment was started from estrous phase only, as the ovarian and uterine activities change markedly from one phase to another phase of estrous cycle. The saline or pethidine was administered intraperitoneally everyday between 10:00 and 11:00 AM.

All the rats were sacrificed on 31st day, 24 h after the last treatment. The ovaries were dissected out immediately and separated out from the adherent tissue and weighed to the nearest mg on an electronic balance. Organ from one side of each animal was fixed in Bouin's fluid, embedded in paraffin wax, sectioned at 5 μm, stained with haematoxylin-eosin for histological studies. Follicular diameter and morphologies were used to classify follicles by using established methods (Hirshfield, 1983; Sanjay and Joshi, 1997) as

Class I: Small preantral follicles (SPAF) (< 90 μm)

Class II: Large preantral follicles (LPAF) (91 - 260 μm)

Class III: Small antral follicles (SAF) (261 - 350 μm)

Class IV: Medium sized antral follicles (MSAF) (351 - 430 μm)

Class V: Large sized antral follicles (LSAF) (431 - 490 μm)

Class VI: Graafian follicles (GF) (> 491 μm)

Follicles under regression were classified depending on the degree of regression as:

Stage IA: Pyknosis in some granulose cells.

Stage IB: Degenerative changes in the entire granulose layer.

Stage IIA : Oocytes with pyknotic nuclei, blocked meiosis in metaphase I (pseudo maturation) and degenerating cumulus cells.

Stage IIB: Characterized by oocytes floating in the antrum with few pyknotic bodies

Morphometric studies of the ovary were made by using stage and ocular micrometer. Organ from the other side was used for biochemical estimations like cholesterol (Peters and Vanslyke, 1946), protein (Lowry *et al.*, 1951) and glycogen (Carrol *et al.*, 1956).

RESULTS

Changes in body weight

There is no significant change in the body weight, after administration of pethidine.

Ovarian gravimetric changes

Administration of 0.5 mg and 0.75 mg pethidine for 30 days has caused highly significant ($P < 0.001$) decrease in the ovarian weight (Table 1).

Biochemical changes

The pethidine administration has shown inhibitory effect on ovarian activities. Cholesterol, the precursor

Table 1. Effect of Pethidine on ovarian gravimetric and biochemical parameters in albino rats

Treatment	Ovary mg/100g BW	Cholesterol µg/mg	Protein µg/gm	Glycogen µg/gm
Saline	55.95 ± 2.04	3.26 ± 0.01	6.14 ± 0.04	2.12 ± 0.04
0.5 mg Pethidine	45.87 ± 2.08**	4.62 ± 0.03*	4.18 ± 0.05**	1.80 ± 0.06*
0.75 mg Pethidine	42.89 ± 1.09**	5.30 ± 0.05**	3.80 ± 0.04**	1.82 ± 0.03*

Dose: mg/100 g body weight; Duration: 30 days; M ± S.E. = Arithmetic mean ± Standard error; *$P < 0.01$, **$P < 0.001$, when compared to saline treated control

for steroid biosynthesis is increased significantly ($P < 0.01$) with 0.5 mg and highly significant ($P < 0.001$) with 0.75 mg pethidine administration. The protein content of ovary is decreased highly significantly ($P < 0.001$) with administration of both the doses. Glycogen, the energy reservoir of female reproduc-tive activities is decreased significantly ($P < 0.01$) with both doses (Table 1).

Ovarian follicular kinetics (Figs. 1-3)

Pethidine administration has caused decreased in the number of healthy follicles and increase in the number of regressing follicles.

The number of SPAF (Class I) and LPAF (Class II) is decreased highly significantly ($P < 0.001$) with both the doses of pethidine. This decrease in the number of SAF, MSAF, LSAF and GF (Class III - Class VI) is significant ($P < 0.01$) with 0.5 mg and highly significant ($P < 0.001$) with 0.75 mg pethidine administration.

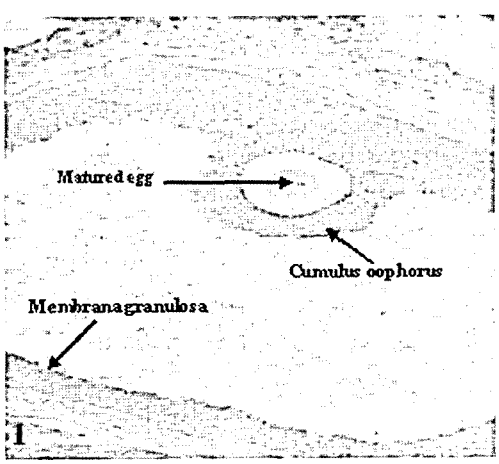

Fig. 1. Control rats with normal ovarian Graafian follicle (× 400).

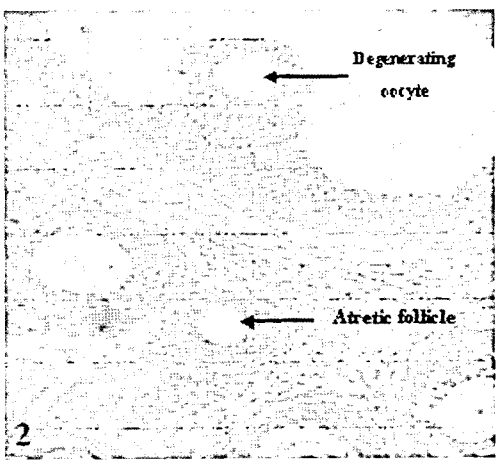

Fig. 2. Treated with 0.5 mg pethidine showing degenerating oocyte and atretic follicles (× 400).

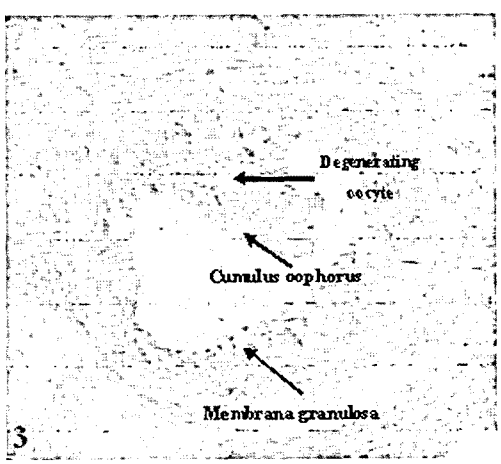

Fig. 3. Treated with 0.75 mg pethidine showing larger follicular atresia with degenerated oocyte, cumulus oophorus and granulosa membrane (× 400).

Table 2. Effect of Pethidine on ovarian follicular kinetics in albino rats

	Number of healthy follicles					
Treatment	Class I < 90 μm	Class II > 91 - 260 μm	Class III > 261-350 μm	Class IV > 351 - 430 μm	Class V > 431 - 490 μm	Class VI > 491 μm
Saline	58.21 ± 2.81	46.67 ± 0.80	11.80 ± 0.66	6.82 ± 0.42	4.08 ± 0.12	2.80 ± 0.02
0.5 mg Pethidine	38.44 ± 0.2**	36.26 ± 1.20**	7.67 ± 0.33*	3.99 ± 0.29*	2.72 ± 0.01*	1.81 ± 0.03*
0.75 mg Pethidine	32.77 ± 0.91**	28.23 ± 1.80**	4.89 ± 0.12**	3.20 ± 0.24**	0.94 ± 0.02**	0.69 ± 0.01**

	Number of regressing follicles			
Treatment	Stage IA	Stage IB	Stage IIA	Stage IIB
Saline	5.88 ± 0.04	3.47 ± 0.08	1.38 ± 0.04	1.03 ± 0.01
0.5 mg Pethidine	10.29 ± 0.88**	7.23 ± 0.60**	2.20 ± 0.09**	2.08 ± 0.05**
0.75 mg Pethidine	13.26 ± 0.94**	8.92 ± 0.24**	3.33 ± 0.04**	3.01 ± 0.11**

Dose: mg/100g body weight; Duration: 30 days; M ± S.E. = Arithmetic mean ± Standard error; *$P < 0.01$, **$P < 0.001$, when compared to saline treated control.

With both the doses of pethidine administration highly significant ($P < 0.001$) increase in the number of regressing follicles from stage IA to IIB is observed (Table 2).

DISCUSSION

It is well known that the recruitment of preantral follicles from primary follicles requires pituitary FSH (Young *et al.*, 1992). Further the growth of follicles from preantral stage upto ovulatory stage depends on both FSH and LH. The entire process of follicular growth and cell differentiation appears to involve progressive changes in the response of theca and granulose cells to LH and FSH respectively (Richards, 1980). The ovarian cycle is a consequence of the dynamic interplay between gonadotrophic stimulation of the ovary and negative and positive feedback actions of ovarian steroids on the hypothalamus and pituitary. Ovulation is triggered through the positive feedback response to estrogen and progesterone involving a midcycle surge of LH (Landgren *et al.*, 1977; Fink, 1979; Crowley and Mac Arthur, 1980).

In mammals endogenous opiod peptides are known to inhibit the release of LH-RH and this action is reversible by its antagonist naloxone (Kalra and Kalra, 1983; Meites, 1986). According to several investigators CNS influencing drugs inhibit the release of pituitary gonadotrophins from pituitary by acting through hypothalamus thereby blocking the neural stimulus to GnRH (Blake *et al.*, 1972; Blake, 1974, 1978; Anderson *et al.*, 1982). Pethidine, a synthetic opiod analgesic having similar actions of pituitary gonadotrophins via hypothalamo hypophyseal portal system (Eisenman *et al.*, 1958).

In the present study the chronic treatment of pethidine for 30 days has caused the reduction in the weight of ovary. The decrease in the number of follicles from SPAF (Class I) up to ovulating follicles or GF (Class VI) and increase in the number of regressing follicles attributes the non availability of pituitary gonadotrophins due to pethidine treatment as pethidine being a CNS influencing drug and synthetic opiod, inhibits the release of pituitary gonadotrophins (Blake *et al.*, 1972; Blake, 1974, 1978; Anderson *et al.*, 1982). As recruitment of SPAF from primary follicles depends on availability of FSH and further folliculogenesis from SPAF to GF requires FSH and LH (Richards, 1980; Young *et al.*, 1992), more follicles in pethidine treated rats undergo regression rather than maturation.

The low protein content of ovary indicates the retarded ovarian growth as FSH is essential for

protein synthesis in gonads (Means, 1975).

High accumulation of cholesterol content in the ovary of drug treated rats may be attributed to the lowered steroidogenesis which is dependent on availability of pituitary gonadotrophins.

The energy source for female reproductive activities is ovarian glycogen, that is estrogen dependent (Walaas, 1952). The supply of glycogen to different reproductive organs in female has been reported to be controlled by the ovarian estrogen and progesterone (Gregoire *et al.*, 1967). The decreased levels of glycogen in the pethidine treated ovary might be due to low ovarian steroidogenesis, which is again attributed to low availability of pituitary gonadotrophins.

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