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EFFICACY OF EXOGENOUS GONADOTROPINS ON THE MAINTENANCE OF SPERMATOGENESIS IN PETHIDINE TREATED ALBINO RATS

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Abstract : An attempt is made to induce the pethidine suppressed gonadal activities by the administration of exogenous gonadotrpins (hCG, PMSG, hCG + PMSG). Administration of 5 IU gonadotropins either separately or in combination to the rats treated with pethidine for 30 days resulted in the significant increase in the weight of testis, diameter of testis and seminiferous tubules. Gonadotropin(s) treatment stimulated the spermatogenic activity which was inhibited by pethidine. Therefore the number of spermatogonia, spermatocytes, spermatids in the seminiferous tubules and spermatozoa in cauda epididymis is increased significantly. Decreased testicular cholesterol, increased protein content and weight of accessory sex organs indicate in rejuvenation of steroidogenesis. Combination of both the gonadotropins is more effective in bringing all these activities.

Key words : exogenous gonadotropins pethidine spermatogenesis cholesterol accessory sex organs

INTRODUCTION

Pethidine is a synthetic, analgesic drug introduced by Eisleb and Schaumann in 1939. Pethidine like other opioids, bind to opiod receptors and exert their chief pharmacological actions on the CNS. Opiods act on the hypothalamus to inhibit the release of gonadotropin releasing hormone (GnRH) and corticotropin releasing factor (CRF), thus decreasing circulating concentrations of luteinising hormone (LH), follicle stimulating hormone (FSH), adrenocorticotrophic hormone (ACTH) and β-endorphin (1). The hypothalamus regulates the release of pituitary gonadotrophins i.e. FSH and LH through the neural signals causing release of gonadotropins releasing hormone - GnRH (2). Morphine an opioid has a well established tonic inhibitory influence on the neuroendocrine gonadal axis (3-7). 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 gonadotropin releasing hormone (8-11). It is well established that both the gonadotropins are essential for the testicular function, and are required for the completion of steroidogenesis and spermatogenesis (12). The studies of

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Eisenman et al (13) have shown that drug addicts have shown suppressed hypophyseal gonadotropins but increased sensitivity to exogenous chorionic gonadotrophins. Our earlier studies have shown that chronic treatment of pethidine, has resulted in the suppression of steroidogenesis and spermatogenesis because of paucity of pituitary gonadotropins (14). Therefore the present investigation is taken up to test the efficacy of exogenous gonadotropins (hCG and PMSG) on the maintenance of spermatogenesis in pethidine treated albino rats.

METHODS

Male albino rats (Wistar Strain) of 90 to 100 days old, weighing 175–200 gms were used for experimentation. The animals were maintained under laboratory conditions with lighting schedule of 12 hrs light and 12 hrs darkness. They were fed with balanced diet as prescribed by CFTRI, Mysore and water *ad libitum*.

The rats were divided into 5 groups of eight animals each and treated as follows.

Group 1: Received 0.2 ml of saline/100 g body wt for 30 days and served as controls.

Group 2: Received 0.5 mg of pethidine/100 g body wt for 30 days in 0.2 ml of saline.

Group 3: The rats treated with 0.5 mg pethidine for 30 days, received 5 IU PMSG (Folligen, intervet, international Boxmere Holland) in 0.2 ml saline.

Group 4: The rats treated with 0.5 mg pethidine for 30 days, received 5 IU hCG (Chorulon, intervet, international Boxmere Holland) in 0.2 ml saline.

Group 5: The rats treated with 0.5 mg pethidine for 30 days, received 5 IU PMSG + hCG in 0.2 ml saline.

All the injections were given intraperitoneally every day between 10.30 to 11.30 A.M. The exogenous gonadotropins were administered (PMSG, hCG, hCG+PMSG) to pethidine treated rats for 7 days. The experimental animals were autopsied by cervical dislocation on the next day of the last injection. The testis, epididymis, seminal vesicle and vas deference were dissected out and weighed upto the nearest mg on the electronic balance. The organ weights were calculated per 100 g body wt by using the formula, Organ weight (mg)/body wt (g) x 100. The left testis was processed for the estimation of cholesterol (15) and protein (16). The portion of right testis was fixed in Bouin's fluid and calcium formal for histological and histochemical studies respectively. They were processed and embedded in paraffin wax, sectioned at 5 μ thickness. The sections were stained with haematoxylene-eosin for histological observations and with Sudan Black B for histochemical localisation of lipids.

The micrometric measurements such as diameter of testis and seminiferous tubules were measured by the help of ocular and stage micrometers under light microscope. The spermatogenic element count (17) was made by randomly selected twenty round sections from each group and the cauda epididymal sperm count was made using the

method described by Kempinas and Lamano-carvalho (18). Statistical analysis was done by using Student's 't' test and the values were judged significant if P< 0.05 (19).

RESULTS

Gravimetric and micrometric changes of the testis (Table I): Pethidine treatment has caused significant reduction in the weight of testis. Administration of exogenous gonadotropins (hCG, PMSG, hCG + PMSG) for 7 days to the pethidine treated rats has caused significant increase (P<0.001) in the weight of the testis. Though, there is an increase in the diameter of testis in all the pethidine treated groups after the administration of exogenous gonadotropins, a significant increase (P<0.001) is seen only with hCG+PMSG. The diameter of seminiferous tubule is significantly increased (P<0.001) with PMSG and hCG+PMSG administration.

Biochemical changes of testis (Table I): The exogenous gonadotropins reversed the inhibitory effect of pethidine on testicular function in albino rats. Cholesterol, the precursor for sex steroid hormone is decreased due to exogenous gonadotrpin treatment, in comparison with pethidine treated controls but it is significant (P<0.001), only with hCG+PMSG treatment. The protein content of the testis is increased significantly (P<0.001) with PMSG and hCG + PMSG treatment.

Histological changes (Table II): Though there is an increase in the number of spermatogonia, it is highly significant (P<0.001) only with PMSG treatment. The number of spermatocytes and spermatids is increased significantly (P<0.001) with PMSG and hCG + PMSG. Though hCG treatment has caused increase in the spermatocytes and spermatids, it is not significant.

TABLE I : Effect of exogenous gonadotropins on gravimetric, micrometric and biochemical changes in pethidine treated albino rats.

(Duration – 7 days)

Treatment mg/100 g body wt.	*Weight of the testis/100 g body wt.*	*Cholesterol (µg/mg)*	*Protein (µg/mg)*	*Diameter of testis (mm)*	*Diameter of tubules (µm)*
Saline	1237.19±48.68	5.40±0.89	14.29±6.4	3.72±0.12	192.78±10.4
0.5 mg Pethidine	1101.51±56.78*	8.16±1.26*	8.92±5.4*	3.21±0.16*	156.40±9.4*
5 IU hCG	1253.83±18.00**	6.52±1.31	10.45±3.4	3.54±0.30	187.24±4.9*
5IU PMSG	1069.30±42.20*	7.46±0.30	13.36±4.2**	3.82±0.19*	198.08±15.40**
5IU hCG + PMSG	1291.02±18.72**	4.96±0.20**	13.90±2.9**	4.34±0.36**	220.21±12.23**

*P<0.05 compared to saline treated group;
*P<0.05; **P<0.001 compared to pethidine treated group.

TABLE II : Effect of exogenous gonadotropins on spermatogenic elements in pethidine treated albino rats.

(Duration – 7 days)

Treatment mg/100 g body wt.	*Spermatogonia*	*Spermatocytes*	*Spermatids*	*Sperm count (million/cauda)*
Saline	111.40±12.28	139.92±13.35	195.80±4.51	1.41±0.06
0.5 mg Pethidine	47.53±4.29·	87.21±2.01·	59.80±1.26·	0.73±0.09·
5 IU hCG	57.75±4.36	95.40±2.09	133.70±3.61*	0.76±0.03
5IU PMSG	107.30±2.59**	108.25±1.67**	146.30±1.78**	1.16±0.02*
5IU hCG + PMSG	98.80±5.18*	131.60±4.36	193.50±6.11**	1.53±0.07**

·P<0.05 compared to saline treated group;
*P<0.05; **P<0.001 compared to pethidine treated group.

The sperm count is significantly increased (P<0.001) with the treatment of PMSG and hCG + PMSG. This clearly indicates that the conversion of spermatogonia to spermatozoa is dependent on PMSG, which is FSH like in action. The accumulation of Sudanophillic lipids in the Leydig cells and seminiferous tubule of pethidine treated rats also indicates the lack of pituitary LH. After the administration of exogenous gonadotrophins the Sudanophilic lipid accumulation is decreased.

Gravimetric changes in the accessory reproductive organs (Table III): Though exogenous gonadotropins treatment at the level of 5 IU increased the weight of accessory reproductive organs, it is

TABLE III : Effect of exogenous gonadotropins on accessory sex organs in pethidine treated albino rats.

(Duration – 7 days)

Treatment mg/100 g body wt.	*Ogran weights mg/100 g body wt.*			
	Epididymis	*Seminal vesicle*	*Prostate*	*Vas deferens*
Saline	385.40±20.95	367.21±16.31	107.72±2.67	57.03±2.94
0.5mg Pethidine	357.99±6.10·	313.93±23.84·	93.96±5.03·	41.83±3.56·
5IU hCG	381.15±11.28**	403.61±11.73**	124.17±4.06	54.91±1.89**
5IU PMSG	303.49±14.10	381.72±13.88*	109.07±2.79*	44.33±2.68
5IU hCG + PMSG	422.12±9.22**	503.13±29.38**	136.38±11.11**	63.84±0.76**

·P<0.05 compared to saline treated group;
*P<0.05; **P<0.001 compared to pethidine treated group.

significant ($P<0.001$) with hCG and hCG + PMSG only. This significant increase in the weights of accessory sex organs can be attributed to the availability of androgens, as hCG being LH like in action might have caused the androgen production by the Leydig cells.

DISCUSSION

In mammals endogenous opioid peptides are known to inhibit the release of LH-RH, an action reversible by the antagonist naloxone (20, 21). It is observed in Lal munia (Bird) that morphine has accelerated the plumage colouration, weight of the testis and reproductive activities, suggesting that endogenous opioid peptides are important constituents of neuroendocrine mechanism that influence the development of the testis (22). These results contradicts the results of many others where morphine has well established tonic inhibitory influence on neuroendocrine gonadal axis (3-7).

Further it is shown that the naloxone which is a opioid antagonist enhances the release of LH-RH or LH (7, 23, 24).

Pethidine a synthetic analgesic opiod, influences CNS and known to alter the secretions and release of pituitary gonadotropins via hypthalamo-hypophseal portal system (13). It is also known to depress the sexual activity (13).

In the present study the treatment of pethidine for 30 days has caused reduction in the weight of testis, regression in spermatogenesis and sperm count, which may be due to the non-availability of pituitary FSH. The increase in the levels of cholesterol and Sudanophilic lipids and decrease in the weights of accessory organs may be due to non-availability of LH for steroidogenesis which has resulted in reduction of circulating androgens, hCG administered alone or in combination with PMSG might have stimulated the steroidogenesis in the Leydig cells (25, 26) as a result of which the cholesterol and Sudanophillic lipids are utilised for steroidogenesis. PMSG being more FSH like has supported the spermatogenesis as a result, the spermatogenic elements are increased after PMSG treatment in the pethidine treated rats (27). However, the administration of combined dose of gonadotrophins (PMSG + hCG) is more effective as already stated, because PMSG acts like FSH and hCG like that of LH and both these gonadotrophins are essential for normal spermatogenesis and steroidogenesis in rats.

Increase in the weight of accessory organs indicates the production of androgens in the testis.

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