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Effects of Ovariectomy or Orchidectomy and Estradiol Valerate or Testosterone Enanthate Replacement on Serum Insulin in Rats

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Abstract: Various clinical observations and experimental data from *in vitro* studies suggest that insulin and sex hormones interact. The main purpose of the present study was to examine the effects of testosterone and estradiol on serum insulin in rats. Seven week old male and female albino (Wistar) rats were used in our study. Testosterone enanthate (50 mg kg⁻¹ day⁻¹) or estradiol valerate (200 µg kg⁻¹ day⁻¹) were injected intraperitoneally or subcutaneously in orchidectomised or ovariectomised rats, respectively. In orchidectomised rats, serum insulin was decreased compared with control animals (p<0.01), on the other hand, decreasing of serum insulin was prevented by testosterone replacement (p<0.001). In ovariectomised rats, serum insulin was also decreased compared with control group (p<0.01) and decreasing of serum insulin was prevented by estradiol replacement (p<0.05). Conclusively, present findings indicated that testosterone or estradiol were serum insulin enhancer hormones in male or female rats, respectively.

Key words: Insulin, orchidectomy, ovariectomy, testosterone, estradiol, rat

INTRODUCTION

Sex steroid hormones exert major effects on insulin secretion and function. Endogenous administration of androgens has been reported to cause hyperinsulinemia (Shoupe and Lobo, 1984). Insulin resistance is also associated with androgens (Stellato *et al.*, 2000; Kapoor *et al.*, 2005; Pitteloud *et al.*, 2005). Estrogens influence insulin resistance (Nagata *et al.*, 2000), insulin sensitivity (González *et al.*, 2002) and β-cell function (Choi *et al.*, 2005). Meanwhile, the findings about the effects of sex steroids on insulin secretion are some conflicting and there are few studies on the effects of exogenous testosterone or estradiol on insulin secretion. This study was carried out to elucidate the effects of testosterone enanthate or estradiol valerate-which are used widely in clinical therapies- on serum insulin in rats.

MATERIALS AND METHODS

Animals: Adult albino (Wistar) rats weighting 200 -250 g were purchased and raised in our colony from an original stock of Pasteur institute (Tehran, Iran). The temperature was at 20-25°C and animals kept under a schedule of 12 h light: 12 h darkness (light on at: 08:00 am) with free access to water and standard laboratory chow. Care taken to examine the animals for general pathological symptoms. Food was withheld for 12-14 h before operation or death.

Materials: Testosterone enanthate, estradiol valerate were obtained from Abureyhan chemical. The commercially available solid phase [¹²⁵I] insulin RIA kit (DSLab inc., Webster) was used for insulin assay.

Protocol of study: This research was conducted in Laboratory Complex of IAU-SR (Tehran, Iran) in 2005. Animals were randomly divided into control, gonadectomised, sham, vehicle or hormone receiving male and female gonadectomised groups of 10 each. Testosterone enanthate (50 mg kg⁻¹ day⁻¹) or estradiol valerate (200 µg kg⁻¹ day⁻¹) were injected intraperitoneally or subcutaneously in orchidectomised or ovariectomised rats, respectively. In vehicle receiving gonadectomised animals, vehicle (olive oil) was also injected as same as testosterone or estradiol. Injection of estradiol, testosterone or vehicle was initiated on the third day after surgery (Yoneda *et al.*, 1998) and continued at daily intervals. Animals of each group were killed 4 weeks after operation. Following serum collection, serum insulin was measured and compared statistically between the groups.

Surgical procedure: Gonadectomy was performed according to the procedures described by Waynforth (Waynforth, 1988). Briefly, rats were anesthetized using ketamine hydrochloride (100-120 mg kg⁻¹) and xylazine hydrochloride (24 mg kg⁻¹) intramuscularly. For ovariectomy, a small midline dorsal skin incision (1-2 cm)

was made just caudal to the 13th ribs. A small cut then was made into the muscle and ovary was pulled out and removed. The incisions were sutured. For orchidectomy, the scrotal sac was cleaned with alcohol and a small incision of approximately 2 cm was made midsagittally at the scrotal septum. The spermatic cord was dissected, tied and cut. The testes were carefully removed from the scrotal sac. The incision was sutured. In sham operations, the incisions were immediately sutured and the gonadal system was not manipulated.

Serum collection: Blood samples were collected in appropriate tubes by cardiac puncture technique. After collection, the blood samples left to clot at room temperature for 15 min and then centrifuged at 2500 rpm for 15 min. The serum layer was then separated and aliquoted into small test tubes and stored at -20°C until insulin determination.

Statistical analysis: Statistical significance was evaluated by one-way analysis of variance (ANOVA) test. Significance was measured using Fishers least significant for the exact p-values and significant differences are noted in the results.

RESULTS AND DISCUSSION

There was not significant difference between serum insulin of sham gonadectomised and control animals. There was not also significant difference between serum insulin of vehicle receiving gonadectomised and hormone receiving animals. Serum insulin was decreased in orchidectomised rats compared with control animals ($p < 0.01$). Testosterone replacement ($50 \text{ mg kg}^{-1} \text{ day}^{-1}$) caused to increasing of serum insulin compared with orchidectomised rats ($p < 0.001$). The level of Serum insulin concentration in male rats was also significantly decreased in ovariectomised rats ($p < 0.01$). Estradiol replacement ($200 \text{ } \mu\text{g kg}^{-1} \text{ day}^{-1}$) caused to increasing of serum insulin compared with ovariectomised female rats ($p < 0.05$) in (Table 1 and 2).

In present study, since there was not significant difference between serum insulin of sham and control animals, the procedure of gonadectomy or injection itself did not influence the serum insulin level. The finding that testosterone enanthate was a serum insulin enhancer is comparable to those reported in the literature that have shown the enhancing effect of endogenous androgens on insulin secretion (Sumiko *et al.*, 2001; Buffington *et al.*, 1988; Holmång and Bjorntorp, 1991). However, in contrast to present finding, some evidences indicated that testosterone has not significantly affected on insulin

Table 1: Serum insulin level 4 weeks after operation in male rats

Animal	Serum insulin level uu mL ⁻¹ ±SEM	p
Control	15.14±0.78	
Sham-orchidectomised	15.94±0.65	NS
Orchidectomised	8.58±0.18	p<0.01
Vehicle receiving orchidectomised	9.35±0.89	NS
Testosterone receiving orchidectomised	17.35±0.89	*p<0.001

Data represent the Mean ±SEM of 10 rats. p<values are versus control and *: p-value is versus orchidectomised group. NS: Indicates non significant difference

Table 2: Serum insulin level 4 weeks after operation in female rats

Animal	Serum insulin level uu mL ⁻¹ ±SEM	p
Control	16.94±0.68	
Sham-ovariectomised	16.02±0.32	NS
Ovariectomised	9.62±0.09	p<0.01
Vehicle receiving ovariectomised	10.57±0.23	NS
Estradiol receiving ovariectomised	20.57±2.23	*p<0.05

Data represent the Mean±SEM of 10 rats, p<values are versus control and *: p-value is versus ovariectomised group. NS: Indicates non significant difference

secretion (Nielsen, 1984; Haffner *et al.*, 1996). The exact mechanism by which testosterone causes to increasing of serum insulin is uncertain; however, it could be due to decrease in metabolic clearance rate of insulin (McCarroll and Buchanan, 1973), impairment of hepatic capacity to extract insulin (Evans and Kissebah, 1984), increasing of insulin secretion by direct effect of testosterone on pancreas (Diaz-Sanchez *et al.*, 1995) or enhancing of insulin gene expression (Sumiko *et al.*, 2001). Testosterone also can be converted to estrogens by aromatization and it might be considered that the effects of testosterone, in part, would be those of estrogens (Holmång *et al.*, 1992). Increasing of serum insulin following estradiol valerate administration is the finding which has also been appeared in men treated with exogenous estrogens (Polderman *et al.*, 1994). The stimulating effect of estradiol on insulin secretion has also been observed in women with breast cancer (Nagata *et al.*, 2000) and ovariectomized diabetic rats (Choi *et al.*, 2005). However, in contrast to present finding, strogen administration in postmenopausal women is followed by decreasing of serum insulin level (Lindheim *et al.*, 1994). Effects of estradiol on serum insulin might be due to direct interaction of the hormone with pancreatic B-cell cytosolic receptors. Identification of nuclear insulin receptors which interact with estrogens (Morelli *et al.*, 2004) and hypertrophic effect of estradiol on individual B-cells (Zhu *et al.*, 1998) are support for direct effects of estrogen on pancreatic B-cells.

Since testosterone enanthate or estradiol valerate administration clearly increases serum insulin, this should be clinically considered in hormone therapies. However, more cellular and molecular researches are required to elucidate the exact mechanism by which testosterone or estradiol affects on insulin secretion.

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