

Full Length Research paper

Effects of ovariectomy and estradiol valerate or progesterone on serum insulin level in rats

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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 progesterone and estradiol on serum insulin in female rats. Seven week old female albino (Wistar) rats were used in our study. Progesterone (20 mg/kg/day) or estradiol valerate (200 µg/kg/day) were injected subcutaneously in ovariectomised and non-ovariectomised rats. After 4 weeks, serum insulin level was measured indicating decreased serum insulin level in ovariectomised rats compared with control group ($P<0.01$). In contrast to progesterone replacement, decreasing of serum insulin in ovariectomised rats was prevented by estradiol replacement ($P<0.05$). Serum insulin level was also increased in estradiol receiving and decreased in progesterone receiving non-ovariectomised rats ($P<0.001$). Conclusively, our findings indicated that estradiol was serum insulin enhancer hormone and progesterone was serum insulin reducer hormone in rats.

Key words: Insulin, ovariectomy, progesterone, estradiol, rat.

INTRODUCTION

Sex steroids and insulin interact in various aspects of physiological domain. Estrogens influence insulin resistance (Alexanderson et al., 2009; Saengsirisuwan et al., 2009), insulin sensitivity (Koricnac et al., 2009) and β -cell function (Choi et al., 2005). Progesterone also plays significant roles in insulin physiology (Ordóñez et al., 2008; Ordóñez et al., 2007; Gonzalez et al., 2002). Mean while, the findings about the effects of sex steroids on insulin secretion are complex and some conflicting and there are few studies on the effects of exogenous progesterone or estradiol on serum insulin level. This work was carried out to elucidate the effects of ovariectomy and progesterone or estradiol valerate – which are used widely in clinical therapies – on serum insulin level in female 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 was taken to examine the animals for general pathological symptoms. Food was withheld for 12-14 h before operation or death.

Materials

Progesterone and 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 work was conducted in Laboratory Complex of IAU – SR (Tehran, Iran) in 2007. Animals were randomly divided into control, ovariectomised, sham, vehicle receiving sham, vehicle or hormone receiving female groups of 10 each. Progesterone (20 mg/kg/day) or estradiol valerate (200 µg/kg/day) were injected subcutaneously. In vehicle receiving animals, vehicle (olive oil) was also injected as same as progesterone or estradiol. Injection of estradiol, progesterone 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.

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Table 1. Serum insulin level 4 weeks after operation in female rats. Data represent the mean \pm S.E.M. of 10 rats. P values are versus control and *p value is versus ovariectomised group. NS indicates non significant difference.

Animals	Serum insulin level \pm SEM (uu/ml)	P
Control	16.94 \pm 0.68	
Sham – Uni-OVX	15.94 \pm 0.45	NS
Sham – Bi-OVX	16.02 \pm 0.32	NS
Uni-OVX	12.41 \pm 0.87	p<0.01
Bi-OVX	9.62 \pm 0.90	p<0.01
Vehicle receiving Bi-OVX	10.57 \pm 0.23	*NS
Estradiol receiving Bi-OVX	20.57 \pm 2.23	*P<0.05
Progesterone receiving Bi-OVX	7.37 \pm 0.93	*NS

Table 2. Serum insulin level 4 weeks after hormone administration in female rats. Data represent the mean \pm S.E.M. of 10 rats. P values are versus control. NS indicates non significant difference.

Animals	Serum insulin level SEM \pm (uu/ml)	P
Control	16.94 \pm 0.68	
Vehicle receiving	17.03 \pm 0.35	NS
Estradiol receiving	32.38 \pm 0.96	P<0.001

Surgical procedure

Ovariectomy was performed according to the procedures described by Waynforth (1988). Briefly, rats were anesthetized using ketamine hydrochloride (100 - 120 mg/kg) and xylazine hydrochloride (24 mg/kg) intramuscularly. 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. 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 level of sham or vehicle receiving and control animals. There was not also significant difference between serum insulin level of vehicle and hormone receiving rats.

Serum insulin level was significantly decreased in ovariectomised rats (p<0.01) compared with control animals. In addition, serum insulin level was lower in bi-ovariectomised (bi-ovx) than uni-ovariectomised (uni-ovx) animals (P<0.01). Compared with bi-ovariectomised animals significant increasing (p<0.05) or non-significant decreasing of serum insulin level was indicated following estradiol valerate or progesterone replacement, respectively (Table 1). In non-ovariectomised rats, serum insulin level was significantly increased in estradiol treated (P<0.001), but decreased in progesterone treated (P<0.001) groups compared with control animals (Table 2).

In the present research, lacking significant difference between serum insulin level of sham or vehicle receiving and control animals indicated that the procedure of ovariectomy or injection itself did not influence the serum insulin level. The reducing effect of ovariectomy on serum insulin level was appeared mainly by reduced estradiol levels following operation. It has been shown that ovariectomy is followed by markedly reduced plasma estradiol – and not progesterone – levels (Nolan and Proietto, 1995). Our results also have shown that contrary to progesterone, estradiol replacement has reversed the reducing effect of ovariectomy on serum insulin level, confirming the potent stimulatory effects of exogenous estradiol on insulin secretion. The stimulatory effect of estradiol on insulin secretion has also previously been observed (Nagata et al., 2000).

Increasing of serum insulin level following estradiol valerate administration in non-ovariectomised rats is the

finding which has also 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 our finding, Leindheim has shown that estrogen administration in postmenopausal women is followed by decreasing of serum insulin level (Leindheim et al., 1994).

Decreased serum insulin level following progesterone treatment in non-ovariectomized animals was the finding that established the reducing effect of the hormone on serum insulin level.

Conflicting data have been reported on the effects of progesterone on insulin physiology. Foster and Balfour have indicated the reducing effect of progesterone on serum insulin level in postmenopausal women (Foster and Balfour, 1997). Conversely, it has been not found appreciable increase or decrease in serum insulin level among users of oral progesterone (Chasan-Taber et al., 1997).

From this study, we have shown that estradiol and progesterone affected conversely on serum insulin level. The opposite effect of estradiol and progesterone on insulin level was also reported in previous studies (Kumagai et al., 1993; Etchegoyen et al., 1998). In addition, it has been shown that estradiol and progesterone have different effects on insulin signaling (Ordóñez, 2007).

Effects of estradiol or progesterone on serum insulin level might be due to direct interaction of these hormones with their B-cell cytosolic receptors. Identification of specific receptors for estrogen and progesterone in pancreatic islets (Green et al., 1978; Tesone et al., 1979) and hypertrophic effect of these hormones on individual B-cells (Zhu et al., 1998) and identification of nuclear insulin receptors which interact with estrogens (Morelli et al., 2004) are support for direct effects of estrogen or progesterone on B-cells. Recent studies also have established the effects of estrogens or progesterone on insulin signaling (Koricnac et al., 2009; Koricnac et al., 2008; Ordóñez, 2007).

The findings that estradiol or progesterone is serum insulin enhancer or reducer, respectively, improve knowledge about the possible risk/benefit ratio when estradiol and progesterone are used in oral contraceptives or hormone replacement therapy taken by menopausal women with controlled type 1 diabetes mellitus.

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