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Original Article

Effect of intrapleural oxytocin injection on blood glucose level in rat (rattus norvegicous)

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Abstract

Background and purpose: The effect of Oxytocin on energy metabolism is still question. The aim of the present study was to investigate the effect of exogenous oxytocin injection in different dose and timetable on blood glucose level in rat.

Experimental approach: In this study 16 adult female rats were divided into 2 groups (Treatment 1(T1) and Treatment 2(T2)). T1 with 8 adult female rats received 0.2 IU/Kg oxytocin via intrapleural (IP) and blood glucose level was tested at 0th, 20th, 40th and 60th min after injection by collecting the blood from jugular vein. In T2 eight female rats received 0.4 IU/kg oxytocin via IP taking blood glucose measure at the same minutes as T1. The experiment tested in three replicates. Blood glucose meter (Model: 3TMSO1G) was used with glucose smart blood glucose monitoring system to the measurement of blood glucose level in rats. Data were analyzed using the GLM procedure of SAS (SAS, version 9) PDIFF was used to compare least square means among treatments adjusting by tukey test.

Key results: There were hypoglycemic tendency in the changes of the blood glucose level in both T1 and T2, 20th min after injection (88.79±3.28, 68.58±3.63, respectively), while in the remaining subjects (40th and 60th min) blood glucose level increased (115.54±4, 79.7±2.09 and 136.33±5.8, 123.54±0.9, respectively). These results showed that blood glucose level in T1 significantly higher than T2 ($p<0.0001$).

Conclusions and implications: These in vivo results showed that exogenous oxytocin can be good choice to decrease the blood glucose level very fast.

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Introduction

Oxytocin (OT) is nonpeptide hormone synthesized in the supraoptic and paraventricular nuclei of the hypothalamus (Sulu et al., 1999). It has peripheral

(hormonal) actions, and also has actions in the brain. The actions of OT are mediated by specific high affinity oxytocin receptors. These last are structurally related to the vasopressin receptor subtypes, V1a, V1b and V2 and together these receptors form a subfamily of G-protein-coupled receptors. V1b receptor is activated only at very high concentrations of OT. Within this receptor subfamily for the neurohypophysial hormones, the oxytocin receptor displays the least specificity. It can bind vasopressin with a tenfold lower affinity than OT, and for this reason the oxytocin receptor has been indicated as a “nonselective” receptor. In addition, its receptors are present in the oviduct, and the hormone in released during mating of female goats (MC Neily and Ducker, 1972).

The peripheral actions of OT mainly reflect secretion from the pituitary gland. OT effects are shown in uterine smooth muscle contraction, parturition and mammary gland muscles during milk ejection (Ayad et al., 1990). During suckling, maternal oxytocin levels are raised by somatosensory stimulation. Also, It has been seen an effect on blood pressure (Tian and Ingram, 1997), if daily OT is repeated over a 5-day period, blood pressure decreased by 10-20 mmHg, the withdrawal latency to heat stimuli is prolonged, cortisol level are decreased and insulin and cholecystokinin levels are increased (Uvnas-Moberg, 1998).

OT has an insulin effect in stimulating glucose oxidation in normal fat cells in rat (Hanif et al., 1982). In mice, it stimulates inositol phosphate metabolism, affects the pancreas directly and starts the release of insulin (Augert, 1988; Gao, 1991). When oxytocin applied to pancreas locally, it increased insulin levels by 210% and glucagon level by 528% in the rat (Stock, 1990). Additionally, oxytocin affects the release of insulin and glucagon during and after birth in sheep (Wallin, 1989). When the oxytocin was applied to islets of Langerhans in the isolated mouse pancreas, it caused release of insulin and glucagon (Gao, 1992; Gao, 1993). The insulin tolerance test (ITT) shows that overweight people release less oxytocin than ones with normal weight (Coiro, 1988; Fain, 1997). Insulin also effects oxytocin and vasopressin release in man (Fisher, 1987). Intracerebroventricular injection of 200 ng OT has significant rise not only of insulin but also of glucagon and glucose levels. Since this dose of oxytocin also caused a substantial rise of circulating oxytocin levels, these effects on glucose and glucagon may have been exerted at a peripheral site (Bjorkstrand, 1996).

Oxytocin rises in hypoglycemia, and this response is partially inhibited by dexamethasone (Chiodera, 1992). The oxytocin rise in response to insulin induced hypoglycemia was reduced in obese men. Pretreatment with the opioid antagonist naloxone enhanced the oxytocin response to hypoglycemia in obese men and suggested an abnormal activity of endogenous opioids in obesity (Coiro, 1990). In women, unlike men, endogenous opioids didn’t modulate oxytocin release during insulin induced hypoglycemia (Johnson, 1990).

There is a lot of evidence that homeostasis and the rate of glucose utilization play a role in generating the hunger signal (Le Magnen, 1985). Numerous studies with ad libitum fed rats have demonstrated that feeding is linked to the complex mechanism that controls the supply of glucose to the tissues and maintains the blood glucose level. However, glucose as a metabolite controlling feeding is valid for monogastrics, but according to Simkins *et al.*, (1965), Baile and Mayer (1970), and Baile and Della-Fera (1981), there is little evidence that glucose concentration or utilization rate has a significant role in controlling feeding in ruminants (Simkins et al., 1965; Baile and Mayer, 1970; Baile and Della-Fera, 1981).

The effects of vasopressin and OT in animals are well known, but researchers have been trying to learn more on the effects of these hormones on energy metabolism. OT and vasopressin intravenously can increase the levels of insulin and glucose in rabbits (Knudtzon, 1983). High concentration of OT and vasopressin has been identified in the human and rat pancreas (Amico et al., 1988), but actual synthesis of the peptides within the organ has not yet been established. Effects of oxytocin on pancreatic endocrine function have been demonstrated in several species. Using in-vitro islet

cell preparations from rat pancreas, Dunning et al., (1984) demonstrated that oxytocin, in concentrations similar to those found in pancreatic tissue, stimulated the release of glucagon, but not insulin, into the medium. These authors also obtained stimulation of plasma glucagon as well as insulin and glucose levels in vivo after the administration of large doses of oxytocin to the rat. Passive immunization with antibody to oxytocin decreased the glucagon response to haemorrhage in rats (Dunning, 1985). Using dogs, Vilhardt et al., (1986) confirmed that infusions of oxytocin caused an increase of blood glucose was secondary to the stimulation of glucagon. Thus, Paolisso et al., (1988) reported that the infusion of oxytocin into normal subjects caused a rise in blood glucose and glucagon which was followed later by an increase in insulin secretion. However, changes in glucose concentrations are accompanied by profound counter-regulatory hormone responses which may mask any subtle modulatory role of OT. This may be dependent upon a particular local concentration of the hormone and a paracrine action of pancreatic oxytocin on glucagon secretion remains a distinct possibility (Page, 1990). Blood glucose concentration has hypoglycemic tendency during first 7th min after OT injection (Filipov and Kasakov, 1978).

Intracerebroventicular administration of OT and an OT agonist significantly decreased food intake in a dose related manner in fasted rats. This results can be hypothesis that oxytocin also can be decrease the blood glucose level in rats (Olson et al., 1991). The aim of the present study was to investigate the effect of exogenous oxytocin injection in different dose and timetable on blood glucose level in rat (Rattus norvegicous).

Material and methods

Animals

The study was performed using sixteen female rats (Rattus norvegicous) ranging from 8-10 month of age, weighting 260-300 kg. Rats were obtained from Urmia University of veterinary medicine animal house. Throughout the experimental periods, the rats were kept at 24±2°C with 12 h light/12 h dark cycle and provided with standard food and water ad libitum. All animals' procedures complied with an approved Urmia university of veterinary medicine animal care and use committee.

For prevention of any stress in animals, separate cages designed per animal.

Drug

Oxytocin hormone provided from Abureihan Pharmaceutical Co, Iran in 5 IU/ ml vial and stored at 4°C.

Experimental design

In this study, 16 adult female rats divided in two groups. 8 rats were selected for treatment 1(T1) and 8 rats for Treatment 2 (T2). Before starting the experiment rat's neck shaved for blood collection. For prevention of stress and easy way to injection, rats placed in rat holder. The rats in T1 received 0.2 IU/Kg oxytocin (5 IU/ml) (Abureihan Pharmaceutical Co, Iran) via intrapleural (IP) injection according to Robinson and Evans (Robinson et al., 1992). For day zero (day of start experiment) blood collected at 0th, 20th, 40th and 60th min after injection (day zero). For second and third replicates (5 and 10 days, respectively) the same rats received 0.2 IU/Kg OT. Blood collection was done at 0th, 20th, 40th and 60th min after injection.

In T2 rats received 0.4 IU/Kg OT (5 IU/ml) (Abureihan Pharmaceutical Co, Iran) via IP injection. In this treatment, same as first treatment three replicates tested in zero (day of start experiment), 5 and 10 days. Blood collected from jugular vein at the same minutes after injection as T1. Heart rate and pulse were controlled during the experiment.

Blood glucose measurement

For collecting the blood from jugular vein needle inserted through skin right around the middle point between the sternum and shoulder area, where should see the vein. Syringe (1 ml) with a 23 gauge needle was used for blood collection. One drops of the blood placed on the kit and inserted in the Glucose meter

to define the level of glucose in blood. Blood glucose meter (Model: 3TMSO1G) was used with glucose smart blood glucose monitoring system to the measurement of blood glucose level in rats.

Statistical analysis

Data were analyzed as a completely randomized design (animal was nested in treatments), using the GLM procedure of SAS (SAS, version 9) PDIFF was used to compare least square means among treatments adjusting by tukey test.

Results

Difference between low and high dose injection of oxytocin on blood glucose level

Blood glucose level has significance difference between treatments. Results showed that T1 has a higher blood glucose level than T2 (123.13±3.35, 107.19±3.38 mg/dl, respectively) (Graphic 1).

Difference in blood glucose level by attention to time

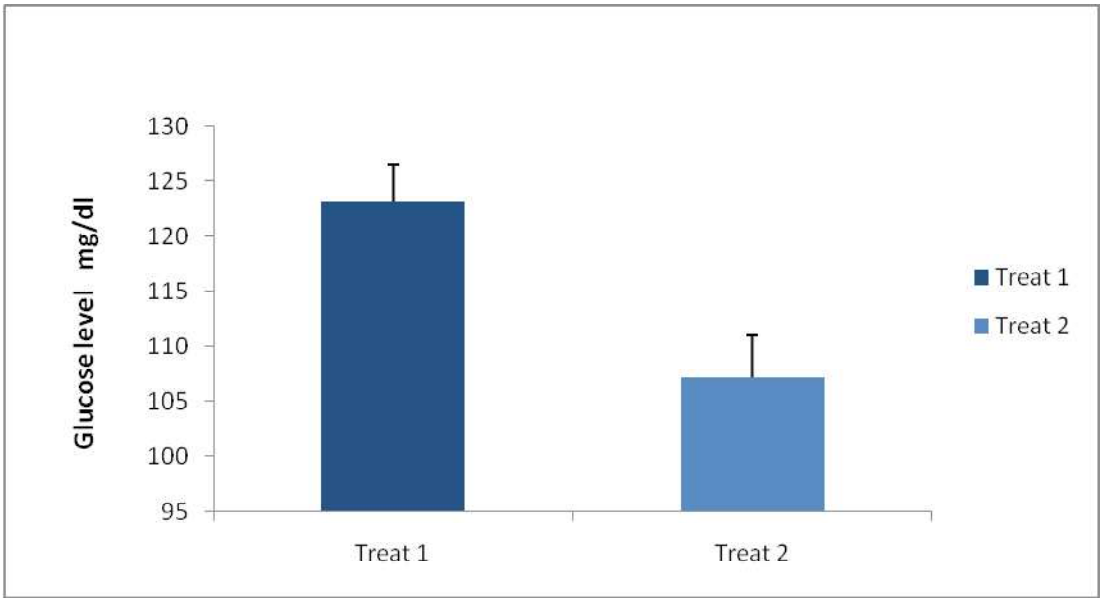
By attention to time dependent effect of oxytocin there is significant difference in time. The results shown that 20th min after injection blood glucose level is in the lowest level, which means in first minutes after injection blood glucose level has significant decrease. These results showed that 40th min after injection blood glucose level start to rise and on 60th min its level almost close to normal level. (Graphic 2)

Interaction between treatments and recolection time

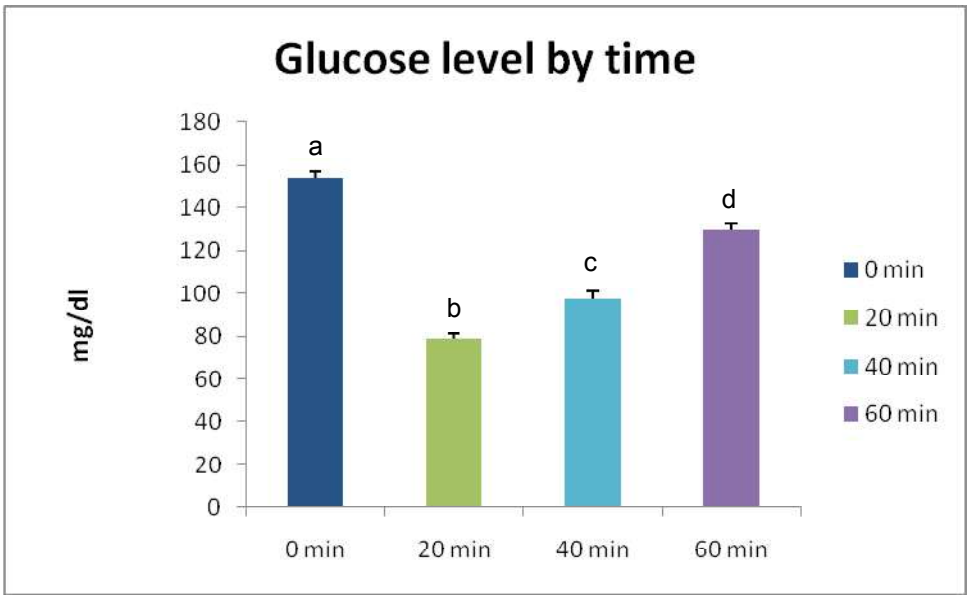
Time of blood test showed that there is significant difference in blood glucose level between treatments ($p<0.0001$). As results shown in T1 level of blood glucose in 0th min is normal, but in 20th min after injection blood glucose level decreased (88.79±3.28 mg/dl) and this followed to 40th min (115.54±4 mg/dl) to show the increase in blood glucose level and in 60th min after injection (136.33±5.8 mg/dl) blood glucose level is almost normal.

In T2 in 0th min (156.95±3.09mg/dl) blood glucose level is normal, 20 min (68.58±3.63 mg/dl) after injection blood glucose level decreased, and followed to 40th min (79.7±2.09 mg/dl) after injection which blood glucose level start to rise and in 60th min (123.54±0.94) significant increase observed.

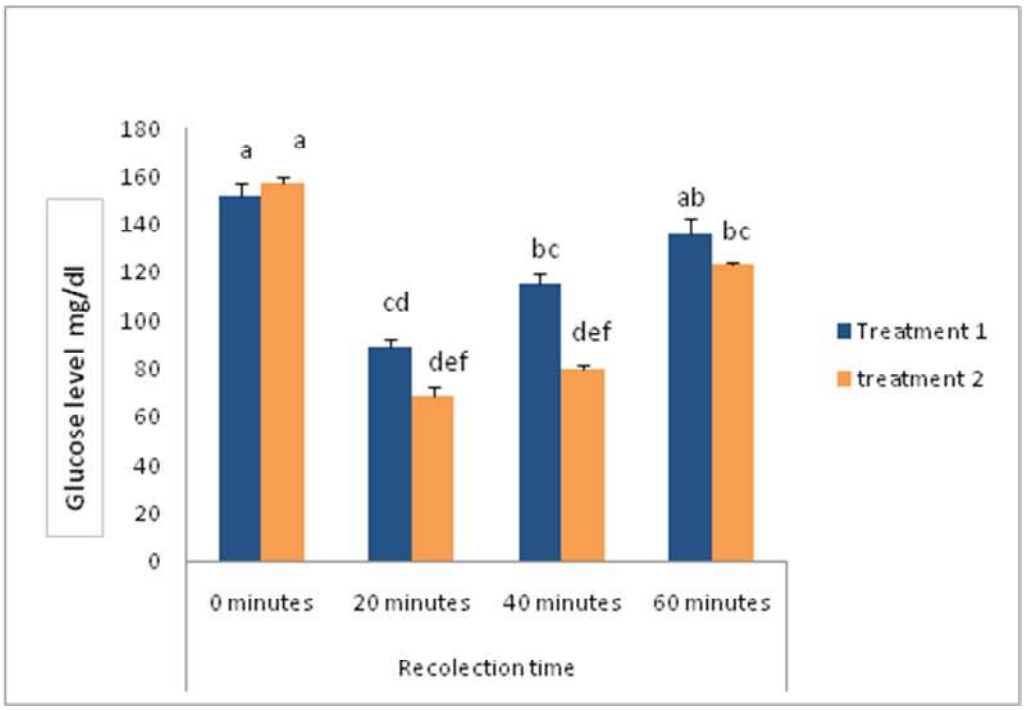
Results showed that there is significant difference between treatments by attention to the time, which



Graph 1: Blood glucose level in treatments 1 and 2, which show the significant difference between two treatments ($p<0.0001$).



Graphic 2 : Glucose level by attention to the time. Blood glucose level in 20th min after injection is the lowest, which show the Oxytocin injection effect in first 20 min.



Graph 3: Inteaction between treatments and recollection time. There is significant difference between treatments ($p<0.0001$).

means there is significant difference in 20th and 60th min after injection in T1. In T2 there is significant difference in 20th, 40th and 60th min. Also there is significant difference in 0th min with all the rest three times in two treatments (Graphic 3).

Discussions

Our results showed that there is significant difference between treatments and time. Knudtzon (1983) applied 0.3 µg OT in rabbit, founding that the glucose

level did not change statistically significant. Besides, Sulu et al., (1999) showed that glucose level in cows was the same during experiments and for control group. In contrast, they found that one of the groups, which used 1.8 mIU/kg/min OT via intravenous, distinct the glucose level dropped at 12 min but this change was not statistically significant. As a result, they believed that the effect of OT on blood glucose level does not depend on dosage. However, in our results we found the dose dependent relation for blood glucose rate which that injection of 0.2 IU/Kg OT has less decrease of blood glucose level than 0.4 IU/Kg.

Even though Filipov and Kasakov (1978) discovered hypoglycaemic tendency in blood level concentration (dose 0.1 IU/kg OT) during the first minutes in 60% of cases, they believed that effect of OT on carbohydrate metabolism is physiologically unimportant. Furthermore, the dosage it could play an important role as our results shown when treatments and 20th and 40th min are compared (Graphic 3).

Other authors highlight the importance of OT. Florian et al., (2010) suggested that OT increases glucose uptake in neonatal rat cardiomyocytes and may thus play a role in the maintenance of cardiac function and cell survival during metabolic stress. Camerino (2009) indicate that OT deficiency can induce a prediabetic state as well as a significantly increased body weight and abdominal fat pad. In addition, the ITT shows that overwight people release less OT than ones with normal weight (Coiro, 1988, Fain, 1997). We believe that also OT may use as an important factor for decrease the blood glucose level in first 20 min, especially in the people who suffer from diabetic.

Growth hormone levels decrease, while insulin levels increase whereas glucagon levels do not change considerably. (Herbein et al., 1985). Paolisso et al., (1988) observed that oxytocin enhanced the glucagon response to insulin induced hypoglycemia, but Page et al., (1990) were unable to confirm this last effect.

Concentrations of glucose in blood of animals may determine rate of steroidogenesis and gonadotropin synthesis and secretion (Lynn et al., 1965; Sen et al.,1979). Secretion of LH in cows was increased by infusion of glucose (Garmedia, 1986) or propionate (Rutter et al., 1983). The results showed that decreasing of blood glucose in first minutes after injection in the female animals could make interfere in the ovulation program.

In ewes, insulin treatment has been associated with both increased (Daniel et al., 2000) and decreased LH concentrations (Clarke et al., 1990), with the direction of the effect apparently dependent on blood glucose levels (Clarke et al., 1990; Arias et al., 1992) and dietary circumstances (Daniel et al., 2000). Then by attention to our results reduction in blood glucose level may influence the LH concentration.

To sum up, administration of 0.4 IU/kg OT showed the lower blood glucose level during the first 20th min maintaining up to 40th min. Moreover, an increment is seen at 60th min after injection. In this study we could show that blood glucose level is depend on the dose can be change and also in primary time after injection we have significant decrease in blood glucose level which most of the researcher mentioned.

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