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Full Length Research Paper

The effects of morphine on the serum level of insulin in adult male Wistar rats

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Morphine is an alkaloid with $C_{17}H_{19}NO_3$ chemical formula that extract from poppy plant and its scientific name is *Papaver somniferum*, it has psychological, analgesia and antidiarrhea effects. Also, millions of humans suffer from diabetes due to either insulin deficiency or insulin resistance. The present study reports the effects of morphine on the serum levels of insulin in adult male Wistar rats. In this empirical research work, we used 50 adult male Wistar rats weighing from 220 to 250 g. The test groups was divided into 3 subgroups containing 10 animals per group received intraperitoneally (IP) either 10, 15 and 20 mg/kg body weight morphine in 1 ml saline for 5 days and the control group received only the saline through the same route for the same period of time. Twenty four hours after the last injections, blood was drawn from the rat ventricles for serum insulin determination. The results showed that morphine administration at various dosages enhances the serum level of insulin and an increase in the dosage further elevates the insulin level significantly. It could be concluded from this investigation that morphine therapy may be beneficial for patients suffering from hypoinsulinism.

Key words: Morphine, insulin, rat.

INTRODUCTION

Morphine is an alkaloid with $C_{17}H_{19}No_3$ chemical formula that extract from poppy plant and its scientific name is *Papaver somniferum*, it has psychological, analgesia and antidiarrhea effects. Yet we identify more than 12 type of endogen peptide that has opioid function and include enkephalin and endorphin. There are more than 8 types of opoid receptor in brain and other organs such as leukocyte membrane that belong to 3 main groups of delta, mu and kappa and involve in different work such as endocrine changes induction (Jaffe and Martin,1992).

Researches show that analgesia effects of opioids apply through peripheral and central nervous system (Bodnar et al., 2004). Opioids control the transformation of pain through presynaptic and post synaptic mechanism and with controlling calcium channels and stimulating potassium channels (Holden et al., 2005). Studies show that opioids stop pain through affecting on limbic system and somatic sensory cortex (Pert and Yaksh, 1975). Studies reveal that patients that use morphine longtime are more sensitive to painful stimuli (opioid-induced hyperalgesia) (Chu et al., 2008).

Studies on animals simple show that opioids leads to the increase of Growth, Prolactin and ACTH hormones secretion and decrease TSH, Testosterone, LH, Stradiol and Oxytocin hormones (Vuong et al., 2010 ; Ogrin et al., 2005; Vescovi et al., 1985). Pancreas is one of the main glands that nervate from both sensational and autonomic nervous system and secretes insulin and glucagon and both are important in adjusting blood sugar (Rossi et al., 2005). Insulin hormone with two polypeptide chains and a molecular weight of 5800 dalton, is secreted with other proteins from the pancreatic beta-cells (Draznin and Roith, 1994; Rutter and Hill, 2006). Different researchers show that various hormonal and non hormonal factors such as Prolactin and Growth hormone (Ropero et al., 2002; Brelje et al., 2004), Estrogen (Ropero et al., 2002; Soriano et al., 2009), Glucocorticoids (Rafacho et al., 2010), Serotonin (Kim et al., 2010), Glucagons and Glucagons-like peptide-1 (Salehi et al., 2010), glucose and faty acids (Rutter and Hill, 2006; Gravena et al., 2002), neuropeptideY and vasoactive intestinal polypeptide (VIP) (Jamal et al.,1991), Somatostatin (Oliver and Kemp, 1980), Orexin (Adeghate et al., 2011), Renninangiotensin system (Leung and Carlsson, 2005), leptin (Bandaru and Shankar, 2004; Kruger et al., 2011; Brown and Dunmore, 2007; Park et al., 2010), ghrelin (Reimer et al., 2003), kisspeptin (Hauge-Evans et al., 2006), opioid and opioid analogs (Vuong et al., 2010) and

adiponectin (Wijesekara et al., 2010), have adjustment roles on beta cell function and insulin secretion.

Different factors involved in creating defect and ultimately the death of pancreatic beta cells and diabetes and we can point to malfunction of reticulum endoplasmic system and mitochondrial and production of reactive oxygen species that operate as connective factor between obesity (Eizirik et al., 2008) and insulin resistance in liver and fat tissue. Studies show that leptin, fatty acid and tumor necrosis factor (TNF) decrease sensitivity of muscles cells, liver and fat tissue to insulin hormone and these material are low in healthy people blood while it increases in fat people and diabetes type 2 patients that affect on beta cells function an insulin secretion (Fujikawa et al., 2010).

Due to a decrease in insulin secretion caused by the destruction of pancreatic beta cells or the resistance of target tissue to insulin which both lead to diabetes (Bouwens and Rooman, 2005) and based on epidemiologic studies which predict that number of 300 million in 2025 (Zimmet et al., 2001) and considering a progressive increase in the number of morphine consumption studying the effects of morphine on the secretion of insulin from the pancreas and the estimation of its serum level becomes of prime importance.

MATERIALS AND METHODS

In this research we used fifty 90-day old adult male Wistar rats weighing from 220 to 250 g obtained from Razi Vaccine and Serum Institute. The animals were divided into 5 groups of 10 animals each including two control and 3 experimental groups. Separate cages were used for each group. All the animals received rat chow and water *ad libitum*. The rat room temperature was 22°C with 12 h of darkness and 12 h of light. This research protocol was approved by the ethics committee of Azad University. In this research, one of the control groups received no treatment and the other one received saline i.p. for 5 consecutive days. The experimental groups received either 5, 10 or 15 mg/kg morphine intraperitoneally for 5 consecutive days and ultimately on the 6^{th} day, the animals were anesthetized mildly with ether and blood were collected from the heart, serum was collected by centrifugation at 5000RPM. Insulin in the sera of control and experimental groups were estimated by an Elisa method. Data were analysed by SPSS software version 13 using one way ANOVA.

RESULTS

Table 1 shows the effects of various dosages of morphine on the serum insulin level in male Wistar rats. Morphine at various dosages increased serum insulin levels significantly and dose dependently.

DISCUSSION

Morphine is one of the main components of poppy plant used by millions of people worldwide through abusing or taking drugs that include this material, which has adverse effects on different organs, particularly on endocrine glands. In this investigation we studied the effect of the intrapertoneal injection of morphine on the serum level of insulin in adult male rats. Results showed that morphine stimulates insulin secretion. Studies reveal that beta-endrophin and enkephalin like immono reactivity increase in diabetic patients (Awoke et al., 1984).

Studies show that enkaphalin and other agonists of opioid receptors of mu and delta increase glucose and insulin hormone in fat and thin rats (Bailey and Flatt, 1987). Researches show that opioid agonists such as morphine and methadone increase glucouse and decrease glicolitic enzyme's function and create insulin resistance like manner (diabetes type 2) in rats (Sadava et al., 1997).

Studies reveal that hypogonadism for long time and sex hormone deficiency cause to increase insulin resistanse and create diabetes and morphine and other opioid agonists decrease sex hormones and hypogonadism (Kapoor et al., 2006).

Studies show that consumption of agonists and opioids such as methadone and morphine for long time cause to blocking function the lper1 (Th1) cytokines and increasing the $lper_2$ (Th2) cytokines, also chronic consumption of morphine lead to decrease Interferon (IFN), Interleukin (IL)-1, TNF-α and increase IL-4 and IL-10 (Amirshahrokhi et al., 2008; Roy et al., 2005, 2004). Some researches show that methadone and other opioid agonists prevent the phagocytosis and cytokinesia function of T Lymphocyte (Li et al., 2002; Budd, 2006).

Studies reveal that mu opioid receptors agonist such as morphine involve in adjusting the function of pancreatic beta cells through controlling immune system and decreasing and increasing some cytokine (Budd, 2006). Studies show that methadone increase insulin secretion in diabetic animals (Roy et al., 2005). Studies show that morphine consumption for long time reduces leptin receptors (Anghel et al., 2008; George et al., 2010). Rats which are lack of leptin receptor or has deficiency in this receptor, hyperinsulinism occurs in them and leptin and TNF- increase insulin sensitivity in rodentia and insulin resistant people (Seufert et al., 2004; Bhansali et al., 2005). Also, leptin and TNF- decrease insulin sensitivity in muscle cells, liver and fat tissue and function of pancreatic beta cells become deficient because of leptin (Fujikawa et al., 2010).

Researches show that morphine and other opioid analogs stimulate the secretion of growth hormone, IGF-1 liver growth factor and prolactin that increase insulin secretion along with multiplying pancreatic beta cells through increasing blood sugar and stimulating insulin synthesizer gen to manifest (Vescovi et al., 1985; Roy et al., 2001; Bandaru et al., 2011).

Elevation of glucose concentration results in the entry of more glucose into pancreatic beta cells and through an increase in the rate of glycolysis, improves insulin secretion.

Table 1. Comparison of different amounts of morphine injected into the peritoneal levels of hormone insulin in the study groups.

Groups	Serum hormone insulin (std. deviation ± mean) (Pg/ml).
Control	200.556 ± 109.201
Witness (1 cc saline)	235.475 ± 156.302
Experimental 1 (minimum dose: 10 mg/kg)	3485.250** ± 713.359
Experimental 2 (average dose: 15 mg/kg)	6691.714** ± 2192.075
Experimental 3 (maximum dose: 20 mg/kg)	1223.565** ± 303.412

** indicate a significant difference in the level (P ≤0.01) with control and witness groups.

Studies show that increased glucose metabolism leads to the production of more ATP in the beta cell cytosol. ATP is the key signal that blocks the ATP dependent potassium channels in the beta cell and an increased cytosolic concentration of calcium leads to insulin secretion (Bouwens and Rooman, 2005). Studies show that opioid increase blood sugar and stimulation of insulin secretion through affecting both sympathetic and parasympathetic nervous system directly (Vuong et al., 2010). Researches show that in diabetic rats by streptozotocin, the plasma level such as TNF-α, IFN-γ and IL-1was increased and methadone consumption decrease the proinflammatory Th1 cytokines and increase the antiinflammatory cytokines such as IL-4 and IL-10 (Amirshahrokhi et al., 2008). Numerous studies show that anti inflammatory and immune system stopping factors could prevent damage beta cells an prevent diabetes (Yang et al., 2003; Maksimovic et al., 2002; Vallejo et al., 2004).

Opioid interfere in immune system function and adjust some parameters of immune system (Budd, 2006; Vallejo et al., 2004). Studies observe that methadone has no meaningful effect on blood sugar and insulin hormone in healthy rats while cause meaningful reduction in blood sugar and meaning increase of insulin hormone in diabetic rats. The protective effect of methadone can be overcome by pretreatment with naltroxane an opioid receptor antagonist (Amirshahrokhi et al., 2008).

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