Full Length Research Paper

The effect of nicotine on the serum level of insulin in adult male Wistar rats

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Nicotine is an alkaloid used by millions of people worldwide through cigarette smoking and has adverse effects on every organ and particularly on the endocrine system. Also, millions of humans suffer from diabetes due to either insulin deficiency or insulin resistance. The present study reports the effects of nicotine on the serum level 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 0.5, 1.0 or 1.5 mg/kg body weight nicotine 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 nicotine 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 nicotine therapy may be beneficial for patients suffering from hypoinsulinism.

Key words: Nicotine, insulin, rats.

INTRODUCTION

Nicotine is an alkaloid extracted from the dry leaves of Nicotiana tobacum (Karlsson and Ahrén, 1998) and millions of people worldwide are exposed to it through smoking cigarettes and also via insecticide inhalation. Nicotine consumption results in changes in cardio-vascular, neural and endocrine functions through effects on the central and peripheral nervous systems (Benowitz, 1988).

Nicotine increases the plasma levels of ACTH, epinephrine and cortisol hormones, dose dependently (Morgan et al., 2004). Through its effects on the nicotinic receptors of acetylcholine in the pancreatic beta-cells, it increases the segregation and reconstruction of inositol phospholipids and therefore improves the calcium sensitivity of these cells (Hamaguchi et al., 2003). Nicotine consumption effectively releases acetylcholine from the synaptic vesicles in the rat brain. Binding of nicotine to the presynaptic nicotinic receptors of cholinergic neural terminals leads to an increased release of acetylcholine (Yoshikawa et al., 2005). It's peripheral effects are through stimulating acetylcholine nicotinic receptors in parasympathetic and sympatho-adrenal

nervous systems (Arendash et al., 1995) It also affects the liver through epinephrine secretion which leads to an increase in the release of glucose from the liver to the blood (Benowitz,1986).

Most of acetylcholine postsynaptic functions are mediated by the nicotinic receptors which are ionotropic neurotransmitters. In the presence of an acetylcholine agonist, an increase occurs in the release of epinephrine and norepinephrine from the central part of the adrenals and also insulin from the pancrease (Iguchi, 1986; Benowitz, 1986).

The endocrine part of the pancreatic parasympathetic fibers originates from the dorsal motor vagus in the brain stem and through activating the postganglionic neurons around the pancreas, results in acetylcholine secretion in this gland and stimulates insulin secretion (Gilon and Henquin, 2001; Aleksander et al., 2009; Rashmi and Rodger, 2009).

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 Le Roith, 1994; Rutter and Hill, 2006).

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

Groups	Serum hormone insulin (std. deviation±mean) Pg/ml
Control	181.562 ± 105.201
Witness (1 cc saline)	212.375 ± 146.303
experimental 1 (minimum dose: 0.5 mg/kg)	1434.214 ± 898.149**
experimental 2 (average dose: 1 mg/kg)	1635.312 ± 413.103**
experimental 3 (maximum dose: 1.5 mg/kg)	2473.437 ± 655.030**

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

Different researchers show that various hormonal and non hormonal factors such as prolactin and growth hormone (Ropero et al., 2002; Brelje et al., 2006), estrogen (Ropero et al., 2002; Soriano et al., 2009), glucocorticoids (Rafacho et al., 2010), glucagon and glucagon-like peptide-l (GLP-1) (Salehi et al., 2010), serotonin (Kim et al., 2010) and glucose (Srinivasan et al., 2005) have adjustment roles on beta cell function and insulin secretion.

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 (Bouwens and Rooman, 2010).

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 membrane and induces membrane depolarization of the cell and an increased cytosolic concentration of calcium leads to insulin secretion (Bouwens and Rooman, 2010).

Due to a decrease in insulin secretion caused by the destruction of pancreatic beta cells or the resistance of target tissues to insulin which both lead to diabetes (Bouwens and Rooman, 2010), and based on epide-miologic studies which predict that the number of diabetic patients would increase in the world to a total of 300 million in 2025 (Zimmet et al., 2001), and considering a progressive increase in the number of cigarette smokers, studying the effects of nicotine on the secretion of insulin from the pancreas and the estimation of its serum level becomes of prime importance.

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 0.5, 1 or 1.5 mg/kg nicotine

intraperitoneally for 5 consecutive days and ultimately on the 6th day, the animals were anesthetized mildly with ether and blood were collected from the heart, serum was collected by centrifugation at 5000 rpm. 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 nicotine on the serum insulin level in male Wistar rats. Nicotine at various dosages increased serum insulin levels significantly and dose dependently.

DISCUSSION

Nicotine is one of the main components of tobacco leaves and millions of consume it through cigarette smoke and also by insecticide inhalation. Nicotine has adverse effects on different organs, particularly on endocrine glands.

In this investigation we studied the effect of the intraperitoneal injection of nicotine on the serum level of insulin in adult male rats. Results show that nicotine stimulates insulin secretion. Studies show that cholinergic agonists such as those of acetylcholine nicotinic receptors enhance the function of sympathoadrenal systems and stimulate the catecholamine hormones secretion leading to hyperglycemia (Ilcol et al., 2002, 2003; Cansev et al., 2007, 2008) and insulin secretion through direct action on the glucose level of pancreatic beta-cells (Ilcol et al., 2007). Nicotine releases acetylcholine from the cholinergic synaptic vesicles effectively and increases the removal of glucose from the liver indirectly through stimulating the muscarinic recep-tors in the nervous system leading to the stimulation of insulin secretion from pancreas (Uyama et al., 2004). Based on some studies, intraperitoneal injection of choline and phosphocholine as precursors of acetyl-choline will result in the stimulation of catecholamine secretion in plasma (Cansev et al., 2007) and cause hyperglycemia (Ilcol et al., 2007) which leads to the stimulation of insulin secretion and its elevation in blood

(Ahrén, 2000; Ilcol et al., 2008).

Binding of nicotine to nicotinic receptors of acetylcholine in the endocrine part of pancreas leads to insulin secretion (Verspohl et al., 1990; Karlsson and Ahrén, 1998). Studies show that administration of choline as a precursor of acetylcholine results in an increase in insulin level in serum through activating nicotinic and muscarinic receptors of acetylcholine (Ilcol et al., 2003; Cansev et al., 2008) thereby increasing the level of acetylcholine agonists in plasma which would have significant effects on the endocrine systems particularly on the secretary function of the endocrine part of the pancreas which produces hormones such as insulin (Gürün et al., 2002; Cavun et al., 2004). Ventrocerebric administration of choline as a precursor of acetylcholine leads to an increase in insulin secretion into blood upon stimulating the cholinergic-nicotinic and the muscarininc pathways and the use of acetylcholine antagonists such as hexamethonium decreases insulin secretion (Cansev et al., 2008). Acetylcholine and its agonists such as nicotine bind to relevant receptors and increase the production of inositol-1,4,5 triphosphate and release calcium from intracellular calcium stores and reinforce the acute effects of glucose on insulin secretion (Boyd,1992).

Nicotine and other agonists of acetylcholine stimulate GLP-1 secretion from the ileum which will result in the enhancement of insulin secretion upon increasing intracellular calcium in pancreatic beta cells (Corrigall, 1994).

Investigations reveal that GLP-1 is a glucose and an insulinotropic dependent hormone which stimulates insulin secretion from pancreatic beta cells and has some application in the treatment of diabetes (Herrmann-Rinke, 1995).

GLP-1 is able to cross the blood brain barrier and to stimulate the dorsal motor vagus resulting in an increase in insulin secretion from the pancreas. Also, GLP-1 binds to relevant receptors in the pancreatic beta cells and enhances cAMP production by increasing adenylate cyclase activity, thereby increasing insulin secretion (Sonoda et al., 2008).

Studies show that acetylcholine and its agonist such as nicotine increase the secretion of CRH secretion from hypothalamus and this neuropeptide induces hyper-glycemia and stimulates insulin secretion through stimulating ACTH and cortisol secretion (O'Carroll et al., 2004).

Binding of CRH to relevant receptors in the pancreatic beta-cells stimulates insulin secretion through an increase in the intracellular calcium concentration and the activation of protein kinase C (O'Carroll et al., 2004). Acetylcholine agonists such as nicotine stimulate AVP secretion from the hypothalamus and this neuropeptide binds to its receptor in the pancreatic beta cells, increases intracellular calcium concentration, leading to the stimulation of insulin secretion (Rashmi and Rodger, 2009). Agonists of acetylcholine nicotinic receptors stimulate the secretion of epinephrine and norepinephrine into blood stream (Cansev et al., 2007). These catecholamines also increase glucagon secretion by stimulating pancreatic receptors (Knudtzon, 1984; Ahrén and Lundquist 1987; Arslan et al., 1991). Glucagon, also, stimulates insulin secretion through stimulating pancreatic receptors in the beta cells.

Nicotine also stimulates prolactin secretion through its effect on the dorsomedial section of the arched nucleus of hypothalamus. Increased prolactin stimulates insulin secretion by increasing glucose concentration in pancreatic beta-cells (Brelje et al., 2004).

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