

Original Article



Long-term administration of intranasal insulin improves peripheral glucose concentration in diabetic male rats

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Abstract

Introduction: Experiments in rodents and humans suggest that short-term intranasal insulin administration, which is known to reach the brain, does not affect peripheral glucose concentration under diabetic conditions.

Methods: In this work, we provide evidence for the effect of intranasal insulin (10 IU/rat/day for 3 or 10 days) on serum insulin and glucose in streptozotocin-diabetic male rats using insulin measurements in the brain and periphery and a serum glucose assay 18 hours after three or ten days of nasal insulin administration.

Results: Our findings revealed peripheral insulin increased and glucose level decreased in the diabetic male rats. Based on insulin kinetics, it seems that brain insulin directly or indirectly regulates serum insulin and glucose metabolism under diabetic conditions.

Conclusion: Our results may suggest an insight into the therapeutic benefits of nasal insulin in diabetes.

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Keywords:

Brain insulin;
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Introduction

Historically, it was assumed that insulin is a hormone that is present only in the periphery and is unable to cross the blood-brain barrier (Havrankova et al., 1978). But over the past few decades it has been considered that insulin is also found in the brain (AJSzabo, 1983; Schechter et al., 1992). Brain insulin is primarily derived from pancreatic insulin and enters the brain via a saturable transport system (Banks, 2004; Banks et al., 1997; Baura et al., 1993; Pardridge et al., 1985; Schwartz et al., 1991). The idea that insulin can be synthesized in the brain is

supported by several studies (Clarke et al., 1986; Deltour et al., 1993; Devaskar et al., 1994; Giddings et al., 1985; Mehran et al., 2012; Schechter et al., 1992; Young III, 1986). It is now well known that brain insulin plays important physiological roles in the central nervous system as well as the periphery (Derakhshan and Toth, 2013).

Peripheral glucose metabolism is regulated by the direct effects of insulin on organs. Furthermore, some studies in rodents have demonstrated that brain insulin suppressed endogenous (especially hepatic) glucose production (EGP) through a brain-liver axis

(Filippi et al., 2012; Koch et al., 2008). In this regards, it has been observed that genetic manipulation of rodent brain insulin receptors can inhibit the suppression effect of brain insulin action on EGP (Koch et al., 2008; Könnner et al., 2007). Therefore, the impaired ability of insulin in glucose uptake and suppression of EGP is a hallmark of type 2 diabetes (T2D) (Filippi et al., 2012; Koch et al., 2008; Lin and Accili, 2011).

Controversial studies reported that brain insulin concentration alters under diabetic conditions. For example, Havrankova et al. (1979) showed that insulin concentrations in the type 1 diabetes (T1D) rat brain were on average two and three times more than the controls, respectively; but the differences were not statistically significant when measured 1 week or 1 month after streptozotocin (STZ) treatment (Havrankova and Roth, 1979). Also, Sakamoto et al. (1980) found that the insulin content of the rat brain was not changed among fasting, T1D and control groups (Sakamoto et al., 1980). In contrast, Ruegsegger et al. (2019) found that brain insulin concentrations were reduced in T1D mice models (Ruegsegger et al., 2019). However, there are no studies on the level of insulin in the brain of T2D. In T2D, the evidence showed that a decrease in the activity of the brain insulin system led to an impairment of energy homeostasis and peripheral insulin sensitivity (Brüning et al., 2000) and was found to affect the metabolism of the periphery (Kullmann et al., 2016). Experimental T2D animal models have shown that brain and systemic insulin resistance are linked (Arnold et al., 2018). So, it is likely that brain and systemic complications of diabetes will be reduced by overcoming this resistance. Intranasal insulin provides an effective noninvasive approach to raise brain peptide concentrations (Born et al., 2002). It has been demonstrated that intranasal insulin administration lowered EGP in healthy humans (Dash et al., 2015). Intranasal insulin may therefore be a valuable treatment in diabetes. Although, it is worth noting that overweight men under hyperinsulinemia conditions did not show EGP suppression with a short-term treatment of intranasal insulin (106 IU) (Heni et al., 2017). Whether longer term administration of intranasal insulin affects peripheral glucose concentration in a T2D model remains to be determined. Thus, in this study we considered if long-

term administration (10 days) of intranasal insulin is able to regulate peripheral glucose in type 2 diabetes. Furthermore, the serum insulin was assessed after using intranasal insulin (10 days) under diabetic and control conditions.

Materials and methods

Animals

Male wistar rats (Two-month-old; weighing 180–210g, n=6/group; total number= 24) were housed 3 per cage at room temperature ($22\pm 2^{\circ}\text{C}$), a humidity of $50\pm 10\%$ and under standard 12h light-dark cycle (the light period started at 7 A.M). Rats had free access to food and water *ad libitum*. Animal maintenance and treatment were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 80-23, revised 1996) and approved by the Animal Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.MSP.REC. 1395.377). After one-week acclimation, the animals were randomly assigned to control+ saline (C+S), diabetic+ saline (D+S), control+ intranasal insulin (C+I) and diabetic+ intranasal insulin (D+I) groups.

Diabetic animal model, measurement of serum insulin and glucose concentrations

Diabetes was induced by a single intraperitoneal injection (45mg/kg body weight) of freshly prepared STZ (Sigma-Aldrich, St. Louis, Missouri, USA) in 0.1M citrate buffer (pH 4.5). Control animals were given an equal volume of citrate buffer. Serum glucose and insulin levels were checked on day 0 (before STZ injection) and also 7 and 14 days after STZ administration. Serum insulin was measured by rat insulin ELISA kit. Serum glucose was determined using the glucose oxidase method (Yuen and McNeill, 2000). Type 2 diabetes was confirmed by fasting serum glucose levels up to 180–200mg/dl and increased serum insulin concentrations (Islam and Choi, 2007).

Intranasal insulin delivery

Intranasal delivery of insulin to the animal brain was performed as previously described by Thorne et al. (2004). Three days after STZ injection, insulin regular (Human, Recombinant DNA origin) was given intranasally at a dose of 10 units/rat/day until day 14. Each rat was placed in a supine position and 50 μl of

insulin was administered in each nostril with an Eppendorf pipette as 10 μ l drops every 1–2min (once a day). Control animals were given an equal volume of saline.

Brain insulin concentration determination

Following decapitation, the animal's brain (6 rats/group) was removed and kept on ice during the whole dissection procedure. Next, a lysis buffer solution (10mM Tris, pH=7.5, 0.5% sodium deoxycholate, 100mM NaCl, 100mM EDTA, protease inhibitor cocktail, 0.01% Triton X 100) was added to the brain tissue. After brain homogenizing, the suspension was centrifuged at 3500g for 10min at 4°C (Balbaa et al., 2017). Then, the supernatant was separated and transferred into a micro-tube and kept at -80°C. Lastly, an ultra-sensitive rat insulin ELISA kit (minimum detection: 0.02 μ g/l; Mercodia, Sweden) was used to measure the brain insulin content.

Statistical analysis

All values from the animals were expressed as mean \pm SEM. Comparisons between the groups were done using a repeated measurement two-way ANOVA (to test serum glucose and insulin concentrations) followed by a Bonferroni post-hoc test and a one-way ANOVA (to test brain insulin levels) followed by Tukey's post-hoc test. $P<0.05$ was considered as statistically significant. All statistical tests reported in this study were done using the GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA).

Results

Serum glucose and insulin concentrations

The rats serum insulin and glucose were measured after 7 and then 14 days of STZ injection. Using a two-way ANOVA, we found that fasting serum glucose levels significantly increased on days 7 and 14 after the injection of STZ compared to the control rats [treatment factor: $F(1, 5)= 351.6$, $P<0.0001$; time factor: $F(2, 10)= 166.2$, $P<0.0001$; drug \times time: $F(2, 10)= 202.8$, $P<0.0001$], [standardised mean difference (SMD)=1.41; 95% CI: -69.24 to -52.54]. The respective values for fasting insulin concentrations was also found to be significantly elevated compared to the control rats [treatment factor: $F(1, 5)= 63.61$, $P=0.0005$; time factor: $F(2, 10)= 15.80$, $P=0.0008$; drug \times time: $F(2, 10)= 16.07$, $P=0.0008$], [SMD=1.41; 95% CI: -1.14 to -0.58] (Fig.1B).

Insulin content in the brain

Brain insulin concentrations in the STZ-treated animals (31 ± 2.7 ng/ml) was significantly increased compared to the control rats (12 ± 0.38 ng/ml), [SMD=1.41; 95% CI: -36.39 to -1.612, $P<0.05$]. We also found that intranasal insulin delivery increased the brain insulin approximately 12.5 and 6.5 times higher in the control (150 ± 8.3 ng/ml), [SMD=1.41; 95% CI: -155.7 to -120.9, $P<0.0001$] and diabetic (203 ± 0.15 ng/ml), [SMD=1.41; 95% CI: -189.6 to -154.8, $P<0.0001$] groups, respectively, compared to the intranasal saline groups (Fig. 2).

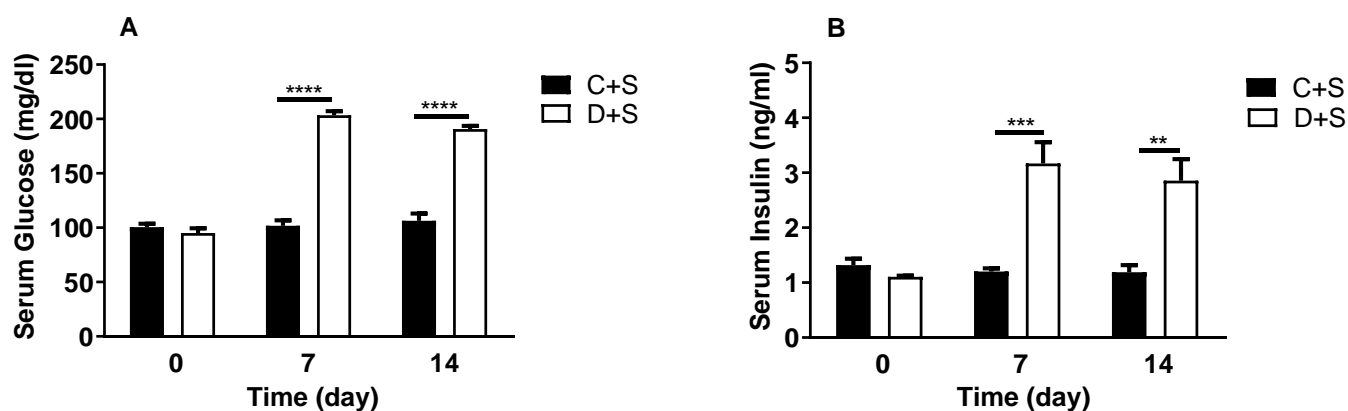


Fig.1. Serum fasting glucose and insulin concentration before the injection, 7 days, and 14 days after the injection of streptozotocin. Data are mean \pm SEM (n=6/group). The statistical test of repeated measures two-way ANOVA showed a significant increase in fasting glucose (A) and insulin (B) concentrations on days 7 and 14 in the diabetic group compared to the control group. ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

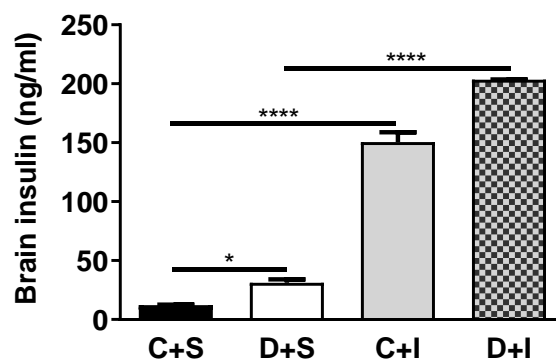


Fig.2. Insulin concentration in the rat brain. The insulin content of the brain in the diabetic group was higher than that of the control group. Intranasal administration of insulin increases levels of brain insulin in the both control and diabetes groups. Data are mean \pm SEM (n=6/group). * P <0.05, **** P <0.0001.

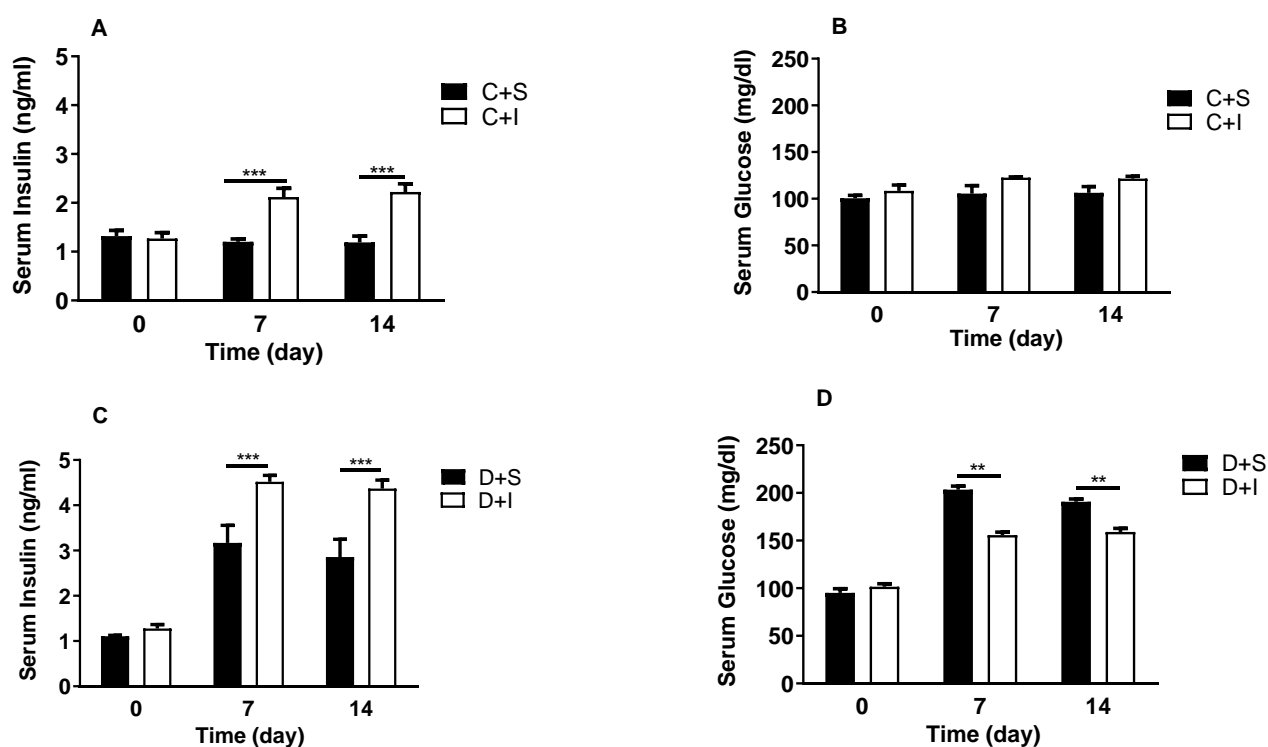


Fig.3. Concentration of insulin and glucose in serum after intranasal insulin delivery. Intranasal insulin administration increased serum insulin levels in the control group (A) but no changes in glucose levels are observed (B). In addition, intranasal insulin delivery in the diabetes group increased serum insulin levels (C) and reduced glucose levels (D). Data are mean \pm SEM (n=6/group). ** P < 0.01, *** P < 0.001.

Effect of intranasal insulin on serum glucose and insulin concentrations

As shown in Figure 3A, intranasal insulin administration in the control group increased serum levels of insulin on days 7 and 14 compared to the intranasal saline group [treatment factor: $F(1, 5)=24.25$, $P=0.0044$; time factor: $F(2, 10)=19.73$, $P=0.0003$; drug \times time: $F(2, 10)=12.95$, $P=0.0017$,

[SMD=1.41; 95% CI: -1.030 to -0.3233]. However, serum glucose levels in these two groups did not show a significant difference [treatment factor: $F(1, 5)=5.550$, $P=0.0651$; time factor: $F(2, 10)=4.039$, $P=0.0518$; drug \times time: $F(2, 10)=0.1113$, $P=0.8957$], [SMD=1.41; 95% CI: -20.68 to 0.9014] (Fig. 3B). Moreover, as shown in Figure 3C, nasal insulin delivery in the diabetic group showed a significant

increase in serum insulin concentration on days 7 and 14 after streptozotocin injection compared to the intranasal saline group [treatment factor: $F(1, 5)=28.30$, $P=0.0031$; time factor: $F(2, 10)=124.2$, $P<0.0001$; drug \times time: $F(2, 10)=10.30$, $P=0.0037$], [SMD=1.41; 95% CI: -1.346 to -0.4690]. We also found that nasal insulin delivery in the diabetic group decreased serum glucose concentrations (~25%) compared to the intranasal saline group [treatment factor: $F(1, 5)=39.02$, $P=0.0015$; time factor: $F(2, 10)=686.1$, $P<0.0001$; drug \times time: $F(2, 10)=9.464$, $P=0.0049$], [SMD=1.42; 95% CI: 7.978 to 19.13] (Fig. 3D).

Discussion

The current findings demonstrate that 1) long-term administration of intranasal insulin decreased peripheral glucose in diabetic rats but not in normal rats; 2) long-term administration of intranasal insulin increases the brain and serum insulin levels in the control and diabetes rats and 3) the brain insulin level increases significantly under early diabetic conditions. We found that the SMD between the groups was ~1.41.

Brain insulin plays the role of a neuroregulatory peptide in feeding behavior, energy storage (Benedict et al., 2008; Hallschmid et al., 2012), cognitive and memory functions (Craft et al., 2012). Insulin effects in the central nervous system (CNS) raise the question of whether CNS locally produces insulin. Rat and mouse brain RT-PCR study revealed the existence of insulin 2 mRNA (Devaskar et al., 1993; Mehran et al., 2012) and insulin gene expression in rabbit brain olfactory bulbs and hippocampal neurons confirmed brain insulin production (Devaskar et al., 1994). The above mentioned studies provided evidence for brain insulin production. It should be mentioned that although brain insulin mRNA expression and insulin peptide production have been reported, other studies have denied the evidence of insulin production in the brain (Adamo et al., 1989; Coker III et al., 1990; Devaskar et al., 1993; Woods et al., 2003). In the current study, brain and serum insulin concentrations were ~12ng/ml and 1.2ng/ml, respectively. Our work reported that rat brain insulin concentration was ~10 folds higher than serum insulin concentration. This is in line with the study by Havrankova et al. (1978) which showed the insulin concentration of the rat brain is 10-100 times higher

than that found in plasma.

Reduced brain cell sensitivity to insulin action has been defined as insulin resistance (Mielke et al., 2005) which could be due to insulin receptor down-regulation, an inability of insulin to bind to receptors or an insulin signaling cascade impairment. In our experimental type 2 diabetes model, we observed that brain and serum insulin levels as compared to control groups were ~31ng/ml (~3 folds increase) and ~3.01ng/ml (~2.5 folds increases), respectively. It has been demonstrated that systemic insulin enters into the brain interstitial fluid through two selective and saturable transport system including the cerebrospinal fluid (CSF) and capillary endothelial cells of the blood-brain barrier. This transport system is impaired by a number of factors, including hyperglycaemia, diabetes mellitus, obesity and inflammation (Banks, 2004; Banks et al., 1997; Banks et al., 2012; Baura et al., 1993; Pardridge et al., 1985; Schwartz et al., 1991). Such findings raise the question of whether impaired insulin transport increases insulin resistance and T2D side effects (Freiherr et al., 2013). Our results indicate that a concentration gradient exists between brain and serum insulin concentrations in early T2D rats, with brain concentration being ~10-fold higher. One possible explanation is that a part of insulin found in the brain is derived from brain insulin production to compensate for impaired insulin signaling and hypometabolism. Clearly, this finding requires follow-up investigation. In this context, intranasal administration of insulin to raise brain insulin is a great hope. Intranasal delivery appears to increase brain insulin concentrations in animals (Ramos-Rodriguez et al., 2017; Salameh et al., 2015) as well as humans (Born et al., 2002). Preliminary reports suggest that intranasal insulin use has a positive effects on memory and pathology in mice models (Chen et al., 2014; Zhang et al., 2016), but not in rat high fat diet-induced T2D (McNay et al., 2010). These results suggest that brain insulin resistance has developed and insulin treatment may not be sufficient to overcome resistance at a cellular level. Our results demonstrate that intranasal insulin delivery (10 IU/rat/day for 3 or 10 days) raises the brain insulin roughly ~12.5 and 6.5 times higher in control and diabetic groups as compared to intranasal saline groups. Born et al. (2002) reported that intranasal regular human insulin (40 international unit)

administration raised the CSF insulin concentration but did not affect serum concentration. In the current study, repeated intranasal insulin delivery increased serum insulin under diabetic condition (~1.5 folds) in the control rats. In contrast, intranasal insulin decreased peripheral glucose concentration in the diabetes groups but not in the control groups. We did not observe a significant difference between 3 and 10 days of nasal insulin administration. It has been reported that a small amounts of insulin absorbs into the circulation shortly after large doses of intranasal insulin administration (Dash et al., 2015; Gancheva et al., 2015; Guthoff et al., 2010; Hallschmid et al., 2012; Heni et al., 2012; Heni et al., 2014), which may contribute in acute regulation of peripheral glucose metabolism and insulin sensitivity (Hallschmid et al., 2012; Heni et al., 2012; Heni et al., 2014). The peak plasma insulin level is seen 30-40min after intranasal administration of insulin and decreases to baseline by 60min (Dash et al., 2015). In the current study, we measured serum insulin and glucose 18h after intranasal insulin. In other words, when brain insulin concentration is high, peripheral insulin level will likely have declined to a basal concentration. Therefore, the increased insulin level in this study may suggest that insulin entered the brain via the nasal route directly or it may indirectly influence peripheral insulin level. In this regard, Heni et al. (2012) found an increase in hypothalamic activity that was related to an increased peripheral plasma level after nasal insulin spray in healthy human (about 20-30%). We showed in this work that nasal insulin decreased peripheral glucose (~25%) under diabetic conditions. In previous works, brain insulin administration did not affect endogenous glucose production and glucose uptake in overweight (Heni et al., 2017) and in insulin resistant men (Xiao et al., 2018). Therefore, a lack of modulation of peripheral glucose might be associated with lowering the sensitivity in central insulin suppression of endogenous glucose production in insulin resistance. On the other hand, the time scales of this studies of intranasal insulin suggest that central insulin does not rapidly affect glucose production in the acute setting. As the current study shows, increasing brain insulin with longer-term (in several days instead of a few hours) administration may improve glucose metabolism under diabetic conditions. During intranasal insulin therapy, we measured the peripheral glucose on day 3. Additional

studies are also needed to evaluate the systemic glucose concentration on the first and second days as well as considering possibly insulin signaling in the liver in the context of brain insulin action under diabetic conditions.

Conclusion

We have provided preliminary evidence that long-term nasal insulin application decreased peripheral glucose concentration in diabetic rats. Our results may provide an insight into the therapeutic benefits of intranasal insulin in diabetes.

Acknowledgments

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Conflict of interest

The authors declare that there is no conflict of interest.

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