

Effect of intranasal insulin on peripheral glucose profile in dexamethasone-induced insulin resistance in Wistar rats

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ABSTRACT

This study evaluates the therapeutic potential of intranasal insulin (INI) in dexamethasone-induced insulin resistance in Wistar rats. In the first phase of the study, thirteen, healthy, untreated male Wistar rats were divided into 2 groups and administered either vehicle (0.9% Normal saline, 20 µl) or insulin (2 IU) intranasally to assess intranasal delivery of insulin in brain. In the second phase of experiments, to evaluate the acute effects of intranasal insulin on peripheral blood glucose, intranasal or intraperitoneal insulin was co-administered with or without dexamethasone 10 mg/kg to 26 male Wistar rats and blood glucose monitored. To evaluate effect of intranasal insulin in peripheral metabolic disease model, insulin or vehicle was administered via intranasal or intraperitoneal (IP) route to control or dexamethasone (Dex)-treated (0.5 mg/kg, IP) female Wistar rats for seven consecutive days. Twenty-four hours after last dose, trunk blood was collected via cardiac puncture. Biochemical assay of glucose, lipid and insulin was performed on serum while enzyme activity – glucokinase and glucose-6-phosphatase (G6Pase) or lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G6PDH) were assayed from liver and brain homogenates respectively. Acute intranasal but not intraperitoneal insulin elevated brain insulin after 30 min. In animals administered single dose of 10 mg/kg dexamethasone, intranasal and intraperitoneal insulin lowered blood glucose within one hour. However, only the former's effect was maintained at the 3rd and fourth hour. Dex-induced hyperglycemia was associated with increased hepatic glucose-6-phosphatase activity and decreased high-density lipoprotein (HDL), these effect were attenuated by subchronic (INI) administration. Also INI did not induce oxidative stress in the brain which suggests no brain damage during the period of study. Subchronic administration of INI was able to reduce the effect of Dex-induced hyperglycemia that is associated with increased hepatic glucose-6-phosphatase activity and decreased high-density lipoprotein (HDL) without damage to the brain. We demonstrate the potential of brain targeting with intranasal insulin in a rat model of insulin resistance and peripheral metabolic disease.

1. Introduction

Since the identification of insulin and insulin receptors in the brain, it has been proposed that the brain plays an important role in peripheral glucose metabolism (Havrankova et al., 1978). The brain integrates neural, hormonal and metabolic factors in its acute and long term regulation of energy homeostasis (Schwartz et al., 2000). While nutrient factors such as fatty acids may be involved in acute control of energy intake, insulin and leptin have emerged as the primary hormones involved in long term regulation of energy intake (Cintra et al., 2007). Peripheral insulin can reach the hypothalamus where it modulates food intake via acting on its putative receptors (Plum et al., 2005), but direct administration of insulin to the brain can modulate

peripheral glucose levels independent of circulating insulin levels. Recent investigations are beginning to describe the neural cells and pathways that govern the brain control of peripheral glucose (Pocai et al., 2005; Derakhshan and Toth, 2013; Blázquez et al., 2014). According to Obici et al. (2002), specialized glucose-sensitive neurons in the hypothalamus are known to affect key metabolic organs such as pancreatic beta cells, liver, adipose tissue via neural and hormonal pathways; and insulin acts on the hypothalamus to inhibit peripheral hepatic gluconeogenesis independent of circulating insulin concentration, hence the vagal and sympathetic nerves serve as channel between these brain cells and peripheral organs (Ott et al., 2012). This route of brain-periphery connection therefore presents an opportunity to modulate the activity of the brain cells to effect changes in function of these

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peripheral tissues.

Although the BBB effectively protects the brain it can prevent important xenobiotics from entering the central nervous system (Miyake and Bleier, 2015), several methods have thus been used to deliver insulin to the brain experimentally including intracerebroventricular and intrathecal administration (Pericles et al., 2014). Both methods are invasive injection techniques of substances directly into the cerebrospinal fluid through the cerebral ventricles bypassing the blood brain barrier (BBB) or through the spinal cord respectively (Glascocock et al., 2011).

Intranasal delivery of neurologic agents to the brain along the olfactory neural pathway was first established in a report by Frey in 1997 as a way of bypassing the blood-brain barrier (Frey, 1997), and in 2001 Frey disclose the method for transporting Insulin-like growth factor-1 (IGF-1); Insulin to the brain by similar pathway for various brain disorders (Frey, 2001). Through the intranasal insulin transfer method established by Frey, several studies have demonstrated that intranasal insulin improves memory in both patients with Alzheimer's disease and in normal adult humans without altering the blood levels of glucose or insulin (Born et al., 2002; Reger et al., 2006; Christian et al., 2007; Schiöth et al., 2012). Intranasal insulin was reported to be of use as antiobesity agent by reduction in body fat in men (Manfred et al., 2004), this route has also been used in the delivery of rat mesenchymal stem cells to the brain in order to minimize distribution of cellular grafts to peripheral organs (Danielyan et al., 2009). Intranasal route conveys insulin (extracellularly) and other drugs to the brain from the nasal cavity via the olfactory and trigeminal nerves (Jogani et al., 2008). The intranasal route comprises the intracellular pathway which involves endocytosis and the extracellular pathway which involves transport of drugs directly into the cerebral spinal fluid (Crowe et al., 2017). Animal models have been developed to study effects and mechanisms of steroid induced metabolic disturbances in rodents. This models resemble the human conditions as hyperglycemia insulin resistance and dyslipidemia and have all been described in these models (Marks et al., 2009; Francis et al 2009; Hanson et al 2013; Zhang et al 2016), it can be expected that insulin acts via its receptors in discrete regions of the brain and several brain regions express insulin receptors particularly the hypothalamus (Baquedano et al., 2016).

In this study we aim to deliver insulin into the brain via the intranasal route to evaluate its effects on peripheral metabolic parameters in dexamethasone-induced insulin resistance in Wistar rats.

We hypothesize that intranasal delivery of insulin would result in sufficient amount of insulin reaching its brain targets and ameliorate dexamethasone-induced metabolic derangements.

2. Materials and methods

2.1. Animals

Adult male and female Wistar rats were obtained and housed in groups of seven and acclimatized for one week to the housing facility of the College of Health Sciences. Housing conditions were temperature of 25 °C, and a 12:12 light:dark cycle with light on by 0700 and off at 1900. Cage dimensions are 610*435*215 mm, floor area 2065 cm² with wood shavings as beddings. Animals had access to formulated rat chow Top Feed Nigeria Ltd. made of (maize, soybean, GNC, corn brown, wheat offal, palm kernel cake and bone) and water *ad libitum*. Animals were monitored at least once daily. No adverse effects were recorded. Initial animal weight was 180 ± 20 g. Animals were further acclimated for another one week to intranasal procedures. The initial exploratory acute insulin experiment was conducted with 14 animals (7 in each group). However, one animal in intranasal insulin group died post anaesthesia and intranasal insulin and was excluded from study. Experimenters who performed intranasal procedures were blinded to the pharmacological treatment. All biochemical assays were also performed by third-party blinded from the whole experiment. However, all

data received was included in eventual data analysis and results. All procedures were carried out following written ethical approval obtained from the University Ethical Committee (UERC/ASN/2017/1004) and in accordance with NIH guidelines on animal handling. All sections of this report follow the ARRIVE Guidelines for reporting animal research. A completed ARRIVE Guideline checklist is included in Checklist S1.

2.2. Drugs

Recombinant human insulin (Actrapid, 100 IU/ml, Novo Nordisk) was purchased from MedPlus pharmacy Ilorin. All biochemical reagents including ELISA kits for rat insulin and corticosterone, assay kits for lipids, lactase dehydrogenase, glucose-6 phosphate dehydrogenase, glucokinase, glucose-6-phosphatase were obtained from Elabscience (US). All other chemicals were of analytical grade and commercially available. Accucheck glucometer and glucose strips used were from Roche (Germany), Micropipette P-10, Eppendorf).

2.3. Intranasal administration

Rats were anesthetized by placing them in tightly sealed transparent glass jars containing isoflurane (5%) obtained from Southern Anaesthesia & Surgical, (West Columbia) for brief periods (< 30 s), removed and restrained in the supine position. A total of 2 IU (1 IU/10 µl; 10 µl/nostiril) of rapid acting insulin or vehicle (0.9% Sodium Chloride, NaCl) was quickly administered intranasally using a micropipette (P-10, Eppendorf). The intranasal administration procedure lasts about 15 s. Animals regain consciousness in another 15–20 s, are monitored and returned to their respective cages.

2.4. Acute insulin study

A total of 13 male Wistar rats were used for the first segment of the first phase of the experiment. Animals were divided into 2 groups (n = 6/7). Group 1 received intranasal insulin (2 IU) while group 2 received intranasal vehicle (0.9% NaCl) as described above. Thirty minutes after drug administration, animals were euthanized by administration of 60 mg/kg pentobarbital intraperitoneally and about 3 mls of blood was collected from the heart through cardiac puncture after which the heart was perfused with 20 mls of phosphate buffered saline. The animals were quickly decapitated and the whole brain carefully excised and placed in ice-cold 4% sucrose solution. The brain was then homogenised using a manual glass homogenizer while blood was allowed to clot for 45 min at room temperature. Blood and brain homogenates were centrifuged (1000 × g for 10 min) after which serum and supernatant respectively were collected using clean Pasteur pipette into dry microcentrifuge Eppendorf tubes. All samples were immediately assayed for insulin and corticosterone content.

Another set of male Wistar rats, were used in the second segment of the first phase of experiments. Since single high dose dexamethasone (dex) could induce hyperglycemia within four hours in rats, we investigated whether INI could modulate this dex effect. Male Wistar rats received single intraperitoneal dose of either 0.9% NaCl or dexamethasone (Hubei Tianyao Pharmaceutical Co Ltd) at a dose of 10 mg/kg body weight. This was immediately followed by either intranasal vehicle or insulin 2 IU (Table 1). Each animal received a maximum of 20 µl (10 µl/nostiril). Blood glucose was monitored at 0, 1, 3 and 6 h after insulin administration. No adverse effects of intranasal administration were observed. Drug administration was carried out between 10:00 and 11:00 hrs. These same set of animals were used after one week to evaluate the use of pentobarbital (30 mg/kg) as sedative as an alternative to isoflurane. However, significant mortality was recorded in animals receiving intranasal administration irrespective of whether animal received vehicle or insulin.

In a similar experiment, twenty-six male Wistar rats were

administered single intraperitoneal dose of either 0.9% NaCl or dexamethasone (Hubei Tianyao Pharmaceutical Co Ltd) at a dose of 10 mg/kg body weight. This was immediately followed by either intranasal vehicle or insulin 2 IU (Table 1). Each animal received a maximum of 20 μ l (10 μ l/nostiril). Blood glucose was monitored at 0 and 4 h only after insulin administration.

2.5. Sub-chronic intranasal insulin during insulin resistance study

After confirming effect of INI on normal and dexamethasone treated animals, we next questioned whether intranasal administration could prevent glucocorticoid-induced hyperglycemia and Insulin resistance. Twenty-six adult Female Wistar rats were randomly allocated (simple randomization) into 5 groups of five animals each. Animals randomized to insulin resistance group received intraperitoneal dexamethasone at a dose of 0.5 mg/kg body weight for seven days, this protocol has previously been shown to induce mild hyperglycemia, dyslipidemia in female wistar rats (Rafacho et al., 2010), and control animals received 0.9% normal saline. Intranasal insulin 2 IU or vehicle was immediately administered to each animal in control and dex-treated animals during the entire seven-day period (Table 1). Drug administration was carried out between 10:00 and 11:00 hrs daily. 24 h after last drug administration, animals were sacrificed by cervical dislocation after an overnight fast of 12 h, blood was collected for biochemical analysis via cardiac puncture, organs such as brain, liver, and pancreas were excised and immediately placed on ice. The brain was homogenized using ice-cold 0.25 M sucrose solution in the brain weight: volume ratio of 1:4 with an electrical homogenizer (Teflon homogenizer, 2700 rpm by Axion medicals), this was centrifuged (5000 $\times$ g for 5 min), the supernatant collected using a micropipette into duplicate clean dry sample bottles and assayed for insulin, lactate dehydrogenase and Glucose-6-phosphate dehydrogenase.

The caudal part of the liver was excised and homogenized with 0.25 M sucrose solution in the liver weight: volume ratio of 1:4 of the weight of the liver to that of the volume of the 0.25 M sucrose solution, the homogenate was centrifuged at 5000 $\times$ g for 5 min, the supernatant collected using a micropipette into duplicate clean dry sample bottles and assayed for glucokinase, glucose-6-phosphatase...

Table 1

Scheme of control and experimental groups. Groups 1, 2, 4 and 5 are controls.

	Intranasal Vehicle	Intranasal Insulin	Intraperitoneal insulin
Intraperitoneal vehicle	Group 1	Group 5	
Intraperitoneal dexamethasone	Group 2	Group 3	Group 4

Group 1 received intranasal vehicle and intraperitoneal vehicle and serve as the vehicular control for all groups. Animals in group 2 received intranasal vehicle and intraperitoneal dexamethasone alone and serve as negative control for group 3. Group 3 is the treatment group and the animals received intranasal insulin and intraperitoneal dexamethasone. Animals in group 4 received intraperitoneal insulin and intraperitoneal dexamethasone and serve as the positive control for group 3. Group 5 controls for the effect of intranasal insulin on normal physiologic state.

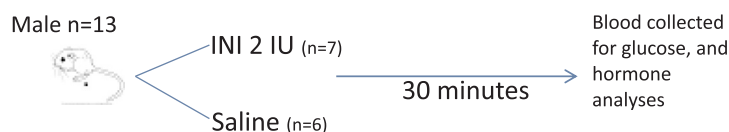
2.6. Hormonal assays

The hormone concentrations were determined with an enzyme linked immunosorbent rat insulin and corticosterone assay (ELISA) kit (Elabscience) and the absorbance measured at 450 nm was detected using a microplate reader. All biochemical assays were performed by a third party completely blinded from the experiments. All data received were included in the results. However, no blinding was done during result assessment.

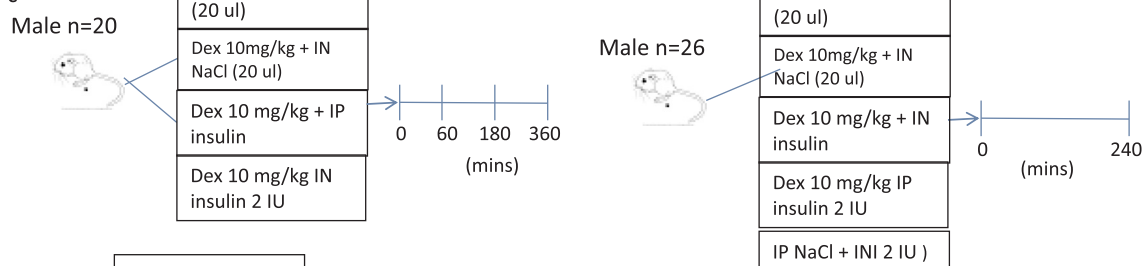
2.7. Statistical analysis

All data are expressed as mean $\pm$ standard error of mean (mean $\pm$ SEM). Statistical group analysis was performed with GraphPad Prism (version 6.0) statistical software using the one-way/repeated ANOVA and Tukeys multiple comparison test. Values of $p \leq 0.05$ were considered significant.

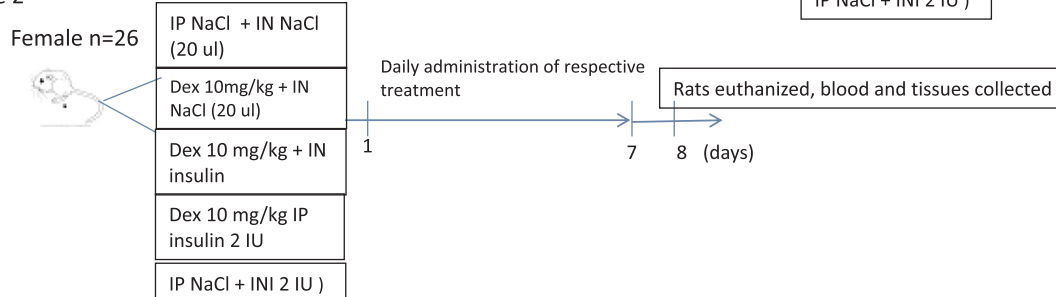
Phase 1a



Phase 1b, c



Phase 2



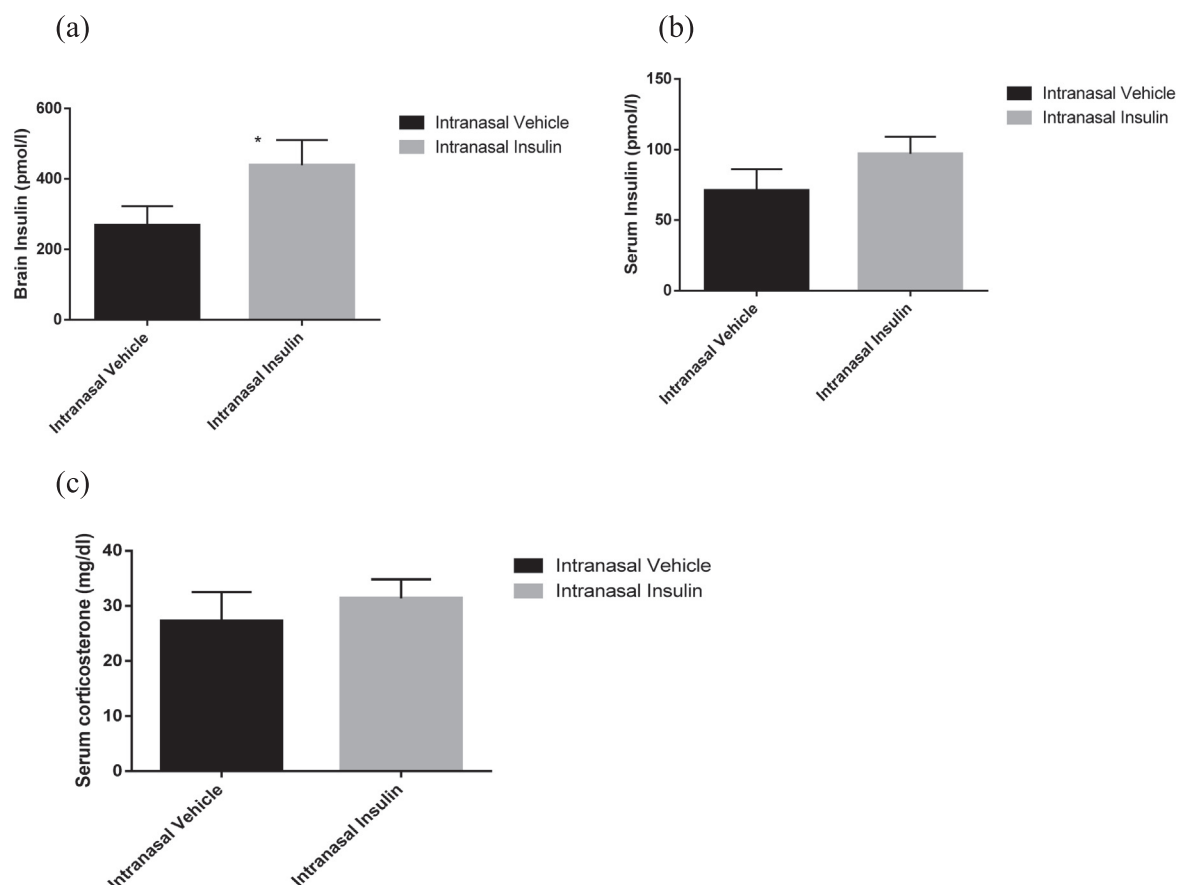


Fig. 1. Insulin levels after single administration of intranasal insulin in (a) Brain insulin, (b) Plasma insulin, and (c) serum corticosterone level. Values are means of 6/7 animals per group. * $p < 0.05$ compared with the control group.

3. Results

3.1. Intranasal insulin (2 IU) elevates insulin concentration in brain homogenate but not serum within thirty minutes.

Insulin concentration was measured in brain and serum thirty minutes after intranasal administration of either vehicle or insulin. Brain insulin concentration was 64% higher following INI compared to intranasal vehicle ($p = 0.04$) (Fig. 1A). We did not observe an increase in serum insulin during this period in any of the groups (Fig. 1B). Since, INI may elevate serum corticosterone, we assayed for this hormone. Intranasal insulin did not significantly elevate serum corticosterone ($p = 0.52$) (Fig. 1C).

3.2. Effects of acute intranasal insulin administration on blood glucose

Following confirmation of insulin delivery to brain via the intranasal route, we then determined whether intranasal insulin (INI) had effect on blood glucose in comparison with intraperitoneal insulin (IPI) in fasted normal animals. Intranasal insulin significantly lowered peripheral blood glucose after four hours of administration ($p = 0.04$) (Fig. 2A). To determine whether effect of INI was retained in dexamethasone treated animals, we compared effects of INI and IPI in dexamethasone treated animals. The results show a time-dependent effect of INI on blood glucose. Both intranasal and intraperitoneal insulin were able to lower blood glucose within one hour suggesting that insulin could reach systemic circulation following intranasal administration and exert effects on blood glucose similar to intraperitoneal insulin. The blood glucose lowering effect of INI was significantly retained for three hours ($p = 0.03$), effect which was absent in the group

that received IPI suggesting that INI effect at that time point is not dependent on systemic insulin (Fig. 2B).

3.3. Effects of repeated dexamethasone and intranasal insulin administration on serum glucose

After confirmation of acute effects of intranasal insulin (INI) in normal and dexamethasone treated animals, we further sought to investigate whether INI has effect on glycemic parameters following repeated administration. Repeated administration of 0.5 mg/kg dexamethasone did not result in a significant increase in blood glucose in Wistar rats. However, INI on its own resulted in significantly elevated blood glucose compared to control animals that received vehicle (Fig. 3).

3.4. Effects of repeated intranasal insulin administration on fasting serum insulin

Intraperitoneal administration of dexamethasone at a dose of 0.5 mg/kg for seven days tended to increase serum insulin levels in dexamethasone-treated female rats (Fig. 4). However, it was observed that the increase was not statistically significant ($p = 0.1602$). Neither intranasal nor intraperitoneal insulin significantly increased basal insulin levels in these animals after seven days of administration. However, intranasal insulin administration both in control and treated rats increased serum insulin during this study.

3.5. Effects of dexamethasone on insulin resistance

By assessing insulin sensitivity using the Homeostatic Model

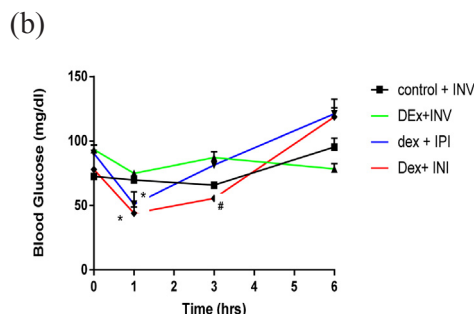
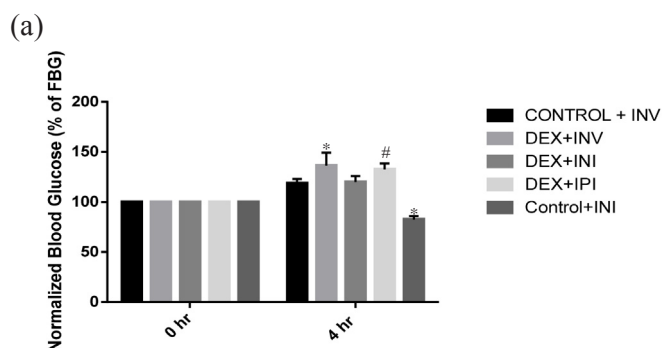


Fig. 2. Effect of dexamethasone on fasting blood glucose levels in Wistar rats (a) 4 h post administration (b) 0, 1, 3, 6 h post administration. Data were analysed by repeated ANOVA and Values are Means $\pm$ SEM; N = 5 for all groups and 6 for Dex. * Significantly different compared to control group $p < 0.05$. Dex, Dexamethasone; INV, Intranasal Vehicle; INI, Intranasal Insulin; IPI, Intraperitoneal Insulin. # Significantly different compared to Dex + IPI. * Significantly different compared to DEX + INV group.

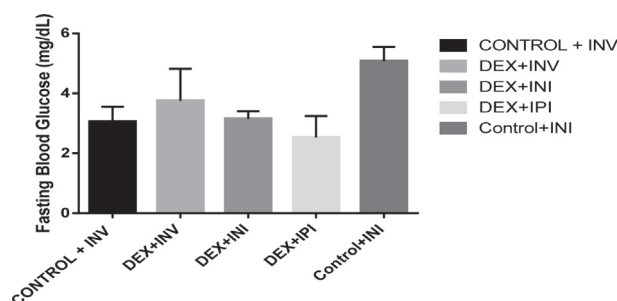


Fig. 3. Effect of intranasal insulin on FBG in rats treated with dexamethasone for seven consecutive days. All values are expressed as Mean $\pm$ SEM; N = 5 animals per group. $p < 0.05$. Dex, Dexamethasone; INV, Intranasal Vehicle; INI, Intranasal Insulin; IPI, Intraperitoneal Insulin.

Assessment of Insulin Resistance (HOMA-IR), we observed a 100% increase in insulin resistance in the dexamethasone treatment group compared to control animals (Fig. 5). This increase was also observed in control animals not receiving dexamethasone but intranasal insulin alone. Interestingly, intranasal insulin prevented hyperglycemia in dexamethasone treated animals.

3.6. Effects of intranasal insulin on lipid profile in dexamethasone treated Wistar rats.

After seven days of intraperitoneal administration of 0.5 mg/kg dexamethasone to Wistar rats, no significant changes were observed in the lipid profiles of the animals. Mean value of total cholesterol (TC) in control animals was 122 mg/dL. TC reduced by 34% in dexamethasone

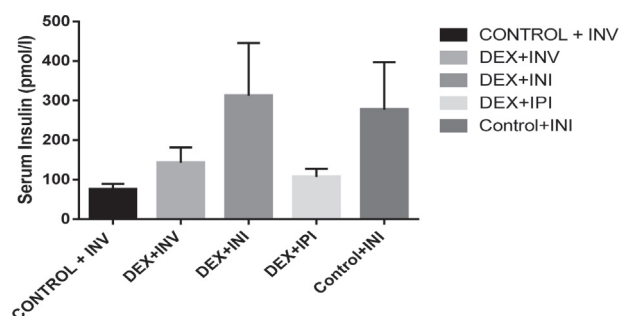


Fig. 4. Effects of dexamethasone on fasting serum insulin levels 24hrs after 7 days treatment. All values are expressed as Mean $\pm$ SEM; N = 5 animals per group. $p < 0.05$. Dex, Dexamethasone; INV, Intranasal Vehicle; INI, Intranasal Insulin; IPI, Intraperitoneal Insulin.

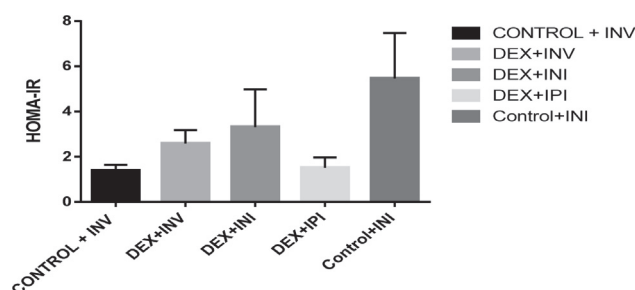


Fig. 5. Effect of dexamethasone on insulin resistance in female Wistar rats after 7 days treatment. All values are expressed as Mean $\pm$ SEM; N = 5 animals per group. $p < 0.05$. Dex, Dexamethasone; INV, Intranasal Vehicle; INI, Intranasal Insulin; IPI, Intraperitoneal Insulin.

treated rats but seemed to be reduced by intranasal but not intraperitoneal administration of insulin. Dexamethasone did not seem to alter total triglycerides in any of the treatment groups. Mean triglycerides value for the control group was 243 mg/dL.

Dexamethasone seemed to lower serum LDL in animals that received intranasal vehicle. Fascinatingly, intranasal insulin further reduced LDL levels compared to dexamethasone control. Dexamethasone reduced HDL concentrations in dexamethasone treated rats by 51% compared to normal controls. However, this decrease did not reach statistical significance. Intranasal insulin however elevated HDL in dexamethasone treated rats, an effect comparable to intraperitoneal insulin administration.

3.7. Effects of intranasal insulin on liver glucose metabolic enzymes in dexamethasone treated animals.

Glucokinase is an enzyme involved in the homeostatic regulation of glucose production and utilization. Dexamethasone did not significantly alter the activity of glucokinase enzyme in the absence of insulin (Fig. 7). However, glucokinase activity was increased in dexamethasone treated animals following intranasal and intraperitoneal insulin administration.

3.8. Effect of dexamethasone and intranasal administration on brain oxidative stress markers

Brain oxidative stress was assessed by the use of Lactate dehydrogenase (LDH) and Glucose-6-phosphate dehydrogenase (G6PDH). Neither dexamethasone nor intranasal administration resulted in a significant change in brain oxidative stress markers as seen in Fig. 8.

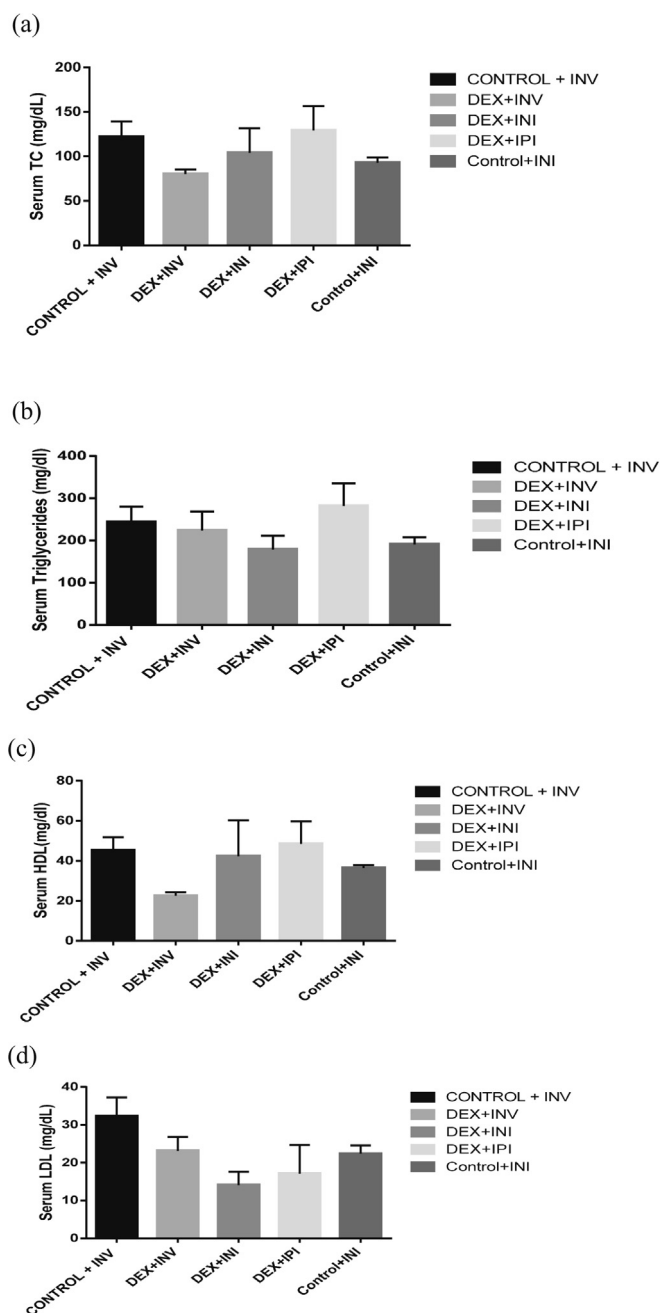


Fig. 6. Effect of Intranasal insulin administration on serum lipid parameters after 7 days of treatment. (a) Serum Total Cholesterol (b) Serum Triglycerides (c) Serum HDL (d) Serum LDL. All values are expressed as Mean $\pm$ SEM; N = 5 animals per group. $p < 0.05$ TC, Total Cholesterol; Dex, Dexamethasone; INV, Intranasal Vehicle; INI, Intranasal Insulin; IPI, Intraperitoneal Insulin.

4. Discussion

Preceding studies have established that intranasal administration proffer an uncomplicated, practical, noninvasive, convenient, cost effective, and an alternative route for rapid drug delivery to the brain/CNS (Liu et al., 2001; Dorman et al., 2002; Wermeling et al., 2002; Vyas et al., 2005). Recent insight into the role of the brain in peripheral glucose homeostasis is opening therapeutic opportunities for targeting the brain in treating metabolic diseases. In the study by Gray and his colleagues in 2014, (Gray et al., 2014) the authors showed the time-dependent delivery of insulin to various parts of the brain, at the earliest time points insulin was delivered to the most proximal parts of the

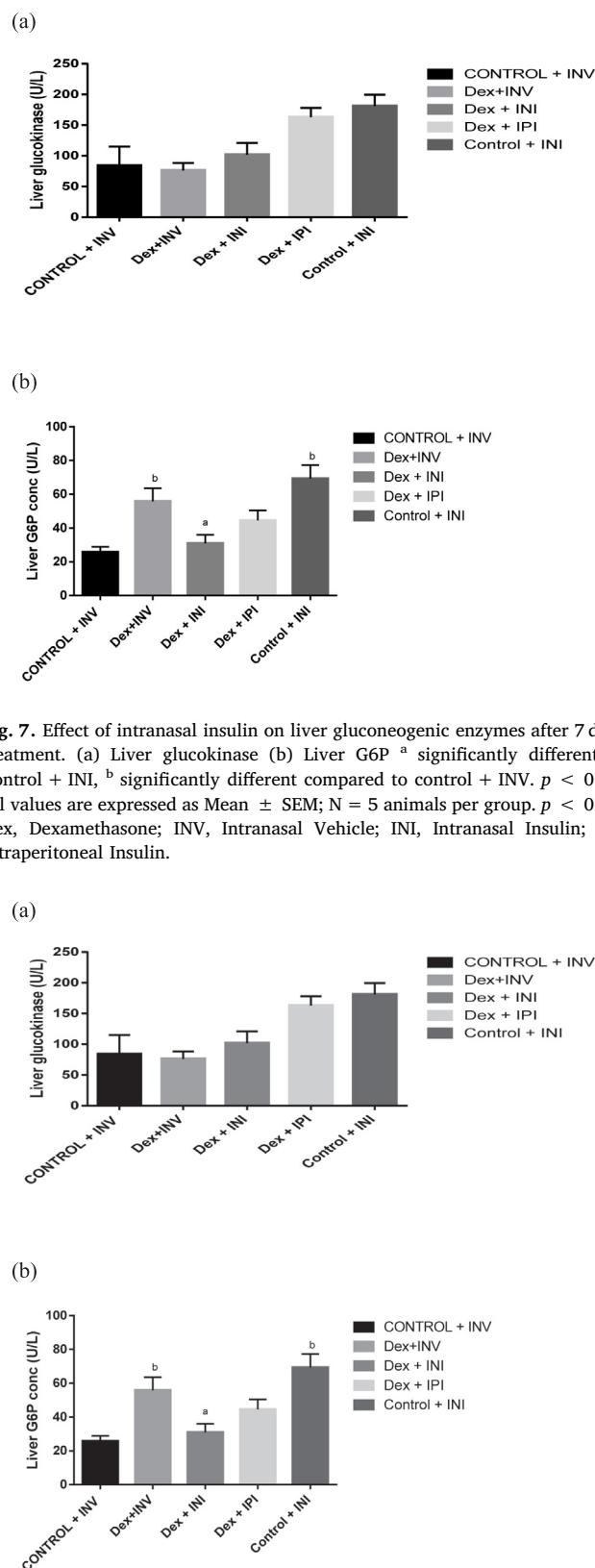


Fig. 7. Effect of intranasal insulin on liver gluconeogenic enzymes after 7 days treatment. (a) Liver glucokinase (b) Liver G6P ^a significantly different to Control + INI, ^b significantly different compared to control + INV. $p < 0.05$. All values are expressed as Mean $\pm$ SEM; N = 5 animals per group. $p < 0.05$. Dex, Dexamethasone; INV, Intranasal Vehicle; INI, Intranasal Insulin; IPI, Intraperitoneal Insulin.

Fig. 8. Effect of intranasal insulin on brain oxidative stress markers after 7 days treatment. (a) Brain G6PDH level (b) Brain LDH level. All values are expressed as Mean $\pm$ SEM; N = 5 animals per group. Data were analysed by one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$. Dex, Dexamethasone; INV, Intranasal Vehicle; INI, Intranasal Insulin; IPI, Intraperitoneal Insulin.

brain including the olfactory lobes, the prefrontal cortex and other distal regions. In the current study, we also confirm the successful delivery of insulin to the brain via intranasal administration of a rapid-acting human insulin preparation. Intranasal delivery of insulin raised brain insulin by three-fold (Fig. 1a), this corroborates with previous reports by Rajasekar, (Rajasekar et al., 2017) showing that different insulin preparations can successfully reach the brain even without the use of enhancers.

Since the administration of insulin is usually accompanied with the risk of hypoglycaemia (McCall, 2012), we evaluated glucose and insulin levels in serum following intranasal administration of insulin. Intranasal insulin significantly lowered blood glucose in dexamethasone-induced hyperglycaemia after four hours of administration in an acute study (Fig. 2a). Although some studies have reported that intranasally administered insulin can reach the systemic circulation, in this study, we did not observe a rise in plasma insulin within 30 min of administering intranasal insulin (Fig. 1b). Several human studies of intranasal insulin have been conducted and reported (Reger et al., 2006; Kamila et al., 2012; Mao et al., 2016; Akintola et al., 2017), the study by Reger et al., in 2006 reported that intranasal insulin did not raise the plasma level of insulin or glucose in trial participants even though it acutely elevate CNS insulin and improve their memory. However, our study did not correlate with this report on plasma glucose levels, with alterations in glucose levels of animals between 0 and 6 h. However, when we monitored plasma glucose over six hours, we observed intranasal insulin reduced plasma glucose within one hour (Fig. 2b) similar to the effect of intraperitoneally administered insulin. This suggests that though insulin does not reach the systemic circulation within thirty minutes, it eventually does at a later time point. Glucose lowering effect of intranasal insulin was subsisting after three hours, an effect not observed with intraperitoneal administration of insulin, this interesting finding suggests that the delayed effect of intranasal insulin observed in this study is not due to insulin that reaches systemic circulation through the nasal route, rather due to actions of insulin on brain that is later relayed to the periphery. In support of this, a previous study (Pocai et al., 2005) reported that there is a lag period of about three hours before the direct effect of insulin in the brain is observed in the periphery. According to (Ott, et al., 2012) this effect is largely due to the fact that insulin takes time to reach certain regions in the brain especially the hypothalamic neurons which are one of the primary insulin sensitive neurons that control peripheral metabolism and in response to insulin modulate autonomic nervous system fibres that project to various peripheral organs including the liver and pancreas. Thus, vagal and sympathetic fibers serve as relay neurons through which insulin in the brain modulates peripheral metabolism.

We again investigated whether the effects of intranasal insulin in normal animals would be replicated in a model of metabolic disease. We evaluated the effects of repeated administration of intranasal insulin on metabolic parameters in animals treated with dexamethasone. Dexamethasone is the most potent, synthetic corticosteroid and acute administration of dexamethasone has been reported to induce metabolic changes within four hours after administration (Samtani and Jusko, 2005). We thus investigated whether intranasal administration of insulin could prevent this effect of dexamethasone, we observed that intranasal insulin prevented increase in glucose following acute administration of dexamethasone. Repeated administration of dexamethasone to experimental animals such as mice and rats has consistently been observed to induce metabolic disorders including fasting and post-prandial hyperglycemia, insulin resistance, dyslipidemia, hyperinsulinemia, hyperglucagonemia amongst others (Rafacho et al., 2010), these effects are dose and duration dependent. Thus, this model may be a valid representation of prediabetes in humans and would be useful in understanding the pathophysiology of prediabetes. In this study, dexamethasone administration for seven days slightly affected several metabolic parameters as shown in (Figs. 3–6). We observed a slight but not significant increase in fasting blood glucose, serum

insulin and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) all pointing to the development of impaired metabolic parameters in animals receiving dexamethasone. Intranasal insulin significantly elevated blood glucose and insulin resistance as assessed by the HOMA-IR in control animals not receiving dexamethasone.

Dexamethasone induced insulin resistance and this was expected as several studies reported similar results (Rafacho et al., 2007; Houtis et al., 2005; Shalam et al., 2006). However, intranasal insulin administered for seven days did not lower insulin resistance as expected but rather increased, though not significantly, in dexamethasone treated animals.

Intranasal insulin does not seem to have significant effect on lipid profile; the total cholesterol was slightly increased in dexamethasone treated animals that received intranasal insulin (Fig. 5a). Intranasal insulin slightly decreased triglyceride levels in animals that were treated with dexamethasone (Fig. 5b). This effect is not significant but gives an insight into the ability of intranasal insulin to decrease serum triglyceride levels hence reducing the risk of metabolic syndrome precipitated by high triglyceride levels induced by dexamethasone, this corroborated with the work of Belaj and his colleagues (Belaj et al., 2014). Intranasal insulin decreases level of LDL in serum though not significantly (Fig. 5d). This shows the potential of intranasal insulin to protect against development of atherosclerosis. LDL deposits cholesterol on artery walls, which in turn, results in the formation of a plaque. Over time, this plaque becomes thicker and results in the narrowing of blood vessels or the plaque could become ruptured, get separated from the arterial wall and result in blood clotting thereby blocking the artery or the clot may become an emboli (Irwin and Tan, 2014). Intranasal insulin increased HDL (Fig. 5c) though not significantly but has the potential to reduce cholesterol levels within the serum as HDL transfers cholesterol to the liver through the process of reverse transfer. Hence, it seems that intranasal administration of insulin may be protective in reversing dexamethasone's effects on high density lipoprotein. These lipid profile results show that intranasal insulin has the potential to prevent hyperlipidemia in Wister rats.

Glucokinase is a glucose metabolic enzyme that facilitates phosphorylation of glucose to glucose-6-phosphate, It does this by storing of glucose within liver cells therefore reducing circulating glucose and enhancing glucose uptake into liver cells to be converted to glycogen (Wilamowitz-Moellendorff et al., 2013). Our result also shows an increase in the activity of glucokinase in the INI treated rats. Dexamethasone is also known to induce glucose production by increasing expression and activity of gluconeogenic enzymes, so modulating its activity in this study may explain the anti-hyperglycemic effect of intranasal insulin.

Glucose-6-phosphatase is a gluconeogenic enzyme that hydrolyzes glucose-6-phosphate, resulting in the creation of a phosphate group and free glucose, it is present in the liver and in the kidney (Ross et al., 2010) Intranasal insulin administration significantly reduced the level of activity of this enzyme in dexamethasone treated animals which could be one of the major ways in which intranasal insulin also acts to lower hyperglycemia. In our study, we report the marked elevation of liver activity of glucose-6-phosphatase (G-6-pase) enzyme in dexamethasone treated animals (Fig. 7). This observation was absent in control animals receiving intranasal insulin thus providing a rational explanation for the observed increase in glucose levels in these animals. However, in dexamethasone treated animals, intranasal insulin markedly reduced dexamethasone mediated increase in G-6-pase activity. This effect of intranasal insulin may explain its ability to prevent the increase in blood glucose mediated by dexamethasone, the effect was absent in the intraperitoneally administered group.

We examined the effect of intranasal insulin on brain lactate dehydrogenase (LDH) and Glucose-6-phosphate dehydrogenase (G6PDH) in order to ascertain the level of oxidative stress caused by intranasal administration to the brain tissue. LDH is an enzyme present in several tissues of the body such as liver, heart, pancreas, kidneys, skeletal

muscles, blood cells and the brain. It catalyzes the reversible conversion of NAD^+ to NADH (Jeng and Loniewska, 2013). The result shows that INI did not have significant effect on brain LDH levels [Fig. 8a]. Interestingly, the levels of Glucose-6-phosphate dehydrogenase (G6PDH) was also not affected, G6PDH an oxidative cytosolic enzyme catalysing the first step in the oxidative division of the pentose phosphate pathway. G6PDH reduces NADP^+ to NADPH while oxidizing glucose-6-phosphate, [Fig. 8b]. Our finding shows that intranasal administration did not induce oxidative stress in the brain during the course of the study, suggesting no damage to the brain tissue during this period.

5. Conclusion

In this study, we have shown that subchronic administration of INI was able to reduce the effect of Dex-induced hyperglycemia that is associated with increased hepatic glucose-6-phosphatase activity and decreased high-density lipoprotein (HDL). Also INI did not induce oxidative stress in the brain which suggests no brain damage during the period of study. Thus, we demonstrate the potential of brain targeting with intranasal insulin in a rat model of insulin resistance and peripheral metabolic disease.

6. Conflict of interest

The authors declare no conflict of interest.

7. Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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