May 24, 2018

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HHS Public Access

Author manuscript

Behav Brain Res. Author manuscript; available in PMC 2018 October 01.

Published in final edited form as:

Behav Brain Res. 2018 October 01; 351: 75–82. doi:10.1016/j.bbr.2018.05.018.

Insulin dependent and independent normalization of blood glucose levels reduces the enhanced rewarding effects of nicotine in a rodent model of diabetes

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Abstract

The rewarding effects of nicotine have been previously shown to be enhanced in rodent models of diabetes. It is presently unclear whether the enhanced nicotine reward observed in the diabetes models are mediated via an insulin or glucose mechanism. This study examined whether the enhanced rewarding effects of nicotine observed in streptozotocin (STZ)-treated rats are insulin-mediated. Male and female rats were treated with STZ and the rewarding effects of nicotine (0.2 mg/kg) were measured using the conditioned place preference (CPP) procedure. Some STZ-treated animals received insulin supplementation via subcutaneous immediately after STZ administration, while other rats received daily injections of dapagliflozin (10 mg/kg), a sodium-glucose cotransporter-2 inhibitor. Both male and female STZ-treated rats displayed hyperglycemia, and their blood glucose levels (BGLs) were normalized to control levels following insulin supplementation or dapagliflozin administration. STZ-treated male rats displayed higher nicotine CPP relative to vehicle-treated controls. This effect was abolished in rats that received insulin supplementation or dapagliflozin administration. STZ-treated female rats displayed reduced levels of nicotine CPP as compared to male rats, regardless of treatment condition. These results suggest that glucose plays a major role in modulating the rewarding effects of nicotine in male rats treated with STZ.

Keywords

Streptozotocin; Insulin; Dapagliflozin; Hyperglycemia; Diabetes; Place Preference

1. Introduction

Diabetes is characterized by hyperglycemia that results from a lack of insulin secretion (type 1) and/or a reduced action of insulin at receptors (type 2) [1]. Diabetes is associated with nicotine use, as active and passive smoking behavior is related to an increased risk of type 2

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Conflict of Interest Statement: The authors report no conflict of interest.

diabetes [2,3]. Moreover, smoking increases insulin resistance [4]. Also, persons with type 2 diabetes who quit smoking experience a long-lasting increase in blood glucose levels (BGLs) regardless of weight gain [5].

Numerous pharmacotherapeutic options are available to normalize BGLs in persons with diabetes. One common method is through insulin supplementation, which leads to an increase in insulin levels and a concomitant decrease in BGLs. Short- and long-term insulin therapies have proven to be useful in preventing the progression of diseases associated with hyperglycemia due to type 1 and type 2 diabetes [6–8]. Aside from insulin, drugs that inhibit the sodium-glucose cotransporter type 2 (SGLT2) promote normal BGLs by preventing renal reabsorption of glucose without altering insulin levels [9]. Among them, dapagliflozin [10] is one of the most potent SGLT2 inhibitor currently used in humans [11]. A single oral dose of dapagliflozin (10 mg/kg) normalizes hyperglycemia in persons with type 2 diabetes [12] without causing hypoglycemia [13]. Similarly, dapagliflozin has been shown to decrease BGLs in diabetic rats [14].

Various rodent models of diabetes have been developed, with the streptozotocin (STZ) administration model being a commonly used approach that represents type 1 and advanced stages of type 2 diabetes [15,16]. STZ is a synthetic antineoplastic agent [17] that induces degranulation of pancreatic beta cells within 48 hours after administration resulting in marginal levels of pancreatic insulin stores [18–20]. Our group has previously shown that STZ-treated rats exhibit an enhancement in the rewarding effects of nicotine, and also experience augmented nicotine physical dependence. Specifically, STZ-treated rats exhibit a greater escalation of nicotine intake, and an enhanced nicotine self-administration as compared to non-diabetic controls [21]. STZ and high-fat diet induced diabetic rats also exhibit an enhanced nicotine conditioned place preference [22,23]. Lastly, STZ-induced diabetic rats exhibit stronger somatic and affective signs during precipitated nicotine withdrawal [22]. Together, these studies suggest that the rewarding effects of nicotine are magnified in a rodent model of diabetes.

As noted above, diabetes is characterized by compromised processing of insulin that leads to a concomitant increase in blood glucose levels. It is presently unclear whether the strong rewarding effects of nicotine in STZ-treated rats is due to the direct effects of insulin or due to the excessive glucose levels. The rewarding effects of nicotine were assessed using CPP procedures in STZ- and vehicle-treated rats. To examine whether the effects are insulin mediated, separate groups of STZ-treated rats received either insulin supplementation, which directly increases insulin levels and leads to a decrease in blood glucose levels; or daily injections of dapagliflozin, which decreases blood glucose levels without altering insulin levels.

2. Methods

2.1. Subjects

The present study used male (N=95), and female (N=83) Sprague-Dawley rats (Envigo, Indianapolis, IN, USA). All rats were housed in groups of 2–3 per cage, in humidity (65%) and temperature (20–22 Cº) controlled room. At the start of the experiment all animals were

adults in their 10^{th}–12^{th} week of life, showed a mean weigh ± SEM of 370±4 grams for males, and 245±3 grams for females. Rats had *ad-libitum* access to standard rodent diet (Teklad 8604) and water in their home cage. The animals were handled for three days before the start of the experiment. All animal care procedures were approved by the Institutional Animal Care and Use Committee at Western University of Health and Sciences.

2.2. Apparatus

The CPP apparatus consisted of two adjacent conditioning chambers (L:35 × W:30 × H:55 cm each) and a small start box (L:19 × W:17 × H:55 cm). One of the conditioning chambers had a metal mesh floor and black and white vertical striped walls, whereas the other chamber had a metal rod floor and black and white horizontal striped walls. The start box was located on the side of the apparatus, and had gray walls and smooth gray floor. The center wall of the CPP arena was a detachable panel shared by both conditioning chambers. A solid panel with a 10 × 10 cm opening was fitted during preconditioning and testing sessions to separate both chambers. A wall panel without an opening was fitted during conditioning sessions to isolate each chamber.

2.3. Drugs

Nicotine hydrogen tartrate salt and STZ were purchased by Sigma-Aldrich (St. Louis, MO, USA). Nicotine was dissolved in 0.9% saline (pH 7.4). Nicotine dose is expressed as free base in this study. STZ was dissolved in a sodium citrate buffer solution (pH 4.5). Dapagliflozin was purchased from Advanced ChemBlocks Inc (Burlingame, CA, USA), and was dissolved in propylene glycol. Insulin and control “blank” implants were purchased from Lin Shin Canada, Inc. (Toronto, Ontario, Canada).

Drug dose selection was according to previous work in our group, in the literature or as recommended. Previous work from our group has shown an optimal nicotine CPP achieved with 0.2 mg/kg of nicotine [23,24]. STZ dose (45mg/kg) was chosen based on our previous work demonstrating efficacy in producing hyperglycemia [25]. Insulin pellets supplementation was according to manufacturer recommendation. Dapagliflozin dose (10 mg/kg) was chosen according to prior published work in rats and humans demonstrating efficacy at this dose [12,13].

2.4. STZ diabetes induction

Table 1 shows the different groups and sample sizes used in the study. BGLs were taken the day before administration of STZ. To measure blood glucose levels, a lancet was used at the tip of the tail to excise a small drop of blood. Glucose values were then assessed using strips and glucometer designed for veterinary use and calibrated for rat blood (AlphaTRAK, Abbott Laboratories, Abbott Park, IL). Male rats displayed a mean baseline BGL of 116±1 mg/dl, and female rats displayed a mean ±SEM of 113±1 mg/dl. Diabetes was induced in some of the animals (46 males and 36 females) by a single injection of STZ (45 mg/kg, i.p.). Control animals were injected with vehicle. In turn, some of the STZ-treated rats were then separated into 2 groups that received insulin pellets or dapagliflozin administration. Each of the experimental groups that received STZ had a corresponding control group that received vehicle and then insulin pellets or dapagliflozin administration. An additional control group

received vehicle administration and saline during conditioning. The weight of the animals, and their BGLs, were measured 24 hours and 14 days after STZ administration.

2.5. Insulin supplementation

Table 2 shows timeline of the experimental treatments. Insulin supplementation consisted of surgical implantation of subcutaneous insulin pellets. The animals were anesthetized with isoflurane (4% induction, 2% maintenance) on the day of STZ injection. Insulin or blank pellets were implanted under the dorsal skin of the animals using a 13G needle. The insulin pellets are designed to release approximately 2 U/24 hour/pellet for >40 days. Rats with body weight of greater than 300 g received two pellets, and rats with body weight of less than 300 g received a single pellet implant, as recommended by the manufacturer. On the day of pellet implantation, male rats that received insulin pellets weighted 285–441g. All male rats received two insulin pellets except for one rat that weighted 285 grams and received a single pellet. Female rats that received insulin pellets weighed 189–273g. The weight of animals and their BGLs were measured 24 h and 14 days after the insulin supplementation in order to track and confirm the effects of insulin supplementation at short and long period after STZ or vehicle treatment.

2.6. SGLT2 inhibitor: dapagliflozin administration

Table 2 shows timeline of the experimental treatments. Treatment with dapagliflozin consisted of a 10 mg/kg daily subcutaneous injection. Controls were injected with the vehicle. The effect of dapagliflozin was assessed by measuring BGLs 2 h after drug injection. Our preliminary examination on the effects of dapagliflozin has shown that BGLs are reduced to their maximal levels at approximately 2 h after administration in STZ treated rats.

2.7. CPP

CPP procedures started 15 days after STZ administration, according to the procedures described by Richardson et al [23]. An 8-day procedure was used consisting of a pre-conditioning test day, 6 conditioning days, and another post-conditioning test day. On the pre-conditioning test, each rat was placed into the start box of the CPP arena and the door was opened. This door was closed once the animal exited the start box, and time spent in each chamber was recorded for 15 min. The time in each compartment was recorded and quantified using EthoVision XT© video tracking software (Noldus Information Technology, Leesburg, VA, USA). During conditioning, the animals were injected with saline or nicotine (0.2 mg/kg, free base, s.c.) and were immediately placed in one side of the conditioning chamber for 30 min. We used a biased procedure, where nicotine was paired with the initially non-preferred chamber of the conditioning apparatus. On the post-conditioning test, the amount of time spent in each chamber was re-assessed for 15 min. A significant change in the time spent on the nicotine-paired side during pre-conditioning versus post-conditioning indicated CPP.

2.8. Statistical Analysis

BGLs and the weight of the animals were analyzed using three-way ANOVAs. We compared a *Group* and *Sex* between subject factor, and a *Time* repeated measure factor. Results of CPP were analyzed using a three-way ANOVA including the *Group* and *Sex* between subject factors, and *Test Session* as a repeated measure factor. To analyze differences in BGLs through CPP due to nicotine injections we used two-way ANOVAs for each group with the *Sex* between subject factor, and a *Nicotine Sessions* repeated measure factor. Post-hoc analyses were carried out using the Fisher LSD test (Least Significant Difference) for pair-wise comparisons ($p \leq 0.05$). All analyses were conducted using IBM SPSS 24© software.

3. Results

Figure 1 shows the effect of STZ treatment and insulin supplementation 24 h and 14 days after STZ treatment in male and female rats. Figures 1A and 1C show mean BGLs ±SEM for male and female rats. Analysis revealed a *Group* × *Time* × *Sex* interaction effect [$F(6,122)= 17.15$, $p< 0.01$]. Prior to STZ injection (baseline) BGLs did not differ among groups; whereas, STZ administration produced a significant increase in BGLs that was detected at 24 hours and 14 days after treatment in both male and female rats. This effect was sex dependent as STZ produced a greater increase in BGLs in male than in female rats. Insulin supplementation in STZ-treated rats normalized the enhanced BGLs at the 24 h and 14 day time points in both sexes. Moreover, insulin supplementation led to a significant reduction in BGLs when compared to its respective baseline values, as well as the control group at 24 h after STZ in both sexes. At 14 days after STZ, insulin supplementation reduced BGLs as compared to the control group in male rats. In female rats, insulin supplementation did not decrease BGLs as compared to the control group or their own baseline measures.

Figures 1B and 1D show mean changes in body weight ±SEM for male and female rats, respectively. STZ-treated rats displayed significant changes in their body weight as revealed by a *Group* × *Time* × *Sex* interaction effect [$F(3,112)= 7.83$, $p< 0.01$]. Control male and female rats displayed an increase in their body weight on day 14 as compared to the 24 h measurement. STZ treatment led to a significant decrease in body weight in both sexes at the 24 h and 14 day time points. STZ-treated male rats displayed a larger decrease in body weight as compared to female rats. In male rats, insulin supplementation in vehicle control and STZ-treated rats increased body weight at 24 h and 14 days after STZ treatment as compared to the control group. In female rats, insulin supplementation alone increased body weight at 24 h and 14 days after STZ treatment as compared to controls. However, insulin supplementation in STZ-treated female rats increased body weight only14 days after STZ administration. Moreover, vehicle controls and STZ-treated male rats that received insulin supplementation exhibited a larger increase in body weight as compared to their respective female rat groups.

Figure 2 shows the effect of STZ treatment and dapagliflozin administration in male and female rats. Figures 2A and 2C show mean BGLs ± SEM before and after dapagliflozin injections on day 14 after STZ administration for male and female rats, respectively. The analysis revealed a *Group* × *Time* × *Sex* interaction [$F(2,72)= 4.10$, $p< 0.01$]. In both sexes,

STZ treatment increased BGLs as compared to the control group before dapagliflozin administration. Dapagliflozin administration significantly reduced BGLs of STZ treated male and female rats to levels below 200 mg/dl, which is within 'normal range'. Moreover, dapagliflozin administration reduced BGLs to a greater extent in STZ-treated male rats as compared to female rats, translating to a 73.5% reduction in male rats versus 67.5% reduction in female rats. Rats given dapagliflozin alone did not exhibit changes in BGLs as compared to their respective control groups.

Figures 2B and 2D show mean changes in body weight ±SEM for male and female rats, respectively. Previous to dapagliflozin treatment, rats displayed significant changes in their body weight as revealed by a *Group* × *Time* × *Sex* interaction effect [$F(2,72)= 18.33$, $p<0.01$]. Male and female vehicle control rats and dapagliflozin-treated rats displayed an increase in their body weight on day 14 as compared to the 24 h measurement. STZ-treated rats showed a decrease in body weight at 24 h and 14 days after STZ treatment as compared to their respective controls across time. STZ-treated male rats displayed a larger decrease in body weight as compared to similarly treated female rats 24 h and 14 days after STZ treatment. Male control rats and those that received dapagliflozin only displayed a larger increase in body weight compared to similarly treated female rats on day 14 after STZ administration, and prior to dapagliflozin treatment.

Figure 3 shows mean ± SEM time spent in the nicotine-paired side during the pre- and post-conditioning test sessions. Figures 3A and 3B display the results for male and female rats, respectively. The analysis revealed a *Group* × *Sex* × *Test Session* interaction [$F(6,164)= 2.20$, $p< 0.05$]. Time spent on the nicotine-paired side was similar for all groups during the pre-conditioning test session. Nicotine conditioning in male rats in the vehicle control group led to an increase in the time spent in the nicotine-paired side during the post-conditioning test day as compared to the pre-conditioning day, indicative of a nicotine-induced CPP. In male rats, nicotine conditioning in STZ-treated rats led to a further increase in time spent in the drug-paired side as compared to the control group, indicative of an enhancement in the nicotine-induced CPP. Insulin supplementation or dapagliflozin administration in STZ-treated male rats led to a decrease in the postconditioning time spent on the nicotine paired side as compared to rats treated with STZ. Nicotine conditioning in male rats receiving insulin supplementation led to an increase in the post- versus pre-conditioning test session, similar control rats. In female rats, nicotine conditioning in the control group, and in rats receiving insulin supplementation only, led to an increase in the time spent on the post- versus pre-conditioning test day. However, differences in time spent between the pre- versus post-conditioning days were not detected for STZ-treated female rats or any group.

Figure 4 displays the mean change in BGLs ±SEM after each of the CPP conditioning sessions with nicotine, and one saline session for male and female rats belonging to control group. The analysis revealed a *Nicotine Sessions* main effect [$F(3,102)= 9.22$, $p< 0.01$]. Both male and female rats displayed a significant increase in BGLs during the first conditioning session with nicotine as compared to the saline session. The effect of nicotine on BGLs was attenuated during the second and third administration of nicotine during conditioning as compared to rats that received saline. Statistically significant differences were not obtained for all other treatment groups (data not shown).

4. Discussion

Our previous work demonstrated that diabetic rats display an enhancement in the rewarding effects of nicotine [23,25]. The present study expands this work by examining whether normalization of BGLs via insulin-dependent or independent mechanisms decrease the strong rewarding effects of nicotine observed in STZ-treated male and female rats. To normalize BGLs, a dual approach was applied by either treating rats with insulin supplementation or dapagliflozin administration. Insulin independent effects were assessed using dapagliflozin, which blocks renal glucose reabsorption and decreases BGLs without altering insulin levels. To summarize, the present study revealed that STZ-treated male rats displayed enhanced nicotine-induced CPP relative to vehicle controls. Both insulin supplementation and dapagliflozin administration reduced the rewarding effects of nicotine in STZ-treated rats to vehicle control male rats. STZ-treated female rats did not exhibit an enhancement of nicotine CPP as compared to control female rats. In both male and female rats, nicotine administration increased BGLs during the first nicotine conditioning session.

Both insulin supplementation and dapagliflozin administration were effective in reducing the BGLs of male and female rats. Dapagliflozin normalized BGLs to control levels, and insulin supplementation decreased BGLs below control levels, an expected result since hypoglycemic episodes can sometimes result from insulin administration [26,27]. In addition, a significant increase in body weight of animals supplemented with insulin was observed, an effect that is due to the appetite stimulating effects of insulin. In fact, it has been reported that chronic insulin supplementation doubles daily food intake and ultimately produces insulin-induced obesity [28]. Insulin supplementation has been described as a potential cause of weight gain in patients with type 1 diabetes [29].

It has been reported that nicotine increases BGLs [30–31]. In our study, we found a significant increase in the BGLs of male and female rats after the first nicotine injection during CPP. However, during subsequent nicotine conditioning sessions, BGLs reduced to levels similar to that observed with saline treatment. The decrease in BGLs may be due to a conditioned tolerance to the effect of the nicotine dose, where a progressive habituation to the nicotine-paired context gradually lowered the nicotine-induced increase in BGLs. Similar reductions in BGLs have been reported in studies assessing classical conditioning [32–34], and/or pharmacological tolerance to nicotine [35].

Sex differences have been previously reported where nicotine produces a stronger CPP in male rats as compared to female rats [24,36]. The present findings agree with past studies as nicotine CPP was more robust in male than in female rats. The precise mechanism for this sex difference has yet to be determined. Male and female rats have different nicotine pharmacokinetic profiles after a single intravenous dosing, with male rats exhibiting a shorter half-life for nicotine and female rats exhibiting a larger volume of distribution, which results in nicotine plasma clearance to be similar between the sexes [37]. Therefore, mechanisms underlying sex differences in nicotine CPP are likely due to pharmacodynamic effects.

Nicotine CPP was enhanced in male rats treated with STZ, consistent with our report [22]. However, the STZ-induced enhancement of nicotine CPP was not detected in female rats. At least three reasons could explain the lack of enhancement of nicotine CPP in STZ-treated female rats. First, the modest rewarding effects of nicotine in female rats may not have been sufficient to produce an enhancement by STZ treatment. Second, female rats are less sensitive to insulin actions in the brain as compared to males [38]. In fact, intra-cerebroventricular (ICV) administration of insulin resulted in a notable decrease in food intake and body weight in male, but not female rats [38]. This effect is likely due to the properties of gonadal hormones, as ovariectomized rats exhibited similar brain insulin sensitivity as males and led to an ICV insulin-induced decrease in food intake and body weight similar to male rats [39]. Reduced sensitivity to insulin has also been reported in humans, as intranasal insulin administration resulted in a decrease in body weight in men, but not women [40]. Lastly, STZ-induced hyperglycemia could alter functioning of glucose-sensing neurons in the hypothalamus and ventral tegmental area which may in-turn impact striatal dopamine release and the rewarding effects of nicotine [41–44], although sex differences in glucose-sensing neurons are not well understood.

The present findings expand our previous work by demonstrating that the strong rewarding effects of nicotine observed in STZ-treated rats may be related to changes produced by chronic and excessive levels of glucose, versus an effect produced by hypoinsulenemia. Indeed, the association between BGLs and nicotine reward has been previously shown, as STZ-treated rats with very high BGLs demonstrated higher nicotine intake as compared to rats with moderately high glucose levels [23]. It is also important to consider whether the enhancement in nicotine reward is dependent on changes in body weight. An increase in body weight has been previously suggested to decrease nicotine reward [45]. If an increase in body weight decreases nicotine reward, then by extension, a decrease in body weight would be expected to increase nicotine reward. However, this view is not supported by our findings, as we are able to separate the changes in body weight from the rewarding effects of nicotine. In the current findings, STZ treated rats had a decrease in body weight and an increase in nicotine CPP; whereas STZ treated rats given dapagliflozin had a decrease in body weight but did not exhibit nicotine CPP. Moreover, previous findings from our laboratory have dissociated body weight and nicotine CPP in rats given a high-fat diet regimen [23]. In that study, rats given a high-fat diet gained weight (~12–13% increase in body weight) and were separated into two subgroups depending on whether they were insulin resistant. Insulin resistant rats exhibited an enhancement in nicotine CPP, while rats given a high-fat diet but not insulin resistant did not exhibit a nicotine CPP. Collectively, these findings suggest that an increase in the rewarding effects of nicotine is independent of changes in body weight.

The modulatory effects of glucose and insulin on consummatory behavior have been extensively studied. The rewarding effects of glucose have been shown to be independent of its sweet taste. For example, intragastric or intravenous glucose administration induces preference for a glucose solution in a two-bottle choice test [46]. Glucose administration has also been shown to increase dopamine release in the nucleus accumbens, a terminal region of the mesolimbic reward pathway [46,47]. Although we are not aware of studies examining actions of glucose directly in mesolimbic circuitry, direct administration of glucose into the

substantia nigra increased dopamine release in the dorsal striatum [48], suggesting that a similar response from dopamine neurons in the ventral tegmental area may be possible. In addition to glucose, insulin has been shown to reduce reward processing. For instance, ICV insulin administration decreases sucrose self-administration and CPP produced by palatable foods [49–51]. Furthermore, ICV and intra-VTA infusion of insulin decreases brain reward function [52,53], whereas direct administration of insulin into the nucleus accumbens leads to a concentration dependent change in dopamine release, with low to moderate concentrations increasing dopamine release, and high concentrations reducing dopamine levels [54].

Previous work from our group has shown that STZ treated rats exhibit reduced basal and nicotine-evoked dopamine levels in the nucleus accumbens, likely due to an increase in dopamine transporter expression resulting in greater dopamine clearance [25]. Furthermore, STZ treatment increases D1 receptors and decreases D2 receptors in the nucleus accumbens and dorsal striatum which may contribute to the enhanced rewarding effects of nicotine [25, 55–58]. STZ treatment also modifies cell signaling molecules downstream from dopamine receptors that are known to play a role in the neuroadaptations in drug abuse. For instance, STZ treated rats exhibit an increase in the phosphorylation of the serine/threonine kinase AKT and the dopamine- and cyclic AMP regulated-neuronal phosphoprotein DARPP-32 in the nucleus accumbens [59]. It is presently unclear whether the changes in receptor levels and signal transductions following STZ administration is a result of enhanced BGLs and/or hypoinsulinemia. It is important to decipher the degree of influence of insulin and glucose on the enhanced rewarding effects of nicotine in STZ-treated rats. When considering the present findings along with previous studies, it is possible that reduction of blood glucose is both necessary and sufficient in decreasing the enhanced rewarding effects of nicotine in STZ-treated rats; whereas supplementing insulin is sufficient but not necessary in decreasing nicotine reward in STZ-treated rats.

In conclusion, our results provide evidence of a reduction in the enhanced rewarding effects of nicotine in diabetic rats by means of two common pharmacologic approaches for the treatment of diabetes: insulin and SGLT2 inhibition. These results suggest a potential contribution of augmented glucose levels as the primary modulator of enhanced nicotine reward in diabetic rats. Further confirmation of this effect is warranted in rodent models of type 2 diabetes, as well as examination of interaction between nicotine reward processing and glucose levels within mesolimbic circuitry.

Acknowledgments

Funding Source: This work was supported by National Institutes of Health grants R15DA040130 and R15DA040130-01S1 to AN

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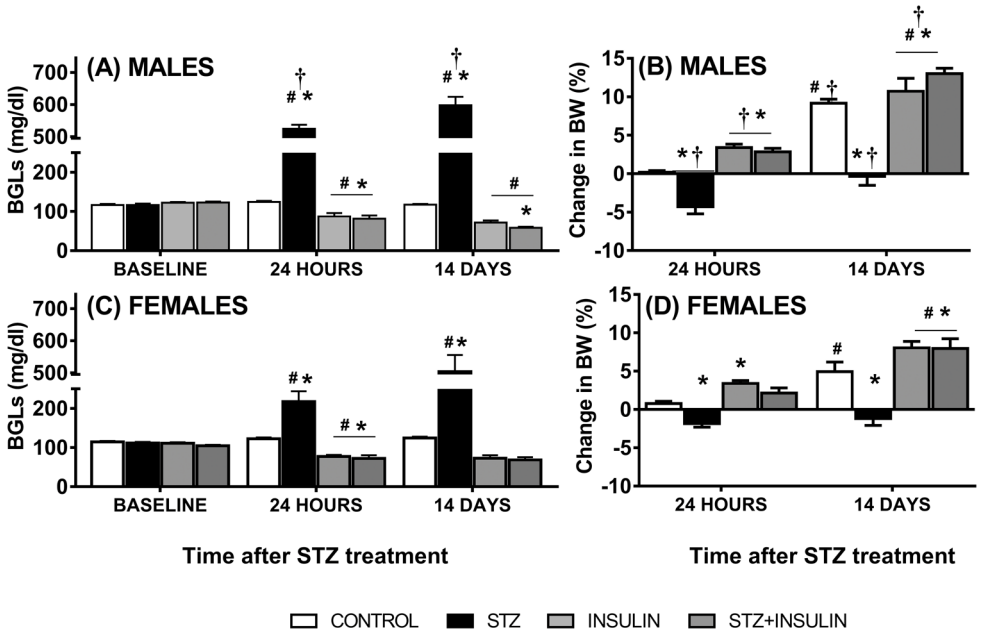

Figure 1.

Effects of insulin supplementation on BGLs and body weight in STZ-treated rats. Figures 1A and 1C display mean ± SEM BGLs 24 h before and 24 h and 14 days after STZ administration in male and female rats respectively. Figures 1B and 1D show mean ± SEM changes on body weight for male and female rats respectively. *= $p < 0.05$ for between groups comparisons. #= $p < 0.05$ for within group comparisons. †= $p < 0.05$ for sex comparisons. The group sizes are as follows, Males: controls n=20, STZ n=18, insulin n=10, and STZ+insulin n=19; Females: controls n=16, STZ n=14, insulin n=11, and STZ+insulin n=11.

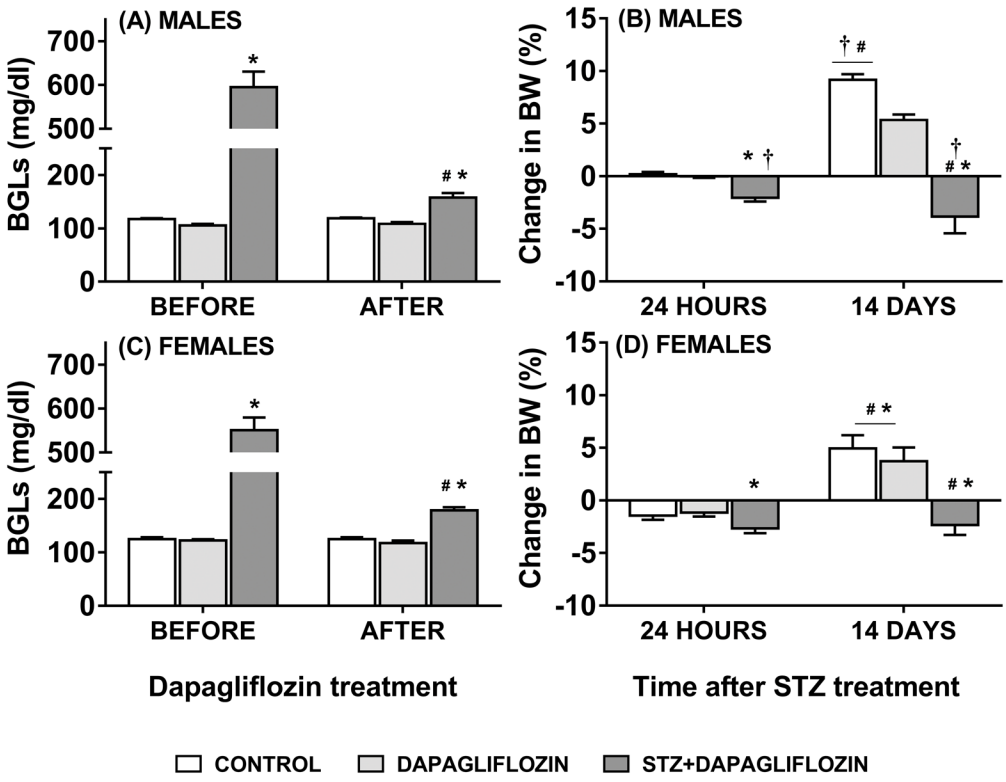

Figure 2.

Effects of dapagliflozin on BGLs and body weight in STZ-treated rats. Figures 2A and 2C display mean ±SEM of the BGLs before and after dapagliflozin administration in male and female rats respectively. Figures 2B and 2D display mean ± SEM changes on body weight for male and female rats respectively. *= p< 0.05 for between groups comparisons. #= p< 0.05 for within group comparisons. †= p< 0.05 for sex comparisons. The group sizes are as follows, Males: control n=20, dapagliflozin n=11, and STZ+dapagliflozin n=9; Females: control n=16, dapagliflozin n=11, and STZ+dapagliflozin n=11.

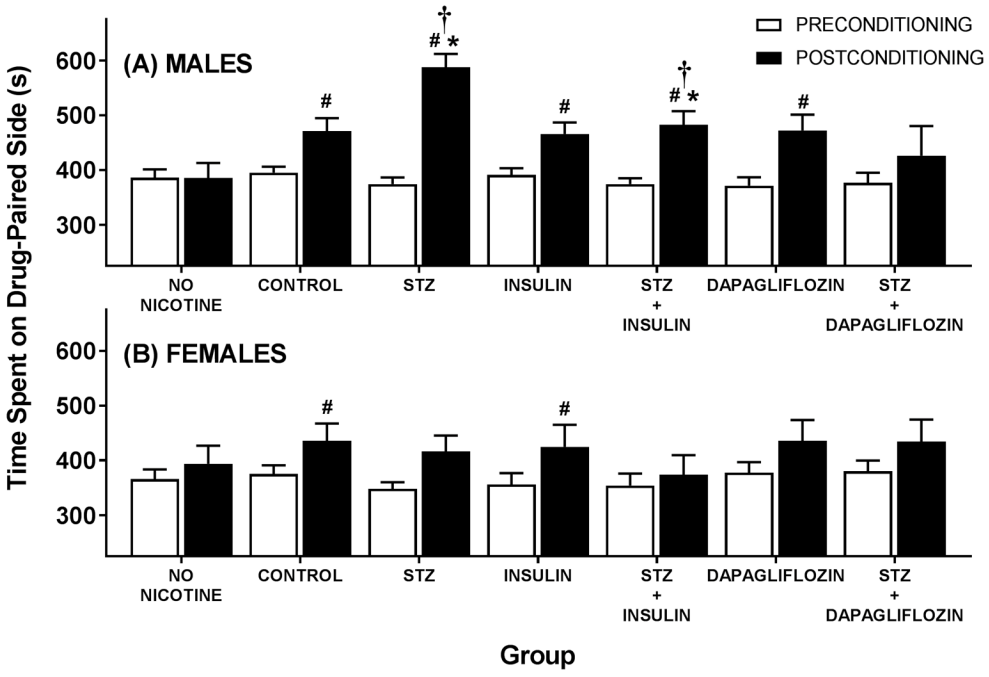

Figure 3.

Enhancement of nicotine-induced CPP in STZ-treated male rats was reversed by insulin supplementation and dapagliflozin administration. Figures 3A and 3B display mean ±SEM of time spent on nicotine-paired side during both preconditioning (white columns), and postconditioining (black columns) for male and female rats respectively. *= $p < 0.05$ for between groups comparisons. #= $p < 0.05$ for within group comparisons. †= $p < 0.05$ for sex comparisons. The group sizes are as follows, Males: control n=20, STZ n=18, dapagliflozin n=11, and STZ+dapagliflozin n=9, insulin n=10, STZ+insulin n=19, and No Nicotine n=8; Females: control n=16, STZ n=14, dapagliflozin n=11, and STZ+dapagliflozin n=11, insulin n=11, STZ+insulin n=11, and No nicotine n=9.

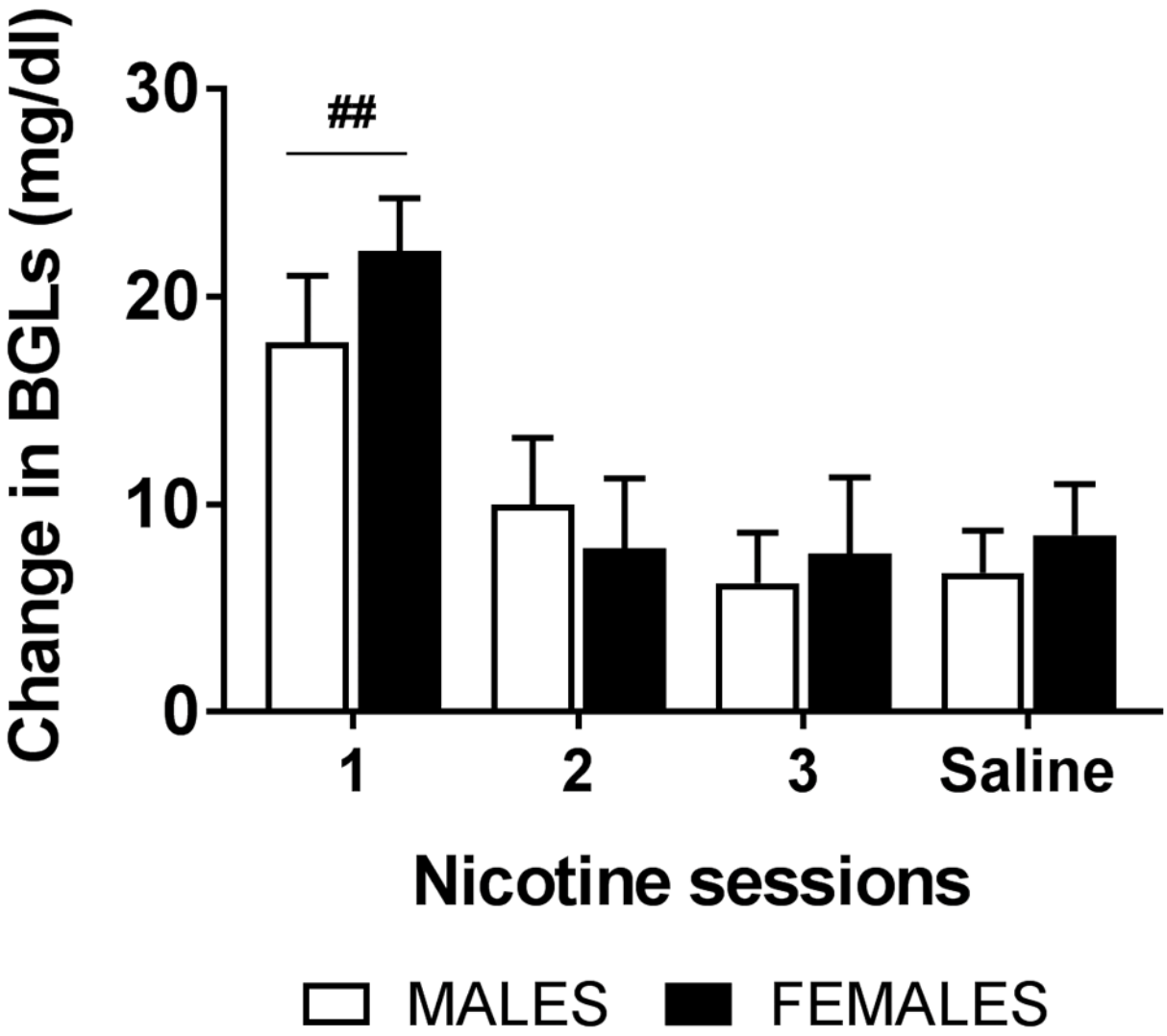

Figure 4.

Effects of repeated nicotine administration on BGLs. mean BGLs variation ±SEM for male and female control rats during the three CPP sessions in which the animals received nicotine, and data of a saline session to provide a baseline for these variations. ##= $p < 0.01$ for within group comparisons. The group sizes are as follows, Males n=20, Females n=16

Table 1

Distribution and sample size of all animals used in the study. Groups are labeled depending on their sex, STZ treatment, and on the treatment used to control the hyperglycemia.

GROUP	DIABETES INDUCTION	TREATMENT	MALES	FEMALES
CONTROL	VEHICLE	VEHICLE	12	12
		CONTROL SUPPLEMENT	8	4
STZ	STZ	VEHICLE	10	10
		CONTROL SUPPLEMENT	8	4
INSULIN	VEHICLE	INSULIN SUPPLEMENT	10	11
STZ+INSULIN	STZ	INSULIN SUPPLEMENT	19	11
DAPAGLIFLOZIN	VEHICLE	DAPAGLIFLOZIN	11	11
STZ+DAPAGLIFLOZIN	STZ	DAPAGLIFLOZIN	9	11
NO NICOTINE*	VEHICLE	VEHICLE	8	9

Table 2

Experimental time-line.

DAYS/GROUPS	0	1	14	15	16–21	22
Insulin or STZ + Insulin	STZ or Vehicle injection & Insulin or blank pellet implantation			Preconditioning	Conditioning	Postconditioning
Dapagliflozin or STZ + Dapagliflozin	STZ or Vehicle injection		Dapagliflozin	Preconditioning & Dapagliflozin	Conditioning & Dapagliflozin	Postconditioning & Dapagliflozin
MEASURES	BGL and BW	BGL and BW	BGL and BW	BGL and BW		