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Alterations in blood glucose levels under hyperinsulinemia affect accumbens dopamine

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Abstract

Dopaminergic systems have been implicated in diabetes and obesity. Notwithstanding, the most basic relationship between dopamine and plasma insulin as well as glucose levels yet remains unknown. The present experiments were designed to investigate the effects of acute hyperinsulinemia on basal dopamine levels in the nucleus accumbens of the rat under chloral hydrate anesthesia using acute microdialysis in combination with the hyperinsulinemic-glycemic clamping procedure. In Experiment 1, each rat was infused with one of three concentrations of insulin (2.4, 4.8, or 9.6 mU/kg per min) while plasma glucose levels were maintained at euglycemia (~5.5 mmol/L). Dopamine, dihydroxyphenylacetic acid and homovanillic acid were not significantly different from baseline during either the clamp or post clamp periods for all insulin concentrations. In Experiment 2, rats were infused with the highest concentration of insulin (9.6 mU/kg per min) and plasma glucose levels were maintained at either hypoglycemia (~3 mmol/L) or hyperglycemia (~14 mmol/L). Dopamine was elevated at 100 min (+113% above basal levels) and 120 min (+117%) in the hypoglycemic condition and at 120 min (+121%) in the hyperglycemic condition. In the hyperglycemic post clamp period, homovanillic acid was decreased below basal levels (approximately -32%). These results together suggest that short-term blood glucose deviations coupled with acute hyperinsulinemia affect the mesoaccumbens dopamine system.

Keywords

Insulin; blood glucose; prediabetes; obesity; overeating; microdialysis

1. Introduction

Alterations in plasma insulin [1,2] or glucose levels [3] or both [4] can have a profound influence on food intake and food preferences. Further evidence also suggests that metabolic alterations in insulin and glucose can influence mesoaccumbens DA [5-7]. That is, lower basal and stimulated DA levels in the ventral striatum have been reported in streptozotocin-induced diabetic rats compared with non-diabetic controls [5]. Intra-accumbens DA injections are also less effective in stimulating food intake in streptozotocin-induced diabetic rats [6]. Additionally, increases in accumbens DA levels have been reported 30 min after a peripheral injection of insulin in non-diabetic rats [7]. The exact contribution, however, of either glucose or insulin on mesoaccumbens DA function remains unclear.

Investigating the influence of plasma insulin levels on DA has attracted considerable attention, in part, because a moderate amount of insulin receptors have been demonstrated in the nucleus

accumbens [8] and have been localized to tyrosine hydroxylase positive cells in the ventral tegmental area (VTA)/substantia nigra (SNpc) regions [9]. Central administration of insulin also has been shown to increase DA transporter (DAT) mRNA in the VTA/SNpc [10]. This increase in transporter likely influences DA signaling, since exogenously administered insulin has been shown in vitro to increase DA uptake in striatal tissue [11,12] and human DAT-transfected cells [11,13]. On this basis, the present experiments focused on the role of acute elevation in plasma insulin on mesoaccumbens DA function.

The hyperinsulinemic-euglycemia clamp technique is an experimental manipulation used primarily to determine whole body insulin sensitivity [14]. Relevant to the present application, this method also has been utilized to control for insulin induced hypoglycemia [15,16]. In the following experiments, acute in vivo microdialysis was used to assess DA and its metabolites in the nucleus accumbens concurrent with the hyperinsulinemic-glycemic clamp. The hypothesis of the first experiment was that increasing levels of hyperinsulinemia would lead a decrease in mesoaccumbens DA. This rationale emerged from the extensive series of studies that have shown that chronic (i.e., several days) or acute [17] third ventricle insulin administration reduces daily food intake and results in a decrease in body weight via insulin's action on the arcuate nucleus of the hypothalamus [18-21]. Based on this research and since hyperdopaminergic conditions have been reported to stimulate food intake [22,23], a similar catabolic role, that is a decrease in DA should be observed in the mesoaccumbens DA system in response to short-term elevations in plasma insulin levels. The second experiment investigated the influence of hyperglycemic or hypoglycemic conditions under hyperinsulinemia on mesoaccumbens DA. Since hypoglycemia and hyperglycemia represent vastly different internal states, the hypothesis of the second experiment was that the two situations of extreme glycemia under acute hyperinsulinemia would have an opposing effect on accumbens DA.

2. Methods

2.1. Subjects

A total of 23 male Sprague Dawley rats (Charles River, Wilmington, MA), with an initial weight of 450-525g were individually housed and placed on a 12/12 h light-dark schedule (lights on at 0700h). All rats received ad libitum standard laboratory chow (Global Diet-2018, Harlan Teklad) and water, unless otherwise noted. Animal protocols were approved by the Animal Care and Use Committee of the Pennsylvania State University College of Medicine, and were in accordance with NIH guidelines.

2.2 Surgeries and hyperinsulinemic clamp procedure

Animals were anesthetized with sodium pentobarbital (50 mg/kg, IP) and were stereotactically implanted with bilateral 21 gauge stainless steel guide cannulae positioned above the posterior nucleus accumbens (A 1.0 mm, L 1.0 mm, and V 4.0 mm from the surface of the skull [24]). One week later, animals were overnight deprived of chow and anesthetized with chloral hydrate (400 mg/kg IP). The depth of anesthesia was maintained with 80 mg/kg chloral hydrate IP and assessed by mandibular tone (i.e., the resistance to the opening of the mouth). Body temperature was maintained at 37°C by a homeothermic heating pad (Stoelting, Co., Wood Dale, IL). Animals were then implanted with catheters for hyperinsulinemic-glycemic clamp procedure. Briefly described, a polyethylene (PE-50) catheter was inserted and secured near to the right atrium via the jugular vein. An additional polyethylene catheter was inserted and secured to the level of the aortic arch via the left carotid artery. Following probe stabilization period of at least 120 min, three 20 min samples were taken to establish a microdialysis baseline sampling. After the baseline period, microdialysis sampling continued for the hyperinsulinemic-euglycemic (Experiment 1), hyperinsulinemic-hypoglycemic (Experiment

2) or hyperinsulinemic-hyperglycemic (Experiment 2) clamping procedure. Microdialysis sampling also continued for 1 h following the clamp phase and this was designated as the post clamp period. The hyperinsulinemic clamping procedure consisted of a continuous venous infusion of insulin (regular human insulin; Humulin R; Eli Lilly & Co.) maintained for 120 min while a variable infusion of blood glucose was adjusted with 50% dextrose to maintain (i.e., to clamp) blood glucose level at ~5.5 mmol/L (Experiment 1; euglycemia), ~3 mmol/L (Experiment 2; hypoglycemia), or ~14 mmol/L (Experiment 2; hyperglycemia). One of three concentrations of insulin (2.4 mU, “low”; 4.8 mU, “moderate”; 9.6 mU/kg per min; “high”) was infused for each rat (n= 4, 5, and 4, respectively) in Experiment 1, whereas only one concentration (9.6 mU/kg per min; n= 5 for both conditions) was infused for each rat in Experiment 2. The insulin concentrations (2.4-9.6 mU/kg/min) were similar to those previously reported in hyperinsulinemia-glycemic studies that assessed the effects of hyperinsulinemia on neurotransmitter levels in the hypothalamus [15,25] and midbrain [16] in rats. Assessment of blood glucose levels were achieved by arterial samples taken every 10 min and measurements were done with a handheld glucometer (~10 µl; Ascenia Elite, Bayer Co; sensitivity 1.1 mmol/L to 33.3 mmol/L). Every 30 min samples were taken to determine plasma insulin levels (~100 µL blood sample; radioimmunoassay, Linco Research, Inc.). Arterial samples were taken before the clamp procedure at the end of the probe stabilization period to establish blood glucose and insulin baseline values. Blood sampling for glucose and insulin continued throughout the clamp and during the post clamp periods.

2.3 Histology

Each animal was exposed to a single hyperinsulinemic-glycemic clamp procedure. Immediately following the 1 h post clamp period, the animals received an overdose of sodium pentobarbital (100 mg/kg, IP) and were perfused transcardially with 0.9% saline followed by 10% formalin. For histological assessment of the microdialysis probes placement, the brains were removed, frozen, and serially sectioned at 50 µm. The sections containing the target area of the microdialysis probes were stained with cresyl violet. The data obtained from rats with misplaced probes were excluded from the statistics and presentation.

2.4 Probe design and microdialysis

The microdialysis probes were 200 µm wide with a concentric design similar to those originally described by Hernandez and colleagues [26]. In our experiment, the probes had a molecular weight cut off of 20k Da and an active 2 mm cellulose tip (Spectrum Co., Ranch Dominguez, CA). The in vitro recovery rate for our probes is ~14% for DA, ~17% for dihydroxyphenylacetic acid (DOPAC), and ~16% for homovanillic acid (HVA). Probes were perfused with an artificial cerebrospinal fluid (145 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl₂, 1.0 mM MgCl₂, 2.0 mM Na₂HPO₄, pH 7.4) at flow rate of 1.0 µl/min. For each rat, 12 samples were collected in individual microcentrifuge tubes that had 5 µL of 10 mM HCl added to each tube to retard sample oxidation. Dialysis samples were either directly measured or stored at -80 °C for future analysis.

2.5 Neurochemical assessment

DA, DOPAC, and HVA were separated from the samples by reverse phase high performance liquid chromatography (HPLC) and measured with coulometric detection (CoulArray; ESA, Inc.). The detection limit for DA was approximately 0.4 pg /20 µl sample. The mobile phase consisted 60 mM sodium phosphate, 100 mM EDTA, 1.24 mM heptane sulfonic acid and 6% (v/v) methanol (Sigma Chem, Co. St. Louis, MO).

2.6 Statistical Analysis

Absolute, basal recovery of DA, DOPAC, and HVA were calculated from the area under the chromatographic peaks and then converted to percentage above the mean of three 20 min baseline samples within subjects. Baseline line samples varied less than 10% for DA and metabolites. Samples were taken bilaterally although all were not used due to probe misplacement, probe malfunction, or sample loss. To be consistent, only unilateral data was used from each rat undergoing a hyperinsulinemic-glycemic clamp procedure. The percentages for DA, DOPAC, HVA and the values for blood glucose and insulin were analyzed by separate ANOVA with repeated measures (time of sample as the within factor, Statistica 6.0, Tulsa, OK) and significance was set at $p < 0.05$. Post hoc LSD planned comparisons were performed when appropriate.

3. Results

3.1 Experiment 1. Hyperinsulinemia under euglycemia

All three concentrations of intravenous infusions of insulin significantly increased plasma insulin levels [$F(6, 36)=15.49$, $p<0.00001$] and the interaction (group X time) [$F(12, 36)=3.00$, $p<0.01$]. As shown in Figure 1 A, the high group had a greater elevation of plasma insulin during clamp period compared with both the low and moderate groups ($p<0.05$). During the clamp procedure all three groups' blood glucose levels were maintained at approximately 5.5 mmol/L, see Figure 1 B. This value based on previous report showing this reading obtained with identical glucometer in an unanaesthetized overnight fasted adult male Sprague Dawley rat [27].

DA, DOPAC and HVA were taken as percentage of difference above basal levels (i.e., 1 h baseline levels for each individual rat) $\pm$ SEM, and expressed for the clamp and post clamp periods (Figure 2 A, B, C, respectively). There was no significant difference between groups or time or the interaction between DA, DOPAC or HVA from baseline levels during the clamp or post clamp periods. There was, however, a great deal of variability in DA during the post clamp period in all three groups with the highest variability observed in the high concentration group ($\sim \text{SEM} \pm 47$). There also was no significant difference in the ratio of DOPAC to DA, which is often used as an index of DA turnover and was calculated for the baseline, clamp and post clamp periods.

3.2 Experiment 2. Hyperinsulinemia under hypoglycemia or hyperglycemia

The highest concentration of insulin (9.8 mU/kg/min) was chosen for the hyperinsulinemia condition in Experiment 2, since the degree of hyperinsulinemia produced by this concentration was significantly greater than the hyperinsulinemia produced by the other two concentrations (see Figure 1 A). In addition, the greatest variability in DA levels was measured during the post clamp period with this concentration hinted that a decrease in blood glucose levels following hyperinsulinemia may alter mesoaccumbens DA. The elevations in plasma insulin levels for the hyperglycemic or hyperglycemic group infused with this insulin concentration are shown in Figure 3 A. In the hyperinsulinemic-hyperglycemic group the blood glucose was maintained at 13.88 ± 1.1 mmol/L, whereas for the hyperinsulinemic-hypoglycemic group it was kept at 3.33 ± 0.4 mmol/L for the clamp procedure, see Figure 3 B. For blood glucose values during the post clamp period in four of the five rats of the hyperinsulinemic-hypoglycemic condition the first three readings were below the low range sensitivity (1.1 mmol/L) of the instrument. In these instances the values were conservatively recorded as 1.0 mmol/L.

DA levels were elevated over time [$F(11, 88)=4.48$, $p<0.005$], despite that there was no group effect or interaction. In the hypoglycemic group there was an elevation in DA above baseline

at 100 min ($+113 \pm 33\%$; $p < 0.05$). This was still apparent at the end of the clamp, that is, at 120 min ($+117 \pm 37\%$; $p < 0.05$). The hyperglycemic group also showed an increase in DA above basal levels during the clamp period at 120 min ($+121 \pm 44\%$, $p < 0.05$; see Figure 5.5 A). Although there was no effect for group, time or their interaction for DOPAC levels (see Figure 4 B), the DOPAC to DA ratio decreased over time and approached significance [$F(11, 88) = 1.8183$, $p = 0.06$]. Also, HVA decreased over the experiment [$F(11, 88) = 3.0622$, $p < 0.005$], HVA was below baseline during the post clamp period at 20 min ($-27 \pm 5\%$, $p < 0.03$), 40 min ($-31 \pm 10\%$; $p < 0.03$) and at 60 min ($-38 \pm 10\%$; $p < 0.05$) for the hyperglycemic condition, see Figure 4 C.

3.3. Histology

In both experiments, the probe tips were located in the caudal region of the nucleus accumbens (anterior-posterior expanse; Bregma 1.6 –1.0; [24]) and the schematic of the active areas (~ 2 mm) of the individual probe membranes are depicted in Figure 5 A (Experiment 1) and Figure 5 B (Experiment 2). In all cases sampling occurred in the region medial to the anterior commissure and in most cases sampling was from both the core and shell regions.

4. Discussion

The major finding of this study was an increase in DA levels in the nucleus accumbens occurring during the later part of a 2 h hyperinsulinemic-glycemic clamp period under conditions of either hypoglycemia or hyperglycemia. In the hyperinsulinemic-hyperglycemic group, levels of the extraneuronal metabolite, HVA were decreased in the post clamp period. It could be speculated that the decrease in HVA and the trend for a decrease in DOPAC in the post clamp period of the hyperinsulinemic-hyperglycemic group resulted from a shift in DA metabolism to DA synthesis following the relatively rapid increase of DA at the end of the clamp period. In the hyperinsulinemic-hypoglycemic group, DA was elevated slightly earlier (at 100 min) and decreased more slowly in the post clamp period.

Despite our hypothesis for the first experiment, which was that different degrees of hyperinsulinemia would decrease accumbens DA, there was no difference in accumbens DA or metabolites in the hyperinsulinemic-euglycemic groups that had different degrees of hyperinsulinemia. The findings of the second experiment suggest that large deviations of plasma glucose levels from baseline levels under hyperinsulinemia, at least acutely, may be required to alter mesoaccumbens DA efflux. This finding is counter to our initial proposition that extreme glycemic conditions should have opposing effects on mesoaccumbens DA. Further experiments, accordingly, should examine if similar fluxes in blood glucose under conditions of hypoinsulinemia or euinsulinemia lead to accumbens DA alterations.

An immediate observation from using the hyperinsulinemic-glycemic technique in both of the experiments is the rather elevated baseline blood glucose levels compared with the values obtained normally in an unanaesthetized rat [27]. This effect was surprising since chloral hydrate has similar characteristics to an anesthetic that has minimal effects on glucose metabolism, sodium thiobarbital [28]. In that particular study, rats injected with chloral hydrate had elevated blood glucose 10 min after the injection and slightly increased glucose tolerance. Similar to sodium thiobarbital though, chloral hydrate did not increase mean arterial blood pressure or the percentage of islet blood flow (islet flow/ total pancreatic flow) and there was no difference in blood glucose levels 120 min after the glucose tolerance test (i.e., both ~ 5 mmol/L) [28]. The observed elevation in baseline blood glucose was corrected in this experiment by maintaining rats at ~ 5.5 mmol/L during the hyperinsulinemic-euglycemic clamp procedure (see results section for details). Notably, chloral hydrate also has been demonstrated to block cocaine induced Fos expression and reduce glutamate levels in the striatum [29]. In the same experiment there were, however, no differences in the basal or evoked

DA levels between chloral hydrate anesthetized and unanaesthetized rats [29]. Additional acute microdialysis studies examining accumbens DA have used chloral hydrate as an anesthetic because it has minimal effects on mesoaccumbens DA efflux over time [30-33] and it has a long effective half life (~6.5 h).

The results from the present study support the findings of Potter and colleagues that showed increased accumbens DA levels in response to a large bolus of peripheral insulin while the hypoglycemic effects remained uncompensated [7]. Interestingly in that study, the dose of peripheral insulin (600 mU) that effectively increased DA levels in the accumbens 30 min post-injection, conversely decreased DA levels in the dorsal striatum 80 min post-injection. Another microdialysis study also showed reduced DA levels in the central nucleus of the amygdala after the administration of a relatively low dose of peripheral insulin (200 mU) and increase in DA levels following an injection of peripheral glucose [34]. This suggests that peripheral insulin or glycemic levels or both differentially regulate DA efflux in dopaminergic terminal regions. In fact, it has been demonstrated that the function [12,35] and the half-life [36,37] of the DAT is regulated quite differently in the dorsal striatum versus the accumbens. Although the microdialysis probes were limited to shell/core regions of the nucleus accumbens in this study, the nigrostriatal DA system has been reported to contain a population of neurons that are responsive to alterations in glucose levels [38,39]. Reverse dialysis of glucose in the SNpc has been shown to lead to alterations in striatal DA efflux [38], whereas increases in plasma glucose levels produce decreases in the firing rate of SNpc neurons [39]. To our knowledge, a similar glucose sensing function has not been extensively reported in the mesoaccumbens DA pathway.

It is generally believed that insulin is not synthesized *de novo* in the adult brain [40,41], but rather is of pancreatic origin [40] and reaches the cerebrum by crossing the blood brain barrier via receptor mediated transcytosis [40,42-45]. The possible direct effects [11-13] of insulin on the mesoaccumbens DA neurons are dependent on this principle. In particular, it has been reported that insulin increases DA uptake in striatal tissue from >24 h fasted rats [12] and following amphetamine incubation in rat striatal synaptosomes and HEK transfected with hDAT preincubated with insulin [11]. Plasma insulin reached supraphysiological levels (~12 ng/ml or 1720 pmol/L) in the present experiments using the high dose of insulin during the clamp procedures, which ensured that insulin receptor were indeed saturated. Based on the protracted time course of hyperinsulinemia and the concurrent provision of either hyperglycemia or hypoglycemia to increase DA efflux, the observed results of the present experiments may be dependent on the engagement of other neural pathways that influence DA function. A distinct possibility is that the hyperinsulinemia conditions stimulate the insulin receptor dense arcuate nucleus [8], which has projections to the lateral hypothalamus [46] with downstream effects on accumbens DA [47]. Since blood glucose alterations were also necessary for the observed effects, coincident inputs from areas that are responsive to variations in plasma glucose, such as the SNpc [39] or ventral medial hypothalamus [48,49] might also be needed. Additionally, the circuitry involved in sympathoadrenal counter-regulatory responses to alterations in blood glucose levels, hyperinsulinemia [50], or physiological stress [51] cannot be discounted.

In summary, the present findings demonstrated that the mesoaccumbens DA system is responsive to changes in blood glucose under acute hyperinsulinemia, but the relationship between this effect and the concurrent metabolic conditions are rather complex.

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Figure 1.

Hyperinsulinemia-euglycemic clamp procedure. Plasma insulin (A) and blood glucose (B) during baseline, clamp, and post-clamp periods. Each rat was exposed to one of three insulin concentrations. The continuous intravenous insulin infusion rate for the “low” group was 2.4 mU/kg/ min, “moderate” dose was 4.8 mU/kg/min and “high” dose was 9.6 mU/kg per min. The high insulin concentration significantly elevated plasma insulin levels compared with the other groups (* $p < 0.01$).

Figure 2.

In vivo microdialysis of the nucleus accumbens during hyperinsulinemic-euglycemic clamping. Dopamine (A), DOPAC (B), HVA (C) during the clamp (left) and post clamp (right) periods. Microdialysis sampling began 1 h prior to the intravenous infusions to establish basal levels, was maintained during the 2 h clamp, and the 1 h post clamp periods. The microdialysis sampling period 1 h before the infusions represented the basal levels for DA and the metabolites.

Figure 3.

Hyperinsulinemia- glycemic clamp procedure. The graphs show plasma insulin (A) and blood glucose (B) during baseline, clamp, and post-clamp periods. The hyperinsulinemia condition was maintained by a continuous intravenous infusion of 9.6 mU/kg/min. During the clamp period hyperglycemia was maintained at approximately 14 mmol/L and hypoglycemia was maintained at approximately 3 mmol/L.

Figure 4.

In vivo microdialysis of the nucleus accumbens during hyperinsulinemic-glycemic clamping. Dopamine (A), DOPAC (B), HVA (C) during the clamp (left) and post clamp (right) periods. Differences in extracellular levels of DA were observed in both group and in HVA in the hyperinsulinemic-hyperglycemic group. Significant changes from basal levels are marked accordingly (* $p < 0.05$; # $p < 0.03$).

Figure 5.

Schematics of coronal sections of the rat brain depicting approximate microdialysis probe sites in the nucleus accumbens. Probe placements for Experiment 1(A) and Experiment 2 (B) is represented by gray bars depicting the extent of the active membrane (0.2 mm X 2 mm). Black areas represent the degree overlap within each experiment. Probes were implanted bilaterally, but data was analyzed from only one probe from each rat. Plates were modified from [24] and approximate anterior position in mm from Bregma are in bold.