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Impaired Striatal GABA Outflow in Leptin Receptor Deficient Rats - The Role of Glucose and K_{ATP} Channels

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ABSTRACT

The leptin receptor (LepR) is considered to be crucial for feedback mechanisms of food intake, body weight and glucose homeostasis. Its activity has been linked to ATP-dependent potassium (K_{ATP}) channels that couple bioenergetic metabolism to membrane excitability. In previous studies, it has been demonstrated that glucose modulates striatal γ-aminobutyric acid (GABA) outflow through K_{ATP} channels in slice preparations of the rat caudate nucleus. Our goal was to assess the impact of the LepR mutation on this mechanism. Slices of the caudate nucleus from heterozygous (Fa/fa) and homozygous (fa/fa) Zucker diabetic fatty rats (ZDF) and control Wistar rats were incubated with two different doses of glucose. GABA outflow was measured by means of high performance liquid chromatography (HPLC) with electrochemical detection. Glucose reduction (from 10 to 7 mM) resulted in a decreased GABA outflow in Wistar rats. In contrast, GABA outflow of striatal slices of LepR $^{Fa/fa}$ and LepR $^{fa/fa}$ rats remained nearly unchanged when extracellular glucose levels were lowered. The K_{ATP} channel blocker glibenclamide (Glb, 10 µM) prevented GABA outflow reduction in control rats at glucose concentrations of 7 mM, implying the involvement of K_{ATP}-channels. However, no change of GABA outflow was observed in LepR mutant rats after Glb addition. Although heterozygous ZDF rats possess one healthy LepR allele, no difference could be observed between lean heterozygous and obese homozygous animals. In summary, we demonstrate for the first time an impaired striatal GABA outflow in response to changing glucose levels in heterozygous and homozygous LepR mutant rats.

INTRODUCTION

ATP-dependent potassium channels (K_{ATP} channels) are an important group of ion channels that couple the cellular metabolic condition to membrane excitability [1]. Their physiological role is most clearly displayed in pancreatic β cells where they couple glucose metabolism to insulin secretion [2]. When glucose levels rise, an increase in the intracellular ATP/ADP ratio leads to closure of K_{ATP} channels, depolarization of the cell membrane and Ca^{2+}-mediated exocytosis of

insulin [1]. Moreover, K_{ATP} channels have been identified in several brain regions, such as the hypothalamus, hippocampus, substantia nigra and striatum [3]. In the brain, glucose responding neurons change their firing rate and neurotransmitter release according to the metabolic conditions [3].

Leptin is an anorexigenic hormone secreted by adipocytes in proportion to body fat. It acts via its receptor (LepR) to regulate the hypothalamic arcuate nucleus circuitry to mediate energy homeostasis and feeding behavior [4]. Animals with a mutation in the LepR or leptin gene exhibit hyperphagia, obesity and sometimes insulin resistance as well as diabetes [5]. Leptin replacement in leptin deficiency has been shown to ameliorate obesity, hyperglycemia and hyperinsulinaemia at doses that do not interfere with food intake, indicating that leptin might play a key role in maintaining glucose homeostasis independently of its role in controlling body fat stores [6]. Spanswick et al (1997) showed that leptin hyperpolarizes glucose responding neurons by activating K_{ATP} channels, a process that is impaired in obese Zucker rats [7]. It was previously hypothesized that γ-aminobutyric acid (GABA) release may be regulated by high and low affinity K_{ATP} channels located on GABAergic neurons [8]. In the present study, we used slices of the caudate nucleus from control Wistar rats, LepR-deficient heterozygous (LepR $^{Fa/fa}$) and homozygous (LepR $^{fa/fa}$) Zucker diabetic fatty rats (ZDF) to investigate the impact of LepR mutation on glucose and K_{ATP} channel-mediated GABA-outflow.

MATERIAL AND METHODS

Animals

Female Wistar rats (n=5) as well as heterozygous (LepR $^{Fa/fa}$, n=5) and homozygous (LepR $^{fa/fa}$, n=5) ZDF rats with an age of 10 weeks were used in this study. All animals were housed individually in transparent Makrolon cages (42 cm×26 cm×15 cm) with food and water *ad libitum* and constant temperature (22 ± 1°C) and humidity (55 ± 5%) under a 12 h light-dark cycle (light on at 6:00 a.m.). Experimental protocols and animal care were reviewed and approved by the "Ministerium für Umwelt, Natur und Forsten des Landes Schleswig-Holstein, Germany", and were conducted in accordance with the NIH guide for the Care and Use of Laboratory Animals.

Superfusion

The animals were euthanized with carbon dioxide and immediately decapitated. Brains were removed, placed in ice-cold Krebs-bicarbonate buffer (pH 7.4), and 250 µm thick caudate nucleus slices from both hemispheres were cut using a McIlwain tissue chopper. Slices were transferred to superfusion chambers and continually superfused (250 µl / min) with artificial cerebrospinal fluid (aCSF) of the following composition (in mM): NaCl 125, KCl 5, $MgSO_4$ x $7H_2O$ 1.14, KH_2PO_4 1.29, $CaCl_2$ x $2H_2O$ 2, $NaHCO_3$ 25, ascorbic acid 0.1, pargyline 0.01, glucose 10 (basal conditions) and glibenclamide 10 µM as indicated. Although GABA measurements were intended, the MAO A inhibitor pargyline was added to the medium to allow comparisons to the results of Steinkamp et al [8]. Slices were equilibrated with 95% O_2 and 5% CO_2, pH 7.4. After a 40 minute washout period, the first four superfusion samples were collected every 10 minutes and taken as basal outflow. Subsequently, glucose concentrations were reduced to 7 or 5 mM in the aCSF and samples were collected every 5 minutes again for 40 min until the end of the experiment. Caudate slices were then removed from chambers and tissue wet weight was measured. Wet weights of slices in each chamber were similar with the mean wet weight of 2.8 ± 0.7 mg, indicating no significances. All superfusion samples were stored at -40 °C and subsequently measured by means of high performance liquid chromatography (HPLC) with electrochemical detection.

HPLC

The HPLC system consisted of a C18 column (Eurospher RP 18, 5 mm, column size 250x4 mm with a pre-column 35x4 mm). GABA values were measured after pre-column derivatization with o-phthaldialdehyde and sodium sulphite for 30 min [9]. Separations were performed under isocratic conditions and the mobile phase (0.1 M sodium phosphate buffer, 0.5 mM EDTA, 25% methanol pH 4.5 and degassed by helium) was pumped at a flow rate of 1.0 ml/min. All chromatographies were performed at 30 °C. The compounds were detected electrochemically using a glassy carbon electrode set at a potential of 900 mV relative to an Ag/AgCl reference electrode (WatersTM 460

electrochemical detector, Millipore Corporation, Eschborn/Ts., Germany). The detection limit of GABA was 2 nM and data was expressed in nmol/l/mg wet weight.

Statistical analysis

GABA outflow was expressed in nmol/l/mg tissue wet weight or as % of control value, as mean ± standard deviation of the mean (SD). The average of four baseline samples was defined as 100%. Differences between the means of treatments and their corresponding controls were tested with one-way analysis of variance and multiple comparisons (ANOVA). Subsequent multiple comparisons were made by Kruskal-Wallis-test and t-test for pairwise comparisons as indicated. Statistical significance was set at $p< 0.05$.

RESULTS & DISCUSSION

Steinkamp et al [8] found increased extracellular dopamine levels under glucose reduction in rat striatal slices. Since their results could be modulated by the GABAA-R antagonist bicuculline they suggested that the dopamine effect was due to reduced GABA activity. The relationship between dopamine and GABA in the case of glucose reduction could now be corroborated by direct GABA measurements described here. Reduction of glucose from 10 mM to 7 mM in the incubation medium resulted in a significant decrease in GABA outflow from 2.5 to 1.1 nmol/l/mg tissue wet weight in control Wistar rats ($p<0.05$, Figure 1). This finding is also consistent with previous reports indicating that GABA release depends on glucose availability in the substantia nigra or the ventromedial hypothalamus in vivo [10, 11]. As shown in Figure 1, further decrease of extracellular glucose to 5 mM did not lead to significant changes of GABA outflow when compared to 7 mM glucose in the superfusion medium. When incubating striatal slices of Wistar rats, lean LepR $^{Fa/fa}$ and obese LepR $^{fa/fa}$ rats with glucose concentrations of 10 mM, GABA outflow was similar between the groups (2.5 nmol/l/mg, 2.8 nmol/l/mg and 3.2 nmol/l/mg tissue wet weight, respectively). However, in contrast to Wistar rats, changes in GABA outflow could not be observed in striatal slices of ZDF rats after glucose reduction (Figure 2). Application of the K_{ATP}-channel blocker glibenclamide (10 µM) to striatal slices of control Wistar rats abolished the decrease of GABA levels induced by glucose reduction (Figure 1 & 2). These results are in line with previous findings that glucose is indeed able to modulate GABA outflow via K_{ATP} channels in striatal slices [11].

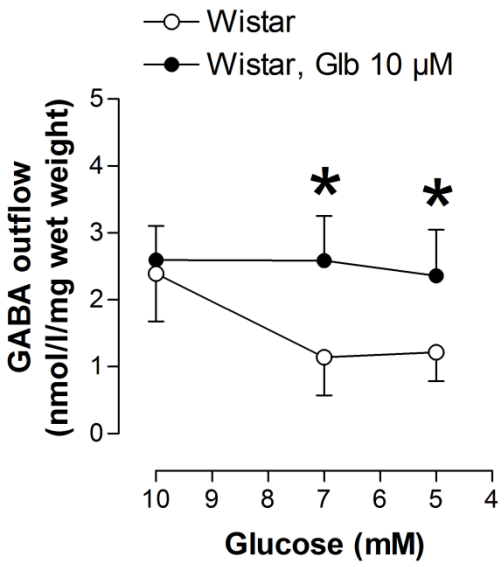

Figure 1: *GABA outflow of caudate nucleus slices taken from control Wistar rats in response to Glucose levels.* GABA outflow was reduced after lowering glucose levels in the superfusion medium from 10 to 7 mM. Decrease to 5 mM glucose did not lead to additional reduction in GABA outflow. Addition of the K_{ATP} channel blocker glibenclamide (Glb) significantly abrogated the decreasing effect (N=10 for each experimental condition, $p<0.05$, t-test).

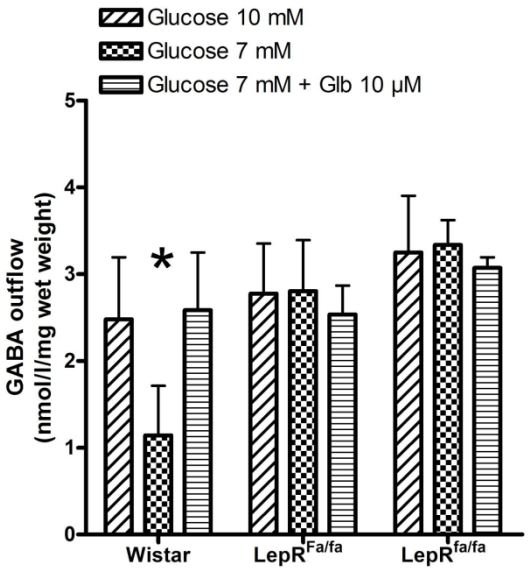

Figure 2: GABA outflow in response to glucose levels and addition of glibenclamide in caudate nucleus slices of control Wistar rats, heterozygous LepR $^{Fa/fa}$ and homozygous LepR $^{fa/fa}$ ZDF rats. GABA outflow at 10 mM glucose was similar between all groups. Reduction to 7 mM glucose led to a

significant decrease in control animals only (N=10 for each experimental condition, $p<0.05$, Kruskal-Wallis-test). This effect was completely abolished by addition of glibenclamide. Neither heterozygous, nor homozygous ZDF rats showed a reduction in GABA outflow for reduced glucose levels, nor had the addition of glibenclamide any measurable effects.

The lack of GABA decrease after reduction of glucose in the caudate nucleus of heterozygous and homozygous ZDF rats, however, is unexpected as former studies indicated that the anti-obesity and anti-diabetic effects of leptin are probably mediated by GABAergic neurons [12-14]. It has been shown that leptin acts on GABAergic neurons by decreasing their inhibitory tone on anorexigenic POMC neurons. Furthermore, deleting LepR in GABAergic neurons is sufficient to mediate most of the LepR mutant obese phenotype [14]. While the direct link between LepR signalling and GABA release in hypothalamic neurons is clearly established, the absence of LepR expression in the striatum precludes direct effects [15, 16]. However, functional imaging in leptin deficient patients demonstrated altered responses to alimentary stimuli and their normalization after leptin replacement therapy [17]. Therefore, our data might reflect extended and indirect effects of LepR signalling on the entire food reward loop [18].

Another interesting fact was the observation of identical striatal GABA levels in the extracellular space after glucose reduction in both lean heterozygous (LepR $^{Fa/fa}$) and obese homozygous (LepR $^{fa/fa}$) rats. Further addition of glibenclamide did not modify striatal GABA levels in both genotypes (Figure 2). While the concept of enhanced glucose sensitivity of homozygous ZDF rats has been established for some time, heterozygous lean rats have been generally considered as unaffected [19, 20]. One possible explanation could be that the mutation of just one allele is sufficient to affect glucose sensitivity in ZDF rats. On the other hand, reduction from 10 to 7 mM glucose might not be efficient enough to show significant differences between both genotypes. Further work needs to be conducted in order to clarify these unexpected results.

In summary, our data confirm that glucose availability influences striatal GABA outflow by using K_{ATP} channels. Furthermore, our study supports the hypothesis that leptin plays an important role in maintaining glucose homeostasis. The steady values of extracellular GABA levels after glucose reduction in ZDF rats indicate that LepR mutation and its inherent insensitivity to glucose lead to hyperinhibition of the system and point to an impaired regulation of GABAergic transmission in the striatum of ZDF rats.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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