Brief Communication

MOLECULAR METABOLISM

Chronic D-serine supplementation impairs insulin secretion

Lisa Suwandhi [1,2], Simone Hausmann [1,2], Alexander Braun [3], Tim Gruber [2,4,13], Silke S. Heinzmann [2,5], Eric J.C. Gálvez [6], Achim Buck [7], Beata Legutko [2,4], Andreas Israel [1,2], Annette Feuchtinger [7], Elizabeth Haythorne [8], Harald Staiger [2,9,10], Martin Heni [2,10,11], Hans-Ulrich Häring [2,10,11], Philippe Schmitt-Kopplin [2,5], Axel Walch [7], Cristina García Cáceres [2,4], Matthias H. Tschöp [2,4,13], Guy A. Rutter [8], Till Strowig [6], Martin Elsner [3,12], Siegfried Ussar [1,2,*]

ABSTRACT

Objective: The metabolic role of D-serine, a non-proteinogenic NMDA receptor co-agonist, is poorly understood. Conversely, inhibition of pancreatic NMDA receptors as well as loss of the D-serine producing enzyme serine racemase have been shown to modulate insulin secretion. Thus, we aim to study the impact of chronic and acute D-serine supplementation on insulin secretion and other parameters of glucose homeostasis.

Methods: We apply MALDI FT-ICR mass spectrometry imaging, NMR based metabolomics, 16s rRNA gene sequencing of gut microbiota in combination with a detailed physiological characterization to unravel the metabolic action of D-serine in mice acutely and chronically treated with 1% D-serine in drinking water in combination with either chow or high fat diet feeding. Moreover, we identify SNPs in SRR, the enzyme converting L-to D-serine and two subunits of the NMDA receptor to associate with insulin secretion in humans, based on the analysis of 2760 non-diabetic Caucasian individuals.

Results: We show that chronic elevation of D-serine results in reduced high fat diet intake. In addition, D-serine leads to diet-independent hyperglycemia due to blunted insulin secretion from pancreatic beta cells. Inhibition of alpha 2-adrenergic receptors rapidly restores glycemia and glucose tolerance in D-serine supplemented mice. Moreover, we show that single nucleotide polymorphisms (SNPs) in SRR as well as in individual NMDAR subunits are associated with insulin secretion in humans.

Conclusion: Thus, we identify a novel role of D-serine in regulating systemic glucose metabolism through modulating insulin secretion.

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Keywords D-serine; Diabetes; Obesity; Insulin secretion

1. INTRODUCTION

Type 2 diabetes (T2D) is a multifactorial metabolic disease, which, as shown by a large number of studies including genome-wide association studies (GWAS) [1—3] as well as numerous large scale metabolite screens [4,5] depends on complex gene and environment interactions. To date, single nucleotide polymorphisms (SNPs) in more than 100 genes have been described to contribute to the development of T2D [3]. Moreover, the list of host or environment derived metabolites contributing to the development of T2D is continuously growing [6—8]. Importantly, many of the identified SNPs and metabolites

associate with alterations in beta cell function and insulin secretion [9—11]. However, some of these proteins and metabolites are also associated with the development of other diseases not directly linked to beta cell function [12]. One of those genes is serine racemase (SRR) [13], which catalyzes the conversion from L-serine to D-serine, an important co-agonist of N-methyl-D-aspartate (NMDA) receptors. SRR function was recently suggested to regulate insulin secretion from pancreatic beta cells [3,14], and chronic as well as acute supplementation of the SRR product D-serine reduced food intake and weight gain upon high fat diet (HFD) feeding in mice [15,16]. However, dysfunction of SRR, the D-serine degrading D-amino acid oxidase

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(DAO), and the main D-serine transporter alanine-serine-cysteine transporter 1 (Asc-1) are implicated in the development of schizophrenia, Alzheimer's disease, and depression [17—21]. However, a more detailed knowledge on the metabolic actions of D-serine could contribute to a better understanding of the strong linkage between obesity, T2D, and schizophrenia [22].

Here, we report positive and negative consequences of long-term treatment with D-serine. We show that D-serine suppresses high fat diet intake resulting in reduced weight gain. Moreover, we demonstrate that highly dosed D-serine induces hyperglycemia, and strongly impairs glucose tolerance due to impaired insulin secretion from pancreatic beta cells. This depends on increased sympathetic activity in the islets of Langerhans rather than direct action on beta cells and can be completely reversed by alpha 2-adrenergic receptor antagonists. Furthermore, we identify novel SNPs in SRR as well as several NMDAR subunits to associate with insulin secretion in humans. Thus, alterations in D-serine levels could contribute to the development of T2D through the modulation of insulin secretion, especially in genetically predisposed subjects.

2. MATERIAL AND METHODS

2.1. Mouse models

C57BL/6N-Rj male mice were purchased from Janvier at age 3 weeks or 7 weeks. Germfree mice were bred at the HZI in Braunschweig. The mice were kept in groups of at least 4 under a 12 h light: 12 h dark cycle and an ambient temperature of 22 ± 2 °C. Mice were fed a standard laboratory chow diet (Altromin 1314) or a 58% high fat diet (Research Diets D12331) *ad libitum*. Animals received either normal water or water supplemented with 1% D-serine (Sigma) or/and 0.3% Dextromethorphan hydrobromide monohydrate (DXM; Sigma) *ad libitum*. To study acute oral uptake of D-serine, eight weeks old male C57Bl/6 mice were gavaged with 100 mg D-serine/kg body weight. For the food preference test, mice were starved for 16 h and afterwards refed with CD and HFD. After 3 h, the weight of the consumed food was determined. Body composition was analyzed with a non-invasive magnetic-resonance whole-body composition analyzer (EchoMRI). Energy expenditure, food and water consumption, locomotor activity, and respiratory exchange quotient (RER) was measured with an indirect calometric system (TSE PhenoMaster). Fecal caloric content was measured from dried fecal pellets using a 6300 Oxygen Bomb Calorimeter (Parr Instrument Technology). Animal experiments were conducted in accordance with the German animal welfare law and performed with permission and in accordance with all relevant guidelines and regulations of the district government of Upper Bavaria (Bavaria, Germany), protocol number 55.2-1-54-2532-52-2016.

2.2. Glucose, insulin, pyruvate tolerance tests and assessment of glucose stimulated insulin secretion

Glucose tolerance tests (GTTs) and insulin tolerance tests (ITTs) were carried out in mice fasted for 4 h. 2 g/kg glucose (20% Glucose solution; Braun) or 0.75 U insulin/kg BW (Actrapid® PenFill® Novo Nordisk) were injected intraperitoneally (i.p.) and glucose concentrations measured in blood collected from the tail before and 15, 30, 60, and 120 min after the injection using a FreeStyle Freedom Lite glucometer (Abbott). Glucose stimulated insulin secretion (GSIS) was assessed in mice fasted for 4 h prior to the i.p. injection of 5 mg/kg BRL44408 (abcam) and 4 g/kg glucose. Blood was collected from the tail vein before and 5, 10, 15, and 30 min after the injection. At the same time points, glucose concentrations were measured. Serum was

prepared by centrifugation (5 min 10.000 × g 4 °C) and serum insulin concentrations were determined using the mouse ultrasensitive insulin ELISA kit (Alpco). For the pyruvate tolerance test (PTT), mice were fasted for 16 h and 5 mg/kg sodium pyruvate (Sigma—Aldrich) were injected i.p. and glucose concentrations were measured before and 15, 30, 60, and 120 min after the injection.

2.3. D-serine measurement

D-serine, L-serine, sodium tetraborate, Marfey's reagent (N_{α}-(2,4-dinitro-5-fluorophenyl)-L-alaninamide), hydrochloric acid, and acetone were purchased from Sigma—Aldrich. Acetonitrile was purchased from Thermo Scientific. LC-MS grade water with 0.1% acetic acid was purchased from Fluka.

Tissues were homogenized in 1 ml 100% methanol using a Polytron and dried at 60 °C under a stream of N_2, and the dried residues were reconstituted in 50 µl milliQ water. Separation of the serine enantiomers was performed with chemical derivatization using Marfey's reagent, generating dinitrophenyl-5-L-alanine-d/L-serine diastereomers (DNPA-D/L-serine), which can be separated by reverse-phase chromatography [23]. To this end, 100 µl methanol, 50 µl 0.125 M sodium tetraborate buffer, and 50 µl 1% Marfey's reagent in acetone were added to the reconstituted sample and heated to 50 °C for 60 min. Derivatization was then stopped by addition of 20 µl 2N HCl. For standard curve calibration, D- and L-serine standards were prepared at 166, 55, 18, 6, 2, 0.7, 0.2, 0.07, and 0.02 mg/l by serial dilution of a 500 mg/l stock solution and derivatized as described above.

Chromatographic separation of DNPA-D- and DNPA-L-Serine was accomplished on an Agilent HP 1200 HPLC system using a Synergi Hydro-RP (C18) 150 mm × 2.1 mm I.D., 4 µm 80°A particles column (Phenomenex) at 25 °C. Mobile phase A was LC-MS grade water with 0.1% acetic acid and mobile phase B was acetonitrile, which was delivered according to the following gradient profile (min/% mobile phase B): 0.0/5, 1.9/5, 12/20, 20/20, 24/90, 30/90. The flow rate was 0.2 ml/min and the injection volume was 10 µl.

The HPLC system was coupled to a Q-Trap MS/MS system (Applied Biosystems). Ionization was accomplished by electrospray ionization in the negative ion mode. Mass spectrometry was carried out in the multiple reaction monitoring mode. To determine the MS/MS fragment pattern with the highest intensity, a single analyte standard containing DNPA-D/L-serine was dissolved in a mixture of water and methanol 50:50 (v/v) at a concentration of 1 mg/l. The standard was infused at a flow rate of 200 µl/min for tuning the compound-dependent MS parameters. The infusion experiment was performed using a syringe pump directly connected to the interface. Optimal detection was provided by scanning for the mass pair 356/356. Declustering potential, collision energy, collision cell exit potential, and entrance potential were optimized to −45, −12, −3, and −6 V. The optimized values for the parameters ion spray voltage, nebulizer gas, auxiliary gas, curtain gas, collision gas and auxiliary gas temperatures were −4500, 40, 35, 20, 3, and 400 °C, respectively. The curtain, collision, turbo, and nebulizer gas was nitrogen generated from pressurized air in a nitrogen generator. To obtain adequate selectivity and sensitivity, the mass spectrometer was set to unit resolution, and the dwell time was 150 ms. Calibration curves were obtained by plotting the peak area of D/L-serine against their concentration. Peak integration was automatically accomplished via Analyst software (version 1.4.2, AB/MDS Sciex). A least squares regression analysis was used to obtain a linear equation over the range of the calibration. The assay finally produced a standard curve that was linear over a range of 0.02—166 mg/l. Samples were measured in triplicates.