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The effects of a *HTR2B* stop codon and testosterone on energy metabolism and beta cell function among antisocial Finnish males

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ABSTRACT

Herein, we examined insulin resistance (IR), insulin sensitivity (IS), beta cell activity, and glucose metabolism in subjects with antisocial personality disorder (ASPD), and whether the serotonin 2B (5-HT2B) receptor and testosterone have a role in energy metabolism. A cohort of subjects belonging to a founder population that included 98 ASPD males, aged 25–30, was divided into groups based on the presence of a heterozygous 5-HT2B receptor loss-of-function gene mutation (*HTR2B* Q20*; n = 9) or not (n = 89). Serum glucose and insulin levels were measured in a 5 h oral glucose tolerance test (75 g) and indices describing IR, IS, and beta cell activity were calculated. Body mass index (BMI) was also determined. Concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid were measured in cerebrospinal fluid, and testosterone levels from serum. An IR-like state comprising high IR, low IS, and high beta cell activity indices was observed among ASPD subjects without the *HTR2B* Q20* allele. By contrast, being an ASPD *HTR2B* Q20* carrier appeared to be preventive of these pathophysiologies. The *HTR2B* Q20* allele and testosterone predicted lower BMI independently, but an interaction between *HTR2B* Q20* and testosterone lead to increased insulin sensitivity among *HTR2B* Q20* carriers with low testosterone levels. The *HTR2B* Q20* allele also predicted reduced beta cell activity and enhanced glucose metabolism. Reduced 5-HT2B receptor function at low or normal testosterone levels may be protective of obesity. Results were observed among Finnish males having an antisocial personality disorder, which limits the generality.

1. Introduction

Comorbidities of psychiatric and metabolic disorders are frequent. For example, more than 25% of individuals diagnosed with diabetes also suffer from depression (Lustman and Clouse, 2005). The combined impact of metabolic and psychiatric disorders decreases daily executive performance and quality of life, causes severe organ complications, and increases mortality, which support a rationale to further examine metabolic pathophysiologies associated with distinct psychiatric disorders. ASPD is a psychiatric disorder, with a prevalence of 1% (Lenzenweger et al., 2007), which

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is linked to an inherently impulsive lifestyle that puts ASPD patients at risk for metabolic disorders because of challenges in maintaining a healthy diet and physical exercise. A Finnish violent offender population saturated with ASPD individuals has been shown to exhibit elevated basal insulin levels (Ojala et al., 2015).

In addition to the clinical hypothesis that patients with ASPD are at an increased risk for insulin-related pathophysiologies, we also hypothesized that the serotonergic pathway could alter insulin secretion and glucose homeostasis. Indeed, preliminary evidence that was mostly obtained from animal study settings suggest that serotonin (5-HT) is involved in the control of islet function and links to insulin resistance (IR) (Bennet et al., 2015; Saunders et al., 2014). Moreover, some studies imply that functional silencing of the serotonin 2B (5-HT2B) receptor may have protective effects on risk for IR and type 2 diabetes (T2D) because the function of the receptor seems to alter islet function and glucose homeostasis (Bennet et al., 2016; Kim et al., 2010, 2015; Tikkanen et al., 2016; Yamada et al., 1998). However, the role of the G_q-coupled serotonin 5-HT2B receptor in human somatic health is poorly characterized, but in psychiatric research studies the 5-HT2B receptor has been recently shown to exert the following tangible effects on human behavior and psychiatric symptoms: increased impulsive behavior, alcohol-related problem-behavior, emotional dysregulation, mood disorders, and anxiety (Bevilacqua et al., 2010; Tikkanen et al., 2015).

To examine the effects of the serotonergic pathway, we studied heterozygous carriers of a 5-HT2B receptor gene mutation (*HTR2B* Q20*) detected in a Finnish young founder population (Bevilacqua et al., 2010). *HTR2B* Q20* is a point-mutation of the 5-HT2B receptor gene, which is located at 2q36-q37. The mutation results in interrupted expression of the 5-HT2B receptor in lymphoblastoid cells, which results in a 50% reduction of the expression of the receptor protein in heterozygous individuals (Bevilacqua et al., 2010). The prevalence of the hereditary *HTR2B* Q20* is relatively high (2.2%) in the Finnish general population. Testosterone was included in analyzes because testosterone levels has been suggested to be elevated in *HTR2B* knockout mice and heterozygous *HTR2B* Q20* carriers in comparison to carriers of the wild type allele (Bevilacqua et al., 2010). Also, reduced testosterone levels have been associated with IR, T2D and obesity (Haffner et al., 1994; Jones, 2010).

2. Material and methods

2.1. Subjects and subgroups

Subjects were obtained from a genotyped cohort that included Finnish alcoholic violent offenders, their relatives, along with healthy controls who were recruited by newspaper advertisements; the cohort included a total of 875 subjects. This cohort was collected to detect biological risk factors for psychiatric disorders and impulsive behaviors. All subjects diagnosed with ASPD that had participated in an oral glucose tolerance test (OGTT) were included without pre-selection, resulting in a total of 98 subjects, all males, who were separated for comparison into the following two groups: heterozygote *HTR2B* Q20* allele carriers ($n = 9$) and subjects homozygous for the wild-type *HTR2B* Q20 allele ($n = 89$). The genotyping procedure has been previously described in detail by Bevilacqua et al. (2010). Genetic and molecular analyzes were performed at the Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, NIH (Bethesda, MD, USA).

2.2. Laboratory tests

Serum levels of glucose, insulin, and testosterone, and gamma-glutamyl transferase were measured after fasting overnight. Insulin was analyzed using a radioimmunoassay method and quantified in antibody-coated test tubes (Count-A-Count, Diagnostic Products Corporation, Los Angeles, CA, USA). Between-assay variation for insulin was 4.6% at 215 pmol/l. All samples were assayed in duplicate. When results of duplicate measurements showed discrepancies of more than 5%, samples were reanalyzed (Virkkunen et al., 1994). For clinical convenience, insulin levels are reported as mU/L. None of the participants had previously used antidepressants or antipsychotics within the two weeks prior to the laboratory tests; lack of drug use status was verified using urine tests. All subjects ate the same food and were not allowed to exercise 24-h prior to measurements. OGTT was administered after 12- to 16-h fasting overnight. At 8 a.m., participants drank a solution containing 75 g glucose (Leiras, Turku, Finland), which was ingested as quickly as possible. From an antecubital vein, nine blood samples (15 mL each) were collected during the 5 h test. For the first 2 h of the test, subjects rested in bed. Thereafter, they were allowed to move about the ward, but resting was encouraged. Blood glucose concentrations were determined enzymatically. Blood samples were stored in tubes that contained sodium fluoride.

Levels of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) were measured from cerebrospinal fluid (CSF) that was obtained by lumbar puncture. A total of 12 mL CSF was collected and 5-HIAA concentrations were analyzed by liquid chromatography and electrochemical detection methods. Prior to puncture between 8 and 9 a.m. the subjects had only been allowed to drink water after 8 p.m. the night before. Moreover, they had eaten the same food and were not allowed to exercise 24 h prior the puncture. No subjects had used a psychoactive medication, such as selective serotonin reuptake inhibitors, within the previous two weeks, which was confirmed by urine tests.

2.3. Measurements of glucose levels, insulin resistance, insulin sensitivity, and beta cell activity

WHO, (2006) definitions for normal and pathophysiological glucose levels were used. Normal fasting glucose levels were defined as 3.9–6.0 mmol/L, impaired fasting glucose tolerance (IFG) was defined as 6.1–6.9 mmol/L, and diabetes was defined as $\geq$7.0 mmol/L. The following glucose reference values at 120 min measurements in the OGTT were used to define impaired glucose tolerance (IGT) and diabetes, respectively: $\geq$7.8 and $\leq$11.0 mmol/L and $\geq$11.1. Additionally, IR, IS, and beta cell activity were calculated using the homeostasis model assessment calculator (Levy et al., 1998) (https://www.dtu.ox.ac.uk/homacalculator, accessed 02.03.16), which utilizes fasting glucose and fasting insulin values. Normal reference values for HOMA2 IR, IS, and beta cell activity are 1.0, 100%, and 100%, respectively. Furthermore, IR and IS were assessed based on the whole body insulin sensitivity index (WBISI) (Matsuda and DeFronzo, 1999) calculated from measurements obtained from the OGTT utilizing glucose and insulin values at baseline, and at 30, 60, 90, and 120 min (http://mmatsuda.diabetes-smc.jp/MIndex.html, accessed 02.04.16). A WBISI value of $\leq$2.5 denoted pathological whole body insulin resistance.

2.4. Area under the curve (AUC)

AUC values were calculated using the trapezoidal method (Purves, 1992) representing the “average curve” because it has been suggested that subtle biological variance might not be detected when presenting the “curve of averages” alone (Allison et al., 1995; Matthews et al., 1990). When the golden standard 2 h AUC was used for calculations of the AUC value, it was subtracted from the baseline value for each subject. Then, mean values were compared between groups.