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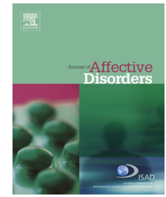

Research report

Fluoxetine-induced pancreatic beta cell dysfunction: New insight into the benefits of folic acid in the treatment of depression

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ABSTRACT

Background: Major depressive disorder is a common psychiatric illness with reported prevalence rates of 12–16% in persons aged 12 and over. Depression is also associated with a high risk of new onset of type 2 diabetes (T2D). This relationship between depression and diabetes may be related to depression itself and/or drugs prescribed. Importantly, the use of selective serotonin reuptake inhibitors (SSRIs), the most commonly prescribed class of antidepressants, increases the risk of developing T2D. However, the mechanism(s) underlying this association remains elusive.
Methods: Here we examine the effects of the SSRI fluoxetine (Prozac®) on beta cell function utilizing INS-1E cells, a rat beta cell line, to elucidate the underlying molecular mechanisms.
Results: Fluoxetine treatment significantly reduced glucose stimulated insulin secretion (GSIS). This decreased beta cell function was concomitant with an increased production of reactive oxygen species and oxidative damage which may contribute to decreased mitochondrial electron transport chain enzyme (ETC) activity. Importantly the fluoxetine-induced deficits in beta cell function were prevented by the addition of the antioxidant folic acid.
Limitations: These studies were conducted in vitro; the in vivo relevance remains to be determined.
Conclusions: These findings suggest that use of SSRI antidepressants may increase the risk of new-onset T2D by causing oxidative stress in pancreatic beta cells. However, folic acid supplementation in patients taking SSRIs may reduce the risk of new onset diabetes via protection of normal beta cell function.
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1. Introduction

Patients with major mental illnesses have an increased risk of metabolic disorders including type 2 diabetes (T2D) (Pan et al., 2012; Renn et al., 2011). Established risk factors for the increased incidence of T2D in patients with depression include: familiarity and genetic predisposition; socioeconomic status; maladaptive behaviors during a depressive episode such as comfort eating and physical inactivity and psychotropic medication's use (McIntyre et al., 2010; Rotella and Mannucci, 2013). Antidepressants are a first-line option for the management of moderate to severe depression, and estimates suggest that in the USA alone, 27,000,000 persons are taking antidepressants (Keller et al., 2005; Olfson and Marcus, 2009). Antidepressant use in Canada is equally astounding; in the Canadian Community Health Survey (Cycle 1.2; 2002) 5.8% of Canadians were reportedly taking antidepressants (Beck et al., 2005). There is now considerable evidence from animal experiments and clinical studies that antidepressant use constitutes a major risk factor for impaired glucose homeostasis and T2D (Bhattacharjee et al., 2013; Rotella and Mannucci, 2013). Although there are a wide variety of medications available for the treatment of depression (Boonstra et al., 2011; Lam et al., 2009; Philip et al., 2008), selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed therapy for adults, children and adolescents (Birmaher et al., 1998; Gelenberg, 2010; Lam et al., 2009). In humans, the use of SSRIs appears to increase the risk of developing T2D. Indeed, a retrospective cohort study identified an increased odds ratio of developing T2D in children and adolescents taking SSRIs ($OR=1.37$; 95% $CI=1.10$–1.71) (Jerrell et al., 2012). Similarly, a recent large case-control study reported that long-term use of SSRIs (i.e., greater than 24 months) in adults, was associated with an increased risk of T2D (incidence rate ratio = 2.06; 95% $CI=1.20$–3.52) (Andersohn et al., 2009). Although the mechanisms underlying the increased risk of T2D in patients with SSRI use have not been fully explored, a recent study has reported that SSRIs can directly impact pancreatic beta cell function (Isaac et al., 2013).

Isaac et al. (2013) demonstrated that high doses of (30 μM) sertraline inhibited glucose-stimulated insulin secretion (GSIS) from pancreatic islets (i.e., impaired beta cell function) and led

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to increased beta cell death (Isaac et al., 2013). The mitochondria plays a central role in regulating beta cell function and survival (Supale et al., 2012); notably SSRI exposure has been shown to cause mitochondrial dysfunction in a variety of cell types (Abdel-Razaq et al., 2010; Agostinho et al., 2011; Han and Lee, 2009; C. S. Lee et al., 2010) although the effects of SSRIs on beta cell mitochondrial function have not been explored. Moreover, SSRIs have also been reported to increase the production of reactive oxygen species (ROS) (Mun et al., 2013) resulting in damage to mitochondrial as well as cytoplasmic proteins, lipids and nucleic acids (Ježek et al., 2012; Supale et al., 2012; Wallace, 2005). Collectively, oxidative stress, an imbalance between the production of ROS and the cellular antioxidant defense system, plays an essential role in the development of T2D (Drews et al., 2010). Indeed, increased ROS production may have profound effects in the endocrine pancreas because pancreatic beta cells have low levels of anti-oxidant enzymes and are therefore particularly susceptible to oxidative stress (Lenzen et al., 1996; Tiedge et al., 1997). Since SSRI exposure has been shown to increase oxidative stress in a number of cell types, it is plausible to suggest that an antioxidant therapy might ameliorate SSRI-induced beta cell deficits. One such therapy is folic acid.

Results from epidemiological studies suggest that low folate may be a risk factor for depression (Gilbody et al., 2007). As a result there has been considerable interest in the use of folic acid as a treatment for depression, alone or as an adjunct to antidepressant use (Taylor et al., 2004). Interestingly, folate deficiency increases ROS production in RINm5F pancreatic beta cells (Hsu et al., 2013) and folic acid administration reduces oxidative stress in patients with type 2 diabetes (Lazalde-Ramos et al., 2012). Taken together, these data suggest that folic acid supplementation might prevent or ameliorate SSRI-induced deficits in pancreatic beta cell function.

2. Methods

2.1. Cell culture maintenance and treatment

INS-1E cells were generously provided by Dr. Claes Wollheim (University of Geneva, Geneva, Switzerland). Cells between passages 60–90 were cultured at 37 °C in a humidified atmosphere of 95% O_2 and 5% CO_2 in RPMI-1640 (RPMI; Sigma Aldrich, Oakville, ON), supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Logan, UT), 1 mM sodium pyruvate, 50 μM β-mercaptoethanol, 1 mM glutamine, 10 mM HEPES, 1 U/ml penicillin, and 1 μg/ml streptomycin (Sigma Aldrich). Unless otherwise noted, experimental protocols were carried out in RPMI media supplemented as described above.

2.2. Cell viability

INS-1E cells were seeded in 96-well plates at a density of 1×10^4 cells/well. Cells were allowed to attach for 24 h. After 24 h, media was removed and cells were treated with vehicle (control) or increasing ½ log doses of fluoxetine hydrochloride ranging from 1×10^{-10} M to 1×10^{-2} M (Toronto Research Chemicals, North York, ON). After 48 h, cell viability was determined using the MTS assay (Promega, Madison, WI) according to the manufacturer's instructions.

2.3. ROS production and oxidative damage

ROS have been shown to act as important signaling molecules in the pancreatic beta cell (Pi et al., 2010). However, if the level of ROS exceeds the antioxidant capacity of the cell, oxidative damage and impaired beta cell function will ensue (Pi et al., 2010). Notably, pancreatic beta cells are particularly susceptible to oxidative damage (Bhattacharjee et al., 2013; Keller et al., 2005; Lenzen et al., 1996; Olfson and Marcus, 2009; Rotella and Mannucci, 2013; Tiedge et al., 1997). To examine if fluoxetine exposure led to increased oxidative damage to pancreatic beta cells, we determined the effect of fluoxetine on ROS production by measuring hydrogen peroxide levels (H_2O_2); H_2O_2 is the most common ROS produced by the mitochondria (Pomytkin, 2012). INS-1E cells were seeded in 96-well plates at a density of 20,000 cells/well. Cells were allowed to attach for 24 h. After 24 h, media was removed and cells were treated with vehicle (control) or 1 μM fluoxetine hydrochloride (Toronto Research Chemicals). This concentration approximates the 90th percentile of human serum fluoxetine concentrations (i.e., 1.2 μM) (Keller et al., 2005; Reis et al., 2009). After 48 h, hydrogen peroxide production in the cell culture supernatant was determined using a commercially available kit (OxiSelect™ Hydrogen Peroxide Assay Kit, Cell Biolabs, Inc. San Diego, CA) according to the manufacturer's instructions.

We further assessed oxidative damage by determining the presence of 4-hydroxy-2-nonenal [4-HNE], a marker of oxidative damage of lipids, by Western blotting. To determine the balance between ROS production and antioxidant capacity of the cell, protein expressions of resident antioxidant enzymes Cu–Zn superoxide dismutase (SOD1), Mn superoxide dismutase (SOD2), catalase and glutathione peroxidase were determined by Western blotting.

2.4. Protein expression of oxidative stress markers

INS-1E cells were seeded in 100 mm dishes until they reached 80% confluence. Cells were then washed with PBS and then incubated with 0 (control) or 1 μM fluoxetine hydrochloride (Toronto Research Chemicals) for 48 h ($N=5$ independent experiments). Following 48 h treatment, cells were pelleted by centrifugation (2000 rpm for 5 min), re-suspended in RIPA lysis buffer (15 mM Tris–HCl, 1% (v/v) Triton X-100, 0.1% (w/v) SDS, 167 nMNaCl, 0.5% (w/v) sodium deoxycholatic acid), with Complete Mini EDTA-free protease inhibitor cocktail tablets (Roche Applied Science). The cellular suspension was sonicated (Microsonix 200) at 7 Hz for 15 sec. Protein content in the supernatants was determined using a BCA protein assay kit (Thermo Scientific), and samples were stored at −80 °C. 20 μg of total protein was subjected to SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) blotting membrane (BioRad Laboratories, Hercules, CA). Membranes were blocked for 2 h in 5% (wt/vol) skim milk in TBST (Tris-buffered saline [TBS], 0.1% [vol/vol] Tween 20) at room temperature on a rocking platform and then incubated with primary antibody at 4 °C overnight (4-HNE mouse polyclonal, 1:5000, Abcam, Toronto, ON; SOD1, rabbit polyclonal, 1:1000, Santa Cruz, Santa Cruz, CA; SOD2, rabbit polyclonal, 1:1000, Santa Cruz; Catalase, 1:500, Abcam; glutathione peroxidase, 1:1000, Abcam). Following washing with TBST, blots were incubated with peroxidase-conjugated secondary anti-rabbit or anti-mouse antibody (1:5000; GE Healthcare, QC, CA) for 1 h at room temperature on a rocking platform. Blots were developed using enhanced chemiluminescence (ECL) (Millipore, Billerica, MA) and quantification was carried out using ImageLab 4.1 software (ImageLab Version 4.1 build 16; Bio-Rad, Hercules, CA). Immunoblots were subsequently incubated with stripping buffer (Thermo Scientific) and re-probed with beta-actin (1:2000, Abcam) to control the protein loading. Blots were quantified as previously described (Bruin et al., 2008).

2.5. Mitochondria as a target of ROS

2.5.1. Electron transport chain activity

There is now considerable evidence to suggest that altered ROS production may have profound changes on mitochondrial function

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