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# Antidepressants induce cellular insulin resistance by activation of IRS-1 kinases

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**Certain selective serotonin reuptake inhibitors (SSRIs) induce the clinical and biochemical manifestations of a metabolic syndrome by as yet unknown mechanism. Here we demonstrate that incubation (1 h) of rat hepatoma Fao cells with the SSRIs paroxetine and sertraline, but not with the atypical antipsychotic drug olanzapine, inhibited the insulin-stimulated Tyr phosphorylation of the insulin receptor substrate-1 (IRS-1) with half-maximal effects at ~10 μM. This inhibition correlated with a rapid phosphorylation and activation of a number of Ser/Thr IRS-1 kinases including JNK, S6K1, ERK and p38 MAPK, but not PKB (Akt). JNK appears as a key player activated by SSRIs because specific JNK inhibitors partially eliminated the effects of these drugs. The SSRIs induced the phosphorylation of IRS-1 on S307 and S408, which inhibits IRS-1 function and insulin signaling. These results implicate selected SSRIs as inhibitors of insulin signaling and as potential inducers of cellular insulin resistance.**


*Keywords:* Antidepressants; Selective serotonin reuptake inhibitors; Insulin receptor substrates; Insulin resistance

## Introduction

Several classes of antidepressants, all affecting brain neurotransmission, are widely used for the treatment of mood and anxiety disorders. Tricyclic antidepressants (TCAs) and tetracyclic antidepressants block the reuptake of both norepinephrine (NE) and serotonin (5-HT), while selective serotonin reuptake inhibitors (SSRIs) (e.g. sertraline and paroxetine) block only the serotonin reuptake transporter (Gram, 1994; Holsboer and Barden, 1996).

Weight gain which is a frequent side effect of treatment with many SSRIs (Fava et al., 2000; Himmerich et al., 2004; Raeder et al., 2006), impairs physical functioning; is associated with negative psychosocial consequences and reduces quality of life (Ganguli, 1999). Weight gain often leads to obesity which is associated with an increased incidence of insulin resistance, diabetes, hypertension, dyslipidemia and coronary artery disease (Despres and Lemieux, 2006). Hence, many patients (up to 50%; Pijl and Meinders, 1996) who suffer from mood and anxiety disorders discontinue treatment, putting them at risk for relapse or lack of improvement.

At the molecular level, increased production of cytokines and adipokines were reported side effects to treatment with selected antipsychotic drugs. These adipokines include leptin and tumor necrosis factor-alpha (TNF-α) (Hinze-Selch et al., 2000; Paterson et al., 2006), both known as inducers of insulin resistance (Zick, 2001). Still, relatively little is known about the pathophysiology of antidepressant-induced weight gain and the metabolic changes induced by these drugs that are directly related to insulin action and insulin resistance.

Insulin resistance is a common pathological state in which target cells fail to respond to ordinary levels of circulating insulin (Kahn and Flier, 2000). Individuals with insulin resistance are predisposed to developing type 2 diabetes, a 21st century epidemic (Reaven, 2005). One of the signaling cascades that have been shown to promote insulin resistance is the c-Jun-N-terminal kinase (JNK) pathway, also known as the stress-activated protein kinase (SAPK) (Zick, 2004). JNK activity is abnormally elevated in obesity (Hirosumi et al., 2002); furthermore, JNK inhibits insulin action by phosphorylating and inactivating the action of the insulin receptor substrate proteins IRS-1 and IRS-2 (Aguirre et al., 2000). IRS proteins are key players in insulin signal transduction and are the best-studied targets of the insulin receptor (reviewed in Zick, 2001; Khan and Pessin, 2002). They contain a conserved pleckstrin homology (PH) domain, located at their amino terminus, that serves to anchor the IRS proteins to membrane phosphoinositides in close proximity to the insulin receptor (Volio-vitch et al., 1995). The PH domain is flanked by a P-Tyr binding (PTB) domain, which functions as a binding site to the NPXY motif at the juxtamembrane domain of the insulin receptor (Eck et al., 1996). The C-terminal region of IRS proteins is poorly conserved. It contains multiple Tyr phosphorylation motifs that serve as a signaling scaffold, providing a docking interface for SH2 domain-containing proteins like

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the p85 regulatory subunit of PI3K, Grb2, Nck, Crk, Fyn and SHP-2, which further propagate the metabolic and growth-promoting effects of insulin (LeRoith and Zick, 2001; Saltiel and Pessin, 2002).

IRS-1 contains >200 Ser/Thr residues (Sun et al., 1991), many of which could be subjected to phosphorylation. Ser/Thr phosphorylation has been increasingly recognized as a negative counter balance to the positive IRS signaling through tyrosine phosphorylation (Zick, 2001). Ser/Thr phosphorylation reduces IRS-1 ability to undergo Tyr phosphorylation by the insulin receptor kinase and serves as a physiological negative feedback control mechanism to turn off insulin's signaling by uncoupling the IRS proteins from their upstream and downstream effectors (Paz et al., 1997; Liu et al., 2001, 2004). Furthermore, activation of IRS kinases is a mechanism utilized by certain inducers of insulin resistance under pathological conditions (Zick, 2001). Several candidate Ser residues were identified as potential targets for IRS-1 kinases. These include Ser24 (Kim et al., 2005), 302 (Werner et al., 2004), 307 (Aguirre et al., 2002), 318 (Moeschel et al., 2004), 408 (Liu et al., 2004), 612 (De Fea and Roth, 1997), 636, 639 (Ozes et al., 2001), 731 (Delahaye et al., 1998) and 789 (Jakobsen et al., 2001). Similarly, a number of kinases, including Erk, PKCζ, IKKβ, JNK, S6K1 AMP-kinase and mTOR, were implicated as potential IRS kinases (reviewed in Zick, 2005). Still, from a molecular perspective it has been difficult to identify discrete sites that are both phosphorylated *in vivo* and when phosphorylated have relevant functional consequences.

We have recently shown that SSRIs activate the JNK pathway in C6 glioma and neuroblastoma cell lines (Levkovitz et al., 2005). These observations prompted us to study the direct effects of SSRIs on insulin receptor signal transduction, with a special emphasis on the role of JNK in this process. Our findings indicate that selected SSRIs such as paroxetine and sertraline activate a number of IRS kinases, JNK included, leading to increased Ser/Thr phosphorylation of IRS-1 with a concomitant reduction in its ability to undergo insulin-induced Tyr phosphorylation. These findings suggest that SSRIs are potential inducers of insulin resistance by directly inhibiting the insulin signaling cascade.

## Results

### *Sertraline inhibits insulin-induced Tyr phosphorylation of IRS-1*

We have previously shown that incubation of rat hepatoma Fao cells with $10^{-7}$ M insulin rapidly stimulates, within few minutes, Tyr phosphorylation of IRS-1, which then gradually declines (Paz et al., 1997; Liu et al., 2001, 2004). To study the effects of the SSRI sertraline on Tyr phosphorylation of IRS-1, Fao cells were incubated with the drug for 2–4 h before being stimulated for 2 min with insulin. Pretreatment with sertraline (50 μM) did not affect the cellular content of IRS-1 (Fig. 1), but it inhibited the insulin-induced Tyr phosphorylation of IRS-1 by 50–70%, with half-maximal effects obtained at 10–20 μM (not shown). This was evident both in total cell extracts (Fig. 1A) and in IRS-1 immunoprecipitates (Fig. 1B). These inhibitory effects were not restricted to Fao cells as sertraline (25 μM) almost completely inhibited insulin-induced Tyr phosphorylation of IRS-1 in the INS-1E beta cell line (Fig. 1D), with half-maximal effects obtained at 10 μM (not shown). Still, not all psychotropic agents acted as activators of IRS-1 kinases. For example, the atypical antipsychotic drug olanzapine, that causes weight gain and the metabolic syndrome, failed to inhibit Tyr phosphorylation of IRS-1 (Fig. 1C).

### *Sertraline activates the IRS-1 kinases ERK and S6K1*

We and others have previously shown that IRS-1 is subjected to Ser/Thr phosphorylation that inhibits it ability to undergo insulin-stimulated Tyr phosphorylation (reviewed in Zick, 2005). Therefore, the ability of sertraline to activate potential IRS-1 kinases was studied. As shown in Fig. 2, a 2-h incubation of Fao cells with sertraline (50 μM) resulted in a marked activation of the Ser/Thr kinases ERK and S6K1, which are known to induce the phosphorylation of IRS-1. ERK and S6K1 are the downstream effectors of MEK and mTOR, respectively. Therefore, the effects of inhibitors of these kinases, PD98059 (for MEK) and rapamycin (for mTOR) were studied to confirm the specificity of the effects of sertraline. Indeed, as shown in Fig. 2A, PD98059 and rapamycin inhibited the activation of ERK and S6K1 by sertraline. Inclusion of insulin for the final 2-min incubation with sertraline did not affect the extent of activation of ERK or S6K1, suggesting that these kinases are already maximally activated by the drug (Fig. 2A). The effects of sertraline (50 μM) appeared to be specific. Unlike insulin (Fig. 2B, lane b), sertraline on its own (Fig. 2B, lane c) failed to activate Akt (PKB), a downstream effector of IRS-1 and PI3K. Furthermore,

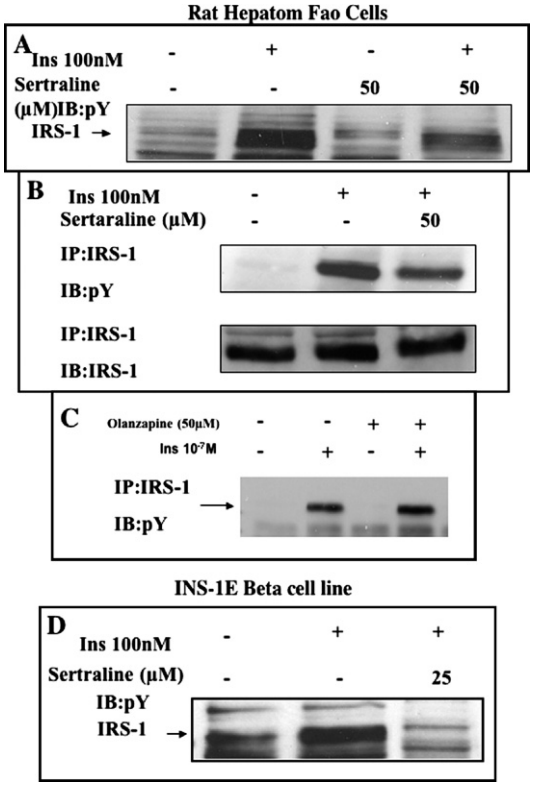


Fig. 1. Effects of sertraline and Olanzapine on insulin-induced Tyr phosphorylation of IRS-1 in Fao and INS-1E cells. Fao cells at 80% confluence (A–C) or INS-1E cells at 70% confluence (D) were deprived of serum for 16 or 2 h prior the experiment, respectively. The cells were incubated at 37 °C with sertraline or olanzapine at the indicated concentrations for 4 h (A), 2 h (B), 4 h (C) or 2 h (D), followed by treatment with 100 nM insulin for 2 min. Cell extracts (50 μg) were resolved by SDS–PAGE and were immunoblotted with anti pY antibodies (A, D). Alternatively, samples (400 μg) of the cell extracts were subjected to immunoprecipitation (IP) with anti IRS-1 antibody (B, C). Immunocomplexes were resolved by SDS–PAGE and were immunoblotted with anti pY or anti IRS-1 antibodies. One representative out of three experiments is shown.