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Effects of different antipsychotics and the antidepressant mirtazapine on glucose transporter mRNA levels in human blood cells

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Abstract

A relationship between cell metabolism and the expression of glucose transporters (GLUT) has been reported. On the other side, treatment with some antipsychotics has been associated with an increased incidence of hyperglycemia and new-onset type 2 diabetes.

We here examined the effects of different concentrations of the conventional antipsychotic haloperidol (400 and 800 μg/ml), of the atypical antipsychotics clozapine (100 and 200 μg/ml) and olanzapine (100 and 200 μg/ml) as well as of the antidepressant mirtazapine (10^{-7} mol) on the mRNA levels of GLUT1–5 in the human leukemic blood cell line U937 after incubation for 48 h.

After experimental treatment, significant increases were detected by ANOVA and appropriate post-hoc tests for mirtazapine in GLUT4 mRNA levels as well as for haloperidol 400 and 800 μg/ml, olanzapine 200 μg/ml, and mirtazapine in GLUT5 mRNA levels. ANOVAs revealed no statistically significant changes in GLUT1–3 and β-actin mRNA levels.

These findings suggest that direct effects of psychotropic drugs on cellular GLUT4 and GLUT5 may be involved in the metabolic dysfunctions occurring during psychopharmacological treatment.

Keywords: Antipsychotic; Glucose transporter; mRNA; Human blood cells; U937 cells

1. Introduction

Treatment with a number of antipsychotic compounds has been associated with an increased incidence of hyperglycemia and new-onset type 2 diabetes (Dwyer and Donohoe, 2003; Zimmermann et al., 2003). For this reason, the interrelation between cellular glucose transport and antipsychotics has been studied for some years, showing meanwhile that antipsychotics may affect glucose transport into target cells under certain conditions: for example, Ardizzone et al. (2001) demonstrated that risperidone, clozapine and desmethylclozapine inhibited glucose transport in a dose-dependent fashion in rat pheochromocytoma (PC12) cells, whereas clozapine and fluphenazine inhibited glucose transport in the rat muscle cell line L6. Dwyer et al. (1999a) detected a significant increase (approximately threefold) in the cellular levels of the glucose transporter (GLUT) 1 and GLUT3 following a 24 h exposure to conventional antipsychotics such as chlorpromazine, fluphenazine and pimozide in adherent-growing PC12 cells. Fluphenazine, chlorpromazine, clozapine and haloperidol inhibited glucose uptake in this system after a short (30 min) preincubation with the drugs. However, no distinct relationship of such effects with regard to dopamine receptors was detectable in these cells after incubation with different types of neuroleptic drugs (Dwyer et al., 1999b). Fischer et al. (1995) found that serotonin, tryptamine, 5-methoxytryptamine and dopamine increased glucose transport in rat cardiac myocytes. The effect was maximal after 90 min, and was

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paralleled by an increase in the amounts of GLUT1 and GLUT4 at the cell surface. In contrast, selective 5-HT receptor agonists failed to stimulate glucose transport. The effect of 5-HT was not affected by 5-HT receptor antagonists, adrenergic receptor antagonists, or by dopaminergic receptor antagonists (including haloperidol). These data suggest that the effects of different antipsychotics and antidepressants on glucose transport and GLUTs seem not to be related to their neurobiological receptor profile.

GLUTs are integral membrane proteins which transport glucose into target cells across the plasma membranes. Meanwhile, the GLUT family consists of 13 members which have been named GLUT1–12 and HMIT (H^+-coupled myo-insositol transporter) and are divided into three subclasses (Wood and Trayhurn, 2003). The tissue-specific transporter isoforms generally differ in their affinity to the natural substrate D-glucose according to the specific functions of the respective organ (Lange, 2001). The similarity of the amino acid sequence of the human GLUT family ranges from 28% to 65% identity if compared with GLUT1. Structurally, the GLUTs contain a 12 membrane-spanning region with intracellularly located amino- and carboxyl-termini (Wood and Trayhurn, 2003).

Up to now, effects of psychotropic agents on GLUTs have been described in very few studies (e.g. Ardizzone et al., 2001; Dwyer et al., 1999a,b; Fischer et al., 1995) and only on the protein level. We here measured for the first time the effects of different concentrations of the conventional antipsychotic haloperidol, the atypical antipsychotics clozapine and olanzapine as well as the antidepressant mirtazapine on GLUT1–5 in a human leukemic blood cell line on the level of gene expression (mRNA).

2. Materials and methods

2.1. Preparation and incubation of U937 cells

The human leukaemic cell line U937 was cultured in Roswell Park Memorial Institute 1640 (RPMI) (Gibco/BRL, Eggenstein, Germany) medium supplemented with 10% fetal calf serum (FCS) (Biochrom, Berlin, Germany) and 1% glutamine as well as 0.5% glutamate (Gibco/BRL, Eggenstein, Germany). For experiments, 225,000 cells were plated in 6 well dishes (Greiner, Nürtingen, Germany). After 24 h, cells were treated with the following agents (concentrations in brackets): haloperidol (400 and 800 μg/ml), clozapine (100 and 200 μg/ml), olanzapine (100 and 200 μg/ml), mirtazapine (10^{-7} mol), and control treatment (H_2O). After another 48 h, the final amount of cells (900,000 cells per dish) was resuspended in 1 ml Trizol (Invitrogen, Dreieich, Germany) and subsequently frozen at −80 °C.

2.2. Extraction and quantification of m-RNA by RT-PCR methods

After defrosting the samples, the mRNA was cooled and mixed with 300 μl chloroform. After 2 min, the samples were centrifuged at 13,000 rpm for 15 min at 2–8 °C. The interphase was discharged and the water-phase mixed with 500 μl isopropanol. After 15 min of incubation at room temperature, the samples were centrifuged with 13,000 rpm at 2–8 °C. The supernatant was discharged and the samples were washed with 75% ethanol. Subsequently, the tubes were centrifuged for 5 min at 10,000*g*, the ethanol was discharged and the samples dried for 10 min and mixed with 20–30 μl of Aqua bidest. The amounts of extracted mRNA were quantified by established optical methods at A_{260}/A_{280} (Bio Photometer, Eppendorf, Hamburg, Germany) and structural integrity was determined by agarose gel electrophoresis (1.5% agarose; Gibco/BRL; Dreieich, Germany). Equivalent amounts of mRNA were used for the reaction with reverse transcriptase (RT) (Superscript II RNase H Reverse Transcriptase, Gibco/BRL, Eggenstein, Germany). For the glucose transporters (GLUT) and β-actin, the following primers were used (final concentration: 20 μM; annealing conditions: 60 °C for 1 min) (MWG Biotech, Ebersberg, Germany):

GLUT1: 5′ primer: 5′-CGG GCC AAG AGT GTG TGC TAA A-3′;
3 ′primer. 5′-TGA CGA TAC CGG AGC CAA TG-3′

GLUT2: 5′ primer: 5′-CGT CTC CTT TGA CAT TTC CTT C-3′;
3′primer: 5′-GGT GGA GAA AAC AGC CTA GAG AT-3′

GLUT3: 5′ primer: 5′-CCA ACT TCC TAG TCG GAT TG-3′;
3′primer: 5′-AGG AGG CAC GAC TTA GAC AT-3′

GLUT4: 5′ primer: 5′-GCA CCG CCA GGA CAT TGT TG-3′;
3′ primer: 5′-CGC AGA ATT CCC CCC TCA GCA GCG AGT GA-3′

GLUT5: 5′primer: 5′-GCA ACA GGA TCA GAG CAT GA-3′;
3′primer: 5′-TCG CAG GCA CGA TAG AAA AT-3′

β-actin: 5′ primer: 5′-GAG GCC CAG AGC AAG AGA GG-3′;
3′primer: 5′-TCA CCG GAG TCC ATC ACG GAT-3′.