Journal of Neuroscience Research 56:72–75 (1999)

Differential Effects of Olanzapine on the Gene Expression of Superoxide Dismutase and the Low Affinity Nerve Growth Factor Receptor

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Neuroanatomical studies of schizophrenia suggest that progressive neuropathological changes (such as neuronal atrophy and/or cell death) occur over the lifetime course of the disease. Early intervention with atypical neuroleptics has been shown to prevent progression of at least some symptoms, although the mechanisms by which neuroleptics may do this remain unknown. In this study, PC12 cells were used to determine the effects of the new atypical antipsychotic olanzapine on the gene expression of superoxide dismutase (SOD1) and the low affinity nerve growth factor receptor (p75). The results show that olanzapine increases SOD1 at concentrations of 10 and 100 μM after 48 hr of incubation in PC12 cultures. The treatment decreases p75 gene expression at concentrations 100 μM after 48 hr of incubation. Since both the upregulation of SOD1 mRNA and the antisense blockade of p75 mRNA have been associated with reduced cell death, our results suggest that olanzapine has neuroprotective potential and thus may be useful in preventing further neurodegeneration accompanying schizophrenia. J. Neurosci. Res. 56:72–75, 1999.

Key words: olanzapine; superoxide dismutase; PC12 cells; p75

INTRODUCTION

Imaging and neuropsychological studies suggest that ongoing neuronal atrophy accompanies schizophrenia (Turner et al., 1986; Waddington et al., 1991; DeLisi et al., 1997) and that such atrophy is associated with poor outcome (Woods et al., 1990; Woods and Yurgelun-Todd, 1991). Clinical studies suggest that some symptoms of schizophrenia worsen over time; and that early intervention in schizophrenia can improve long-term outcome (Wyatt, 1991; Falloon, 1992; Wyatt and Henter, 1998). This not only suggests progressive neurodegeneration in schizophrenia, but that some neuroleptics may in part arrest this process. Olanzapine is a new D2/5-HT2-blocking thienobenzodiazepine derivative that is not only successful in the treatment of both the positive and negative symptoms of schizophrenia (Tran et al., 1997) but produces less motor side effects than haloperidol (Beasley et al., 1997). It also has a much lower risk than clozapine to produce agranulocytosis, and like clozapine, appears to be useful in the reversal of some cases of tardive dyskinesia (O'Brien and Barber, 1998; Littrell et al., 1998). Olanzapine is possibly one of a few select neuroleptics (including risperidone and clozapine) that is a good candidate for safe preventative intervention in first-break schizophrenia.

Although it is known that clinically efficacious antipsychotics block central dopamine D2 and/or serotonergic 5-HT2 receptors, the complete mechanisms by which neuroleptics exert their therapeutic effects and side effect profiles are not fully understood. The molecular mechanisms by which neuroleptics might provide neuronal protection and prevent permanent symptom deterioration are also unknown. Since such an effect may or may not be dependent on the receptor blocking profile of each neuroleptic, we decided to examine the effects of olanzapine on the gene expression of some neuroprotective genes downstream from receptor blockade.

A possible target gene includes includes the Cu++/Zn++ dependent superoxide dismutase (SOD1, E.C.1.15.1.6), an enzyme that reduces cellular oxidative stress and neuronal damage via the inactivation of oxygen free radicals. In vivo studies have demonstrated that

Abbreviations: DA, dopamine; p75, low affinity nerve growth factor receptor; SOD1, superoxide dismutase.

Contract grant sponsor: Saskatchewan Health; Contract grant sponsor: Schizophrenia Society of Saskatchewan; Contract grant sponsor: Ian Douglas Bebensee Foundation.

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Received 3 November 1998; Revised 30 November 1998; Accepted 1 December 1998

upregulation of this enzyme is neuroprotective in ischemia (Wengenack et al., 1997) and glutamate neurotoxicity (Furukawa et al., 1997), whereas mutant dyfunctional SOD1 has been associated with motor neuron degeneration resembling ALS (Kong and Xu, 1998). A second candidate gene includes the low affinity nerve growth factor receptor (p75), a 75 kDa transmembrane protein that constitutively induces neural cell death in the absence of its primary ligand, nerve growth factor (NGF) (Thoenen and Barde, 1980; Levi-Montalcini, 1987; Greene and Kaplan, 1995; Kaplan and Miller, 1997; Muller and Clos, 1997). Both the antisense blockade of its mRNA (Barrett and Georgiou, 1996) and the inhibition of its function by antibodies (Frade et al., 1996; Rabizadeh et al., 1993) have been associated with reduced cell death in PC12 cells. Thus, we expect olanzapine to upregulate SOD1 and/or downregulate p75 mRNA in culture.

MATERIALS AND METHODS

The PC12 cell line was obtained from American Type Culture Collection (Rockville, MD) and cultured in RPMI 1640 medium containing 5% fetal calf serum and 10% horse serum plus 100 U/ml penicillin and 100 µg/ml streptomycin as described in protocols provided by the supplier. The cells were plated in 6-well plates in the presence or absence of 10 or 100 mM olanzapine during 24 or 48 hr. Streptomycin was purchased from Sigma-Aldrich Canada (Oakville, ON). Olanzapine (LY 170053) was kindly supplied by Lilly Research Laboratories (Indianapolis, IN).

SOD1 cDNA was kindly provided by Dr. J. T. Coyle (Harvard Medical School, Boston, MA) and p75 cDNA was the gift of Dr R. Lindsay (Regeneron Pharmaceuticals, Tarrytown, NY). The cDNA probes were labeled by random primer synthesis with [a-32P]dCTP as described earlier (Fainberg and Vogelstein, 1983; Li et al., 1998a).

Total cellular RNA was prepared from treated cells by extraction in GITC buffer and collected by ultracentrifugation through 5.7 M CsCl. The RNA was chloroform-extracted, ethanol-precipitated, resuspended in DEPC treated water, and stored at -70°C until use. RNA was measured spectrophotometrically by absorbance at 260 nm. The total RNA was denatured at 65°C for 15 min in MOPS buffer containing 50% formamide and 2.2M formaldehyde and separated by electrophoresis in a 1.0% agarose gel containing MOPS buffer and 2.2M formaldehyde. Following electrophoresis, the RNA was transferred to nylon membranes and the membranes were cross-linked in a UV Stratalinker 2400.

Filters were prehybridized at 65°C for 2 hr with prehybridization solution containing 10% dextransulfate, 5 X SSPE, 5 X Denhardt's solution, 0.5% SDS, and denatured salmon sperm DNA (200 µg/ml). Hybridization was performed at 65°C for 18 hr. After hybridization, membranes were washed at room temperature twice in 2 X SSPE-0.1%SDS, once in 0.1 X SSPE-0.1% SDS at 60°C and once in 0.1 X SSPE-0.1% SDS at 60°C. The membranes were washed and exposed to X-Omat AR film with intensifying screens at -70°C to obtain autoradiograms from membranes. The autoradiograms were scanned with a computerized densitometer (Du 640, Beckman Inc., Fullerton, CA) for quantitative estimations and the signals were adjusted according to the signals of rehybridization with a cyclophilin probe.

Statistical Analysis

Results were analyzed by one or two way analysis of variance performed using the CLR ANOVA program (Clearlake Research, Houston, TX). In the presence of significant F values, individual comparisons between means were made using Newman-Keuls test.

RESULTS

The PC12 cell cultures were treated with 10 or 100 µM of olanzapine and incubated over 24 or 48 hr at 37°C; at these times and doses, there were no apparent signs of cell death or neurotoxicity. The treatment with olanzapine produced significant increases in the gene expression of SOD1 that were observed with both doses after 48 hr of incubation (as disclosed by one-way analysis of variance; $F_{2,6} = 15.39$, $P < 0.0043$). The increases reached values of 147 and 172 % of their respective saline-treated controls (Fig. 1) . Two-way analysis of variance disclosed an effect of olanzapine treatment ($F_{2,12} = 13.84$, $P < 0.0008$) but no effect of time ($F_{2,12} = 1.10$, $P < 0.31$) or an interaction between dose and time ($F_{2,12} = 0.29$, $P < 0.75$).

The olanzapine-treated cell cultures were also tested for the gene expression of the low affinity nerve growth factor receptor (p75). The results show that the abundance of p75 mRNA was significantly reduced after treatment during 48 hr with 100 µM of olanzapine. The treatment reduced p75 mRNA levels to 21% of its corresponding saline-treated control (Fig. 2) that was statistically significant as shown by one-way analysis of variance ($F_{2,6} = 16.83$, $P < 0.0035$). Two-way analysis of variance disclosed an effect of olanzapine treatment ($F_{2,12} = 17.17$, $P < 0.0003$) but no effect of time ($F_{2,12} = 2.56$, $P < 0.135$) or an interaction between dose and time ($F_{2,12} = 2.49$, $P < 0.125$).

DISCUSSION

PC 12 cells have been widely used as a model for the study of catecholamine synthesis, release, and metabo-

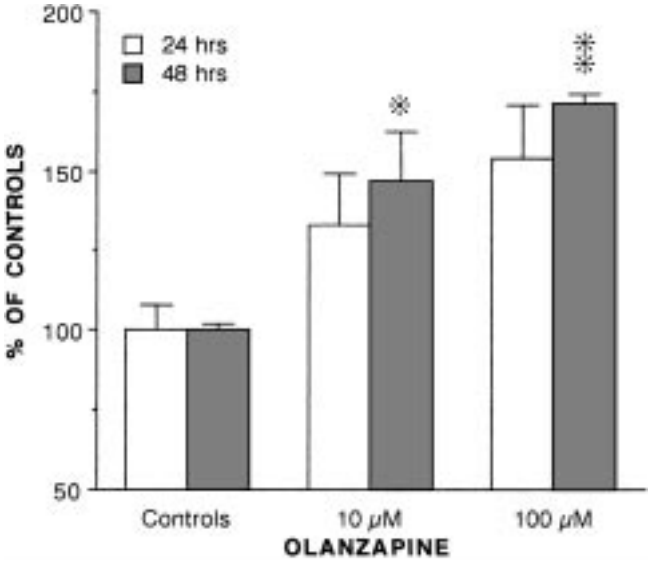

Fig. 1. Effect of olanzapine on SOD1 gene expression in PC12 cells. The cells were incubated with 10 or 100 μM during 24 or 48 hr. Values are means ± S.E.M. (bars) (N = 3), $*P < 0.05$ or $**P < 0.01$ by the Newman-Keuls test with respect to the control group.

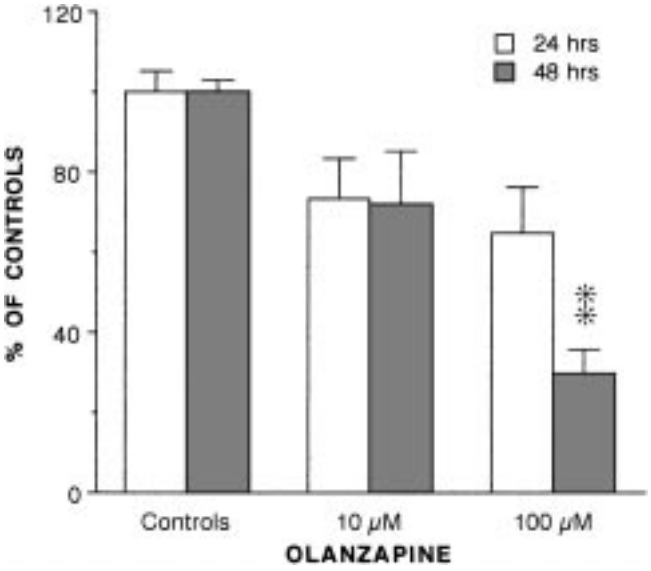

Fig. 2. Effect of olanzapine on p75 gene expression in PC12 cells. The cells were incubated with 10 or 100 μM during 24 or 48 hr. Values are means ± S.E.M. (bars) (N = 3), $**P < 0.01$ by the Newman-Keuls test with respect to the control group.

lism, and neuronal differentiation and cell death (reviewed by Greene and Tischler, 1982; Stefanis et al., 1997). The present investigation shows for the first time olanzapine increases SOD1 and decreases p75 gene expression in PC12 cells. The results support the hypothesis that olanzapine could protect neurons by upregulating the expression of genes coding for a neuroprotective enzyme (SOD1) and downregulating the expression of genes that code for a receptor (p75) associated with programmed cell death. In agreement with the effects of olanzapine, we have recently found that incubation of PC12 cells with clozapine (2.5, 25 μM) also reduces p75 gene expression (Li et al., 1998b).

The etiology of schizophrenia is only partially understood and, consequently, the basis for its treatment rests in the symptomatic response to antipsychotics. Although there have been many reports of reduced brain volume and enlarged ventricles in schizophrenia (indicative of neuronal atrophy), it is difficult to ascertain if the atrophy occurred during neurodevelopment, at the time of onset, or throughout the course of the illness. This has made it difficult to ascertain whether a neurodevelopmental process underlies schizophrenia and whether this is accompanied by a neurodegeneration as well. These difficulties may be due to the existence of various patient subgroups. In addition, it is unknown whether the atrophy is reflective of reduced cell numbers, atrophy of neuronal processes, or both.

There is some evidence to support in the literature for the neurodegenerative hypothesis of schizophrenia (Coyle, 1996; Nair et al., 1997; also see Introduction). Recently, DeLisi et al. (1996) found that ventricular enlargement occurred throughout the course of the illness and correlated with symptom severity. However, using MRI, Jacobsen et al. (1998) found that there was a progressive decrease in temporal lobe structures over the course of the illness in childhood schizophrenia, suggesting that schizophrenia can indeed be accompanied by a degenerative process. Thus, the apparent superior clinical efficacy of these neuroleptics may be due in part to their neuroprotective capabilities.

SOD1 is a ubiquitous enzyme and is widely distributed in the CNS, including many regions purported to be atrophied in schizophrenia such as the hippocampus and cerebral cortex (Jeste and Lohr, 1989; Selemon et al., 1995). It is, therefore, conceivable that upregulation of this enzyme by antipsychotics could prevent further free radical induced neurotoxicity/neurodegeneration in schizophrenia. The expression of p75 receptor mRNA in adults is primarily limited to the medial septal nucleus and the nucleus of Broca's diagonal band (Vazquez and Ebedal, 1991). These nuclei of the basal forebrain contain cholinergic neurons that project to the hippocampus and cerebral cortex (Ebendal, 1992). The atrophy of these neurons would result in decreased input to the hippocampus and cortex, which could either exacerbate symptoms of schizophrenia, or contribute to the degeneration and death of target neurons. Interestingly, the expression of p75 in cholinergic neurons is associated with Alzheimer's disease (Woolf et al., 1989) and β-amyloid toxicity (Rabizadeh and Bredesen, 1994).

Thus, the early and chronic use of such neuroprotective neuroleptics in schizophrenia may slow development of symptoms either by: (1) preventing neuronal death via downregulation of the p75 receptor, or (2) preventing neuronal damage related to the earlier stages of degeneration by upregulating antioxidant enzymes such as SOD1. In addition, it is possible that olanzapine could be used in other neurodegerative disorders that are accompanied by free radical damage (such as Alzheimer's disease) or involve cell death via the p75 receptor. The cell culture model employed in the present study may be useful to screen the neuroprotective potential of antipsychotics, and thus be of value in future antipsychotic development and assessment.

ACKNOWLEDGMENTS

We thank Ms. G. Stegeman for excellent technical assistance.

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