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Choline acetyltransferase expression in rat prefrontal cortex and hippocampus after acute and chronic exposure to amisulpride, haloperidol, and risperidone

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HIGHLIGHTS

- Short-term and long-term administration of haloperidol reduced ChAT expression in PFC and/or HIP.
- Short-term and long-term administration of risperidone reduced ChAT expression in PFC and/or HIP.
- Long-term administration of amisulpride reduced ChAT expression in PFC and/or HIP.
- Reduced ChAT expression in PFC and/or HIP may produce detrimental effects on cognitive function.

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ABSTRACT

Recently, there has been an increasing concern that atypical antipsychotics as well as typical ones may cause detrimental effects on cognitive function. Supporting evidence comes from many preclinical studies demonstrating that long-term administration of haloperidol, risperidone, and ziprasidone reduced choline acetyltransferase (ChAT) expression in rat hippocampus (HIP). However, to the best of our knowledge, no studies have examined the effects of amisulpride on ChAT expression in rats. Therefore, the aim of this study was to investigate the effects of acute and chronic administration of amisulpride, haloperidol, and risperidone on ChAT expression in the rat prefrontal cortex (PFC) and HIP. Animals received daily intraperitoneal (i.p.) injections of amisulpride (5 or 100 mg/kg), haloperidol (1 or 2 mg/kg), risperidone (1 or 2 mg/kg) or vehicle for 7 or 45 days. One day after the last injection, rats were sacrificed. ChAT immunoreactivity was assessed with immunofluorescence staining. Target areas of brain were PFC and HIP (CA1, CA3 and DG). The short-term administration of haloperidol and risperidone produced significant decrease of ChAT immunoreactivity in the PFC and HIP compared to vehicle whereas amisulpride had no effects on ChAT immunoreactivity in the PFC and HIP. In long-term study, haloperidol and risperidone decreased ChAT-positive cells and/or fiber pixel density in the PFC and HIP whereas amisulpride decreased ChAT-positive cells in the PFC and had no effects on fiber pixel density of ChAT in the HIP. The results suggest that both short-term and long-term administration of haloperidol and risperidone, and long-term administration of amisulpride may produce detrimental effects on cognitive function by reducing ChAT expression in the PFC and/or HIP.

1. Introduction

Recently, there has been an increasing concern that atypical antipsychotics as well as typical ones may cause detrimental effects on cognitive function. Behavioral studies using animals consistently show that SGAs actually exert detrimental effects on memory-related task performance following long-term administration [29]. Some molecular studies reported that atypical antipsychotics stimulate neurogenesis in the hippocampus and prefrontal cortex of adult rats [12,31]; however, it should be noted that these studies used a relatively short duration of drug administration (3 weeks or less). One of the hypothesized mechanisms underlying the detrimental cognitive effects of long-term administration of SGAs is cholinergic dysregulation [29]. Chronic treatment (more than 45 days) with either risperidone [28] or ziprasidone [30] resulted in a significant decrease in choline

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acetyltransferase (ChAT) immunoreactivity in the cortex and hippocampus. These findings have important clinical implications, considering the significant correlation between reduced ChAT levels and impaired cognition in schizophrenia, as well as decreased nicotinic and muscarinic acetylcholine receptors in post-mortem brains of schizophrenic patients [6].

Amisulpride is a selective and potent antagonist at dopamine (DA) D_2/D_3 receptors with no affinity for D_1, serotonin, histamine H_1, muscarinic, or alpha-adrenergic receptors. At low doses ($\leq$10 mg/kg), amisulpride preferentially blocks presynaptic dopamine autoreceptors and increases DA release in the nucleus accumbens, striatum, and olfactory tubercle [26], in addition to inducing significant cerebral glucose utilization in cortical areas and several limbic structures [4]. These mechanisms may explain the beneficial effects of amisulpride on negative schizophrenia symptoms [19] and cognitive function [18]. However, to the best of our knowledge, no studies have examined the effects of chronic amisulpride on ChAT expression in rats. We hypothesized that chronic administration of amisulpride would produce differential dose-dependent effects on ChAT. The aim of this study was to investigate the effects of acute and chronic haloperidol, risperidone, and amisulpride on ChAT expression in the rat prefrontal cortex (PFC) and hippocampus (HIP).

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (250–280 g) were obtained from Orient Bio, Inc. (Seoul, Korea). The experimental procedure was approved by the Animal Care and Use Committee of Chonbuk National University Graduate School of Medicine.

2.2. Drug administration

Haloperidol (Sigma Chemicals, St. Louis, MO, USA) and risperidone were dissolved in a 0.1 M solution of tartaric acid. Amisulpride was dissolved in a 2% glacial acetic acid. Rats were divided into acute (7 days) and chronic (45 days) experimental groups, with separate groups of animals receiving haloperidol (1 or 2 mg/kg/day, i.p.), risperidone (1 or 2 mg/kg/day, i.p.), amisulpride (5 or 100 mg/kg/day, i.p.), or vehicle (n = 5–6/group). The haloperidol and risperidone doses were chosen based on previous studies [14]. The low and high amisulpride doses were chosen to have primarily pre- or post-synaptic DA antagonist effects, respectively, based on prior studies [4,26].

2.3. ChAT immunofluorescence

Animals were sacrificed by decapitation 24 h following the last injection. After perfusion, brains were handled in a proper way and stored at −80 °C. Frozen coronal sections (30 μm) were cut and anterior–posterior coordinates of sections relative to the bregma were +2.70 mm for the prelimbic cortex (PFC), −3.80 to −4.30 mm for CA1, CA3, and the dentate gyrus (DG). After the incubation with PBS containing 0.3% hydrogen peroxide and blocking with 5% bovine serum albumin, sections were incubated overnight at 25 °C in the goat anti-choline acetyltransferase (Millipore, Temecula, CA, USA) diluted 1:100 in same buffer. The sections were washed three times in PBS and incubated for 2 h with Alexa Fluor® 488 donkey anti-goat IgG (Invitrogen, Eugene, OR, USA) diluted 1:600 in PBS.

2.4. Image analyses

Four sequential sections from preselected regions in each brain were obtained, and the average of eight values per region (bilateral values in 4 sections) was used for analysis. The number of ChAT-positive cells in the PFC was obtained by counting individual positive cells with acquired images of dimensions 683 μm × 512 μm at a magnification of 200×. Quantitative data for ChAT-immunostained fibers are expressed as a measure of fiber pixel intensity that was taken as the average pixel density for each image. One and two rectangles with dimension of 345 μm × 259 μm for the PFC and HIP respectively were delineated and examined at a magnification of 400×. Images were collected using a Carl Zeiss Axioskop-2 plus microscope® (Carl Zeiss Light Microscopy, Gotingen, GER) and Axiovision 4.8 software program (Carl Zeiss MicroImaging GmbH, Jena, GER) and analyzed using i-Solution software program (IMT i-Solution Inc., Vancouver, BC, CAN).

2.5. Statistical analyses

The data are expressed as means ± SEM. General linear model three-way ANOVA was conducted on the number of ChAT-positive cells and/or fiber pixel density values (dependent factors) for each brain region with the between group factors of drug, drug dose, and duration of treatment. One-way analysis of variance (ANOVA) was further carried out to compare the difference of dependent factors between vehicle and treatment groups within each treatment duration. When significant differences were observed, a pairwise multiple comparison post hoc test (Tukey's) was performed. Statistical significance was set at $p < 0.05$.

3. Results

The general linear model three-way ANOVA revealed no significant interaction for drug × dose × duration [number of ChAT-positive cells in PFC ($F_{2,80} = 0.39$, $p = 0.676$); fiber pixel density in PFC ($F_{2,80} = 0.13$, $p = 0.880$), CA1 ($F_{2,76} = 0.81$, $p = 0.449$), CA3 ($F_{2,73} = 0.044$, $p = 0.957$) and DG ($F_{2,76} = 0.74$, $p = 0.482$)] but significant interactions for drug × dose [fiber pixel density in CA1 ($F_{2,76} = 5.35$, $p = 0.007$) and DG ($F_{2,76} = 8.05$, $p < 0.001$)] and dose × duration [fiber pixel density in PFC ($F_{1,81} = 4.25$, $p = 0.043$)]. In addition, significant main effects were identified for drug [number of ChAT-positive cells in PFC ($F_{2,80} = 3.88$, $p = 0.025$); fiber pixel density in DG ($F_{2,76} = 3.55$, $p = 0.034$)], drug dose [number of ChAT-positive cells in PFC ($F_{1,81} = 5.83$, $p = 0.018$); fiber pixel density in CA3 ($F_{1,74} = 6.19$, $p = 0.016$) and DG ($F_{1,77} = 7.24$, $p = 0.009$)] and duration of treatment [number of ChAT-positive cells in PFC ($F_{1,81} = 331.20$, $p < 0.001$); fiber pixel density in PFC ($F_{1,81} = 66.72$, $p < 0.001$), CA1 ($F_{1,77} = 51.63$, $p < 0.001$), CA3 ($F_{1,74} = 36.62$, $p < 0.001$) and DG ($F_{1,77} = 57.61$, $p < 0.001$)] (Table 1).

Separate one-way ANOVA revealed that short-term administration of antipsychotics produced treatment effects in the PFC [number of ChAT-positive cells ($F_{6,35} = 2.79$, $p = 0.025$); fiber pixel density ($F_{6,35} = 2.28$, $p = 0.058$)] and HIP [fiber pixel density in CA1 ($F_{6,31} = 1.95$, $p = 0.104$), CA3 ($F_{6,28} = 4.60$, $p = 0.002$) and DG ($F_{6,31} = 4.63$, $p = 0.002$)]. Post hoc comparisons indicated that in the PFC, haloperidol 2 mg/kg and risperidone 2 mg/kg decreased the number of ChAT-positive neurons significantly as compared to vehicle. In the HIP, haloperidol 2 mg/kg and risperidone 2 mg/kg decreased fiber pixel density significantly in the CA3 and DG as compared to vehicle. In the DG of HIP, fiber pixel density of ChAT was found to be lower after treatment with haloperidol 2 mg/kg when compared to amisulpride 100 mg/kg (Fig. 1).

Long-term administration of antipsychotics produced significant treatment effects in the PFC [number of ChAT-positive cells ($F_{6,34} = 18.69$, $p < 0.001$); fiber pixel density ($F_{6,32} = 3.13$, $p = 0.018$)] and HIP [fiber pixel density in CA1 ($F_{6,34} = 7.85$, $p < 0.001$), CA3 ($F_{6,34} = 3.27$, $p = 0.012$) and DG ($F_{6,34} = 7.16$, $p < 0.001$)]. The results