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Short communication

Duration-dependent increase in striatal glutamate following prolonged fluphenazine administration in rats

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Abstract

Chronic neuroleptic administration has been shown to selectively increase striatal extracellular glutamate concentration. In the current study, age-matched female rats were administered chronic oral fluphenazine or no drug via their drinking water for 3 or 32 weeks. Microdialysis probes were inserted into the ventrolateral caudate putamen and the medial nucleus accumbens and dialysis samples were analyzed for glutamate and γ-aminobutyric acid (GABA) concentrations. Glutamate levels were significantly increased only in the ventrolateral caudate putamen after 32 weeks. No significant effects were seen for GABA levels. Neuroleptic-induced enhancement of striatal glutamate levels thus appears to increase with chronic exposure and this increase may relate to late onset motor side effects.

Keywords: Fluphenazine; Microdialysis; Caudate putamen; Nucleus accumbens; Glutamate; GABA (γ-aminobutyric acid)

1. Introduction

Chronic administration of neuroleptics can lead to the development of late onset motor disorders, such as tardive dyskinesia. While a variety of pathophysiological mechanisms have been proposed (See and Chapman, 1994a), the cause of tardive dyskinesia remains unclear. Recent data suggest that neurotoxic mechanisms may play a role in the development of late onset motor side effects, including evidence for increased oxidative metabolism after chronic neuroleptic administration (See, 1991) and increased free radical production in patients with tardive dyskinesia (Lohr et al., 1990). In addition, studies have shown that chronic haloperidol, but not the atypical drug clozapine, increases striatal glutamate levels (See and Chapman, 1994b; See and Lynch, 1995). This effect displays regional selectivity to the caudate putamen in that chronic haloperidol fails to affect basal or stimulated glutamate release in the medial prefrontal cortex (See and Lynch, 1995).

To further characterize temporal characteristics of regional changes in amino acid neurotransmitters produced by chronic neuroleptic treatment, the present study focused on the effects of subchronic (3 weeks) and chronic (32 weeks) administration of fluphenazine, a widely used neuroleptic with a high incidence of motor side effects. Extracellular glutamate and γ-aminobutyric acid (GABA) concentrations were examined in the ventrolateral caudate putamen, an area previously studied following chronic haloperidol (See, 1991; See and Chapman, 1994b; See and Lynch, 1995). In addition, the effects of chronic fluphenazine were studied in the medial nucleus accumbens. The nucleus accumbens was chosen since it is a primary terminal region of the mesolimbic dopamine system and previous studies have shown clear differences in the effects of chronic neuroleptic treatment on these two nuclei (See et al., 1992; Yamamoto and Cooperman, 1994).

2. Materials and methods

2.1. Drug administration

Female, Sprague-Dawley rats were single housed and maintained on a 12 h light/dark cycle with continuous access to food and water. Animals were divided into subchronic fluphenazine (3 weeks), chronic fluphenazine (32 weeks) and control groups. Fluphenazine hydrochloride (Sigma) was administered via the drinking water (0.02 mg/ml). Both the fluphenazine solution and control solu-

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tion contained dextrose (700 mg/l) for masking the flavor and all animals were started on solutions at the same time. Three weeks prior to microdialysis, the subchronic group was switched from control solution to fluphenazine solution. Drug intake was measured twice a week and ranged from 1.15 to 2.02 mg/kg/day over 32 weeks for the chronic group and from 1.19 to 1.88 mg/kg/day over 3 weeks for the subchronic group. The animals were cared for in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals.*

2.2. Microdialysis

After 31 weeks of continuous oral administration, rats were anesthetized with Equithesin (0.07 ml/100 g), ketamine (100 mg/kg) and xylazine (2 mg/kg) and stainless steel guide cannulae (20 gauge) were implanted into the ventrolateral caudate putamen (from bregma: A +0.2, L ±3.6, V −4.8) and the medial nucleus accumbens (from bregma: A +1.6, L ±1.0, V −5.6) according to the atlas of Paxinos and Watson (1986). Dialysis probes were constructed with dialysis membrane (250 μm outer diameter, 3 mm exposed length at the tip). One week after surgery (week 32 of treatment), dialysis probes were inserted into both regions and left in place 18 h prior to beginning sample collection. Fluphenazine solution was replaced with regular tap water at the time of probe insertion. In vitro recovery of GABA and glutamate was determined prior to probe insertion by placing each probe in dialysis perfusion solution (37°C) containing known concentrations of the analytes. The next morning, perfusion began 2 h prior to collecting samples. Probes were perfused (2 μl/min) with a modified Ringer's solution (in mM: NaCl, 147; $CaCl_2$, 1.2; KCl, 2.7; $MgCl_2$, 1.2; Na_2HPO_4, 0.5; pH 7.4) and perfusate samples collected in microcentrifuge tubes (0.5 ml) containing 10 μl of 0.1 M hydrochloric acid. Probe placement was later verified by examination of coronal sections stained with cresyl violet.

2.3. High-performance liquid chromatography (HPLC)

Samples were split and analyzed on two separate HPLC assays. Pre-column derivatization of samples was accomplished using *o*-phthaldialdehyde solution based on previous methods (Bourdelais and Kalivas, 1991). Samples for the detection of glutamate were injected onto a C18 column (Ultrasphere, ODS 3 μm, 75 × 4.6 mm, Beckman Instruments) at a flow rate of 1.4 ml/min. Mobile phase consisted of 0.05 M dibasic sodium phosphate buffer containing 0.1 mM Na_2EDTA, 10% methanol and 9% tetrahydrofuran at a final pH of 6.3 (Murai et al., 1992). Homoserine served as an internal standard. Samples for the detection of GABA were injected onto a C18 column (Phase II, 3 μm, 100 × 3.2 mm; BAS) at a flow rate of 0.8 ml/min. Mobile phase consisted of 0.1 M sodium acetate buffer containing 42% acetonitrile at a final pH of 4.57 (Bourdelais and Kalivas, 1991). Aminovaleric acid served as an internal standard. Detection of amino acid derivatives was accomplished with a coulometric detector (model 5100A with a model 5014 analytical cell; ESA), with the working electrode set at +0.70 V (glutamate) or +0.40 V (GABA), pre-column guard cell electrode set at +1.0 V (glutamate) or +0.70 V (GABA) and a pre-oxidation electrode set at +0.25 V (GABA). Chromatograms were manually scored and converted to concentrations using standard curves.

2.4. Data analysis

Data are represented as means ± S.E.M. Extracellular concentrations were analyzed using analysis of variance with significant differences between groups defined at $P < 0.05$. If the value of F was significant at $P < 0.05$, specific post-hoc comparisons were made (Newman-Keuls test).

3. Results

Fig. 1 illustrates basal levels of GABA (top) and glutamate (bottom) in both the caudate putamen and the nucleus accumbens. A significant between groups difference was seen in the caudate putamen for glutamate ($F(2,19) = 3.85$; $P < 0.05$) and pair wise comparisons showed a significant increase in glutamate in the chronic group compared to control ($P < 0.05$). While there was a trend towards increased glutamate concentrations in the subchronic group and for both fluphenazine groups in the nucleus accumbens, there were no significant differences found when compared to control values. Likewise, no significant differences were noted for basal GABA levels in either brain region after 3 or 32 weeks of fluphenazine administration.

4. Discussion

The present results extend our previous findings of increased striatal glutamate after chronic haloperidol in rats (See and Chapman, 1994b; See and Lynch, 1995). As seen in a previous report (See and Lynch, 1995), this effect is limited to the ventrolateral caudate putamen, a region which serves as a substrate for mediating orofacial motor activity (Kelley et al., 1988). The fact that this increase is also seen after fluphenazine suggests that this effect may be common to typical neuroleptics, but not atypical compounds, such as clozapine (See and Chapman, 1994b). In addition, increases in extracellular glutamate appear to be time dependent, in that subchronic fluphenazine treatment did not produce a significant rise in glutamate levels. While it has been shown that acute neuroleptic administration fails to alter striatal glutamate release (Daly and Moghaddam, 1993), two previous studies have reported a

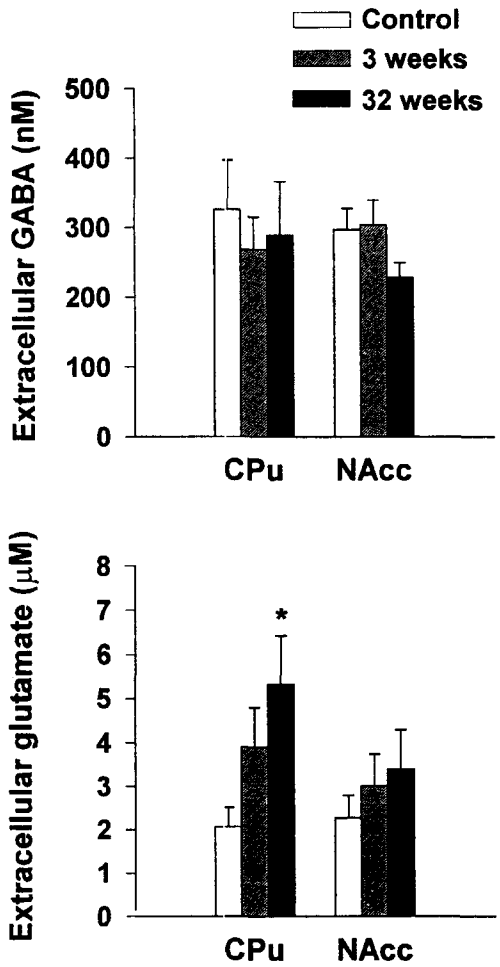

Fig. 1. Extracellular concentrations of GABA (top) and glutamate (bottom) in the ventrolateral caudate putamen (CPu) or medial nucleus accumbens (NAcc) for control, subchronic fluphenazine (3 weeks) or chronic fluphenazine (32 weeks) groups. All groups were age matched (11 months old at the time of dialysis sampling). Data are expressed as means ± S.E.M. ($n = 7–8$ per group) and are corrected for probe recovery. Significantly different from control: $^{*}P < 0.05$, Student Newman-Keuls test.

significant increase in basal striatal glutamate after subchronic (3 weeks) daily haloperidol administration (Moghaddam and Bunney, 1993; Yamamoto and Cooperman, 1994). The differences with the present results may relate to the drug utilized, treatment regimen and/or sampling procedures. However, since we did find a nonsignificant increase in striatal glutamate levels after 3 weeks, we suggest that increased glutamate may occur earlier during repeated exposure, but continues to increase with the passage of time and more prolonged neuroleptic exposure.

In a previous report, we found that 8 months of treatment with fluphenazine decanoate or haloperidol decanoate reduced extracellular GABA in the nucleus accumbens, but not in the caudate putamen (See et al., 1992). While there were no significant differences in the present study, there was an evident trend towards a GABA decrease in the nucleus accumbens of the chronic group. Recent evidence that acute neuroleptic administration alters pallidal GABA levels suggests that striatopallidal GABA pathways may be a primary site of chronic drug effects (Chapman and See, 1996).

The importance of glutamatergic mechanisms in neuroleptic motor side effects is becoming increasingly evident (Gunne and Andren, 1993). Given that recent clinical data implicate altered excitatory amino acid function in both the therapeutic action and side effects of neuroleptics (Tsai et al., 1995), it will be critical to continue exploration of regional effects of neuroleptic action on glutamate neurochemistry.

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