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Short communication

Chronic haloperidol treatment impairs glutamate transport in the rat striatum

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Abstract

High-affinity, Na^+-dependent transport of glutamate into neurons and glial cells maintains the extracellular concentration of this neurotransmitter at a sub-toxic level. Chronic blockade of dopamine D_2 receptors with haloperidol elevates extracellular glutamate levels in the striatum. The present study examines the effect of long-term haloperidol treatment on glutamate transporter activity using an assay based on measuring the uptake of D-[^{3}H]aspartate in striatal synaptosomes prepared from male Wistar rats. The maximal rate of glutamate transport in the striatum is reduced by 63% following 27 weeks of haloperidol treatment. This impairment of glutamate transport may be important in chronic neuroleptic drug action.

Keywords: Glutamate; Haloperidol; Transport; Striatum; Synaptosome

1. Introduction

Haloperidol, a classical antipsychotic drug, is believed to exert its effects by blocking dopamine D_2 receptors (Seeman and Lee, 1975; Creese et al., 1976). The highest densities of both dopamine D_1 and D_2 receptors are found in the striatum (caudate/putamen and nucleus accumbens). These nuclei are generally considered to be sites of both efficacious and undesirable effects of antipsychotic drug treatment (Crow et al., 1975; Deutch et al., 1992). The entire striatum receives major glutamatergic projections from the cortex in addition to dopaminergic inputs from the substantia nigra pars compacta and ventral tegmental area. It has previously been shown that chronic haloperidol treatment raises the concentration of extracellular glutamate (See and Chapman, 1994; See and Lynch, 1995; Yamamoto and Cooperman, 1994). However, the basis for this elevation is presently unknown.

A family of high-affinity Na^+-dependent glutamate transporters are essential for terminating the postsynaptic action of glutamate by rapidly removing it from the synaptic cleft (Fonnum, 1984; Takahashi et al., 1997). Within this family, the GLT-1 glutamate transporter, which is located on glial cells, accounts for approximately 70% of the total glutamate transported (Rothstein et al., 1996). Recent work by Schneider et al. (1998) has shown that GLT-1 mRNA in the striatum was significantly reduced following chronic haloperidol treatment. However, it was not determined whether the changes in these mRNA levels alter the functional activity of the glutamate transporters. In the present study, we have measured glutamate transporter activity and show that administration of haloperidol for 27 weeks significantly impairs the capacity of these transporters to take up glutamate in the striatum.

2. Materials and methods

Male, albino, Wistar rats (Bioresources, Trinity College, Dublin, Ireland) initially weighing 300–350 g were housed four per cage, with a constant temperature of 21°C, on a 12-h light and dark cycle with free access to food and water. Animals were divided into two groups. The control group ($n = 4$) was treated with sesame (Sigma, Poole, Dorset, UK) oil as vehicle while the haloperidol group ($n = 4$) received the depot neuroleptic drug, haloperidol decanoate (Janssen Pharmaceutical, Little Island, Cork, Ireland) at a dose of 28.5 mg kg^{-1}, the equivalent of 1 mg kg^{-1} day^{-1} of unconjugated haloperidol. Injections were

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given intramuscularly every 3 weeks for 27 weeks in a volume of 1 ml kg^{-1}. All procedures for the treatment of these animals were in strict compliance with European Community Directive, 86/609/EC and Cruelty to Animals Act 1876, with protocols approved by the Research Committee of the Royal College of Surgeons in Ireland.

Following the treatment period, rats were killed by stunning and cervical dislocation. Each brain was removed and the paired striata, including caudate/putamen and nucleus accumbens, were dissected out, and homogenised in ice-cold gradient medium, which was composed of 0.32 M sucrose, 1 mM EDTA and 0.25 mM dithiothreitol, pH 7.4. The homogenate was centrifuged for 10 min at 1000 *g* at 4°C (Sorvall centrifuge, SS-34 rotor) and the supernatants were collected and the synaptosomes were purified using a modification of the method of Dunkley et al. (1988). Briefly, Percoll diluted in gradient medium was layered into 10-ml polycarbonate tubes using a peristaltic pump and a flow rate of 1 ml min^{-1}, starting with the most dense (23% v/v), followed by, in order, 15% v/v, 10% v/v, and 3% v/v. A 2-ml sample of the supernatant was gently layered, using a Pasteur pipette, onto the top of the gradients and centrifuged at 32,500 *g* for exactly 5 min at 4°C. Synaptosomes in the 15%/23% Percoll interfacial fraction (layer 4) were carefully removed using a Pasteur pipette. They were washed twice in sodium-free Krebs' bicarbonate medium (choline chloride 116.8 mM, KCl 4.72 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, Tris 8.1 mM, glucose 11 mM and $CaCl_2$ 2.5 mM) pH 7.4 and resuspended in a final volume of 1.25 ml of sodium-free Krebs' medium. An aliquot was removed for protein determination by the method of Markwell et al. (1978) and the remaining synaptosomes used in the transport assay. They were incubated for 4 min at 25°C in normal Krebs' bicarbonate medium (NaCl 109.6 mM, KCl 4.72 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 25 mM, glucose 11

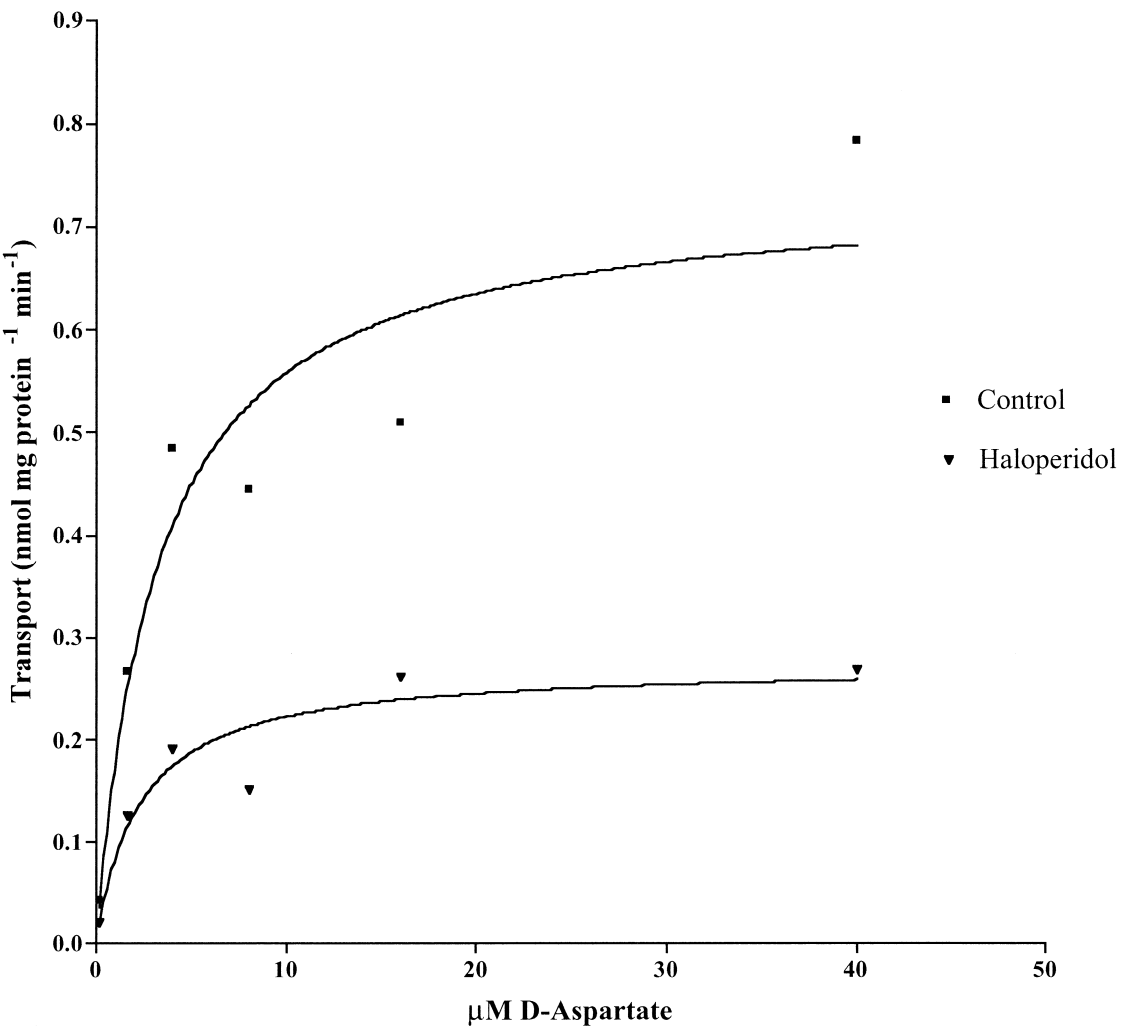

Fig. 1. Effect of chronic haloperidol treatment on kinetic parameters of glutamate transport. Synaptosomes were incubated at 25°C for 4 min and uptake of D-[^{3}H]aspartate at the specified concentrations was measured ($n = 2$ individual experiments, each performed in triplicate). The data were analysed by non-linear regression analysis and show a significant decrease in V_{max} (0.73 ± 0.08 nmol mg protein^{-1} min^{-1} for controls and 0.27 ± 0.03 nmol mg protein^{-1} min^{-1} in chronic haloperidol-treated rats ($P < 0.01$)).

mM and $CaCl_2$ 2.5 mM) pH 7.4 buffer, containing D-[^{3}H]aspartate (4.6×10^{-5}–1.15×10^{-4} MBq mmol^{-1} specific activity). The reaction was stopped by the addition of 200 μl of ice-cold 1 mM D-aspartate followed immediately by centrifugation for 10 min at 13,000 rpm at 4°C. The pellet was retained, washed twice with ice-cold gradient buffer and solubilised overnight in a 2% sodium dodecyl sulphate solution. The quantity of radioactivity in each sample was determined by liquid scintillation spectroscopy, and the rate of transport was plotted as a function of substrate concentration.

The transport assay was performed at 25°C, rather than 37°C, in order to slow down the activity of the transporters to a measurable rate in vitro and previous studies have determined that the transport of D-aspartate was linear between 0 and 20 min at 25°C (McBean, 1994). D-aspartate was used in preference to L-glutamate in these experiments because it is a non-metabolisable substrate for the glutamate transporters (Davies and Johnston, 1976; Fyske et al. 1992). In a separate set of experiments, synaptosomes were prepared from untreated male, albino Wistar rats ($n = 6$). Haloperidol (Sigma) was dissolved in a minimal amount of glacial acetic acid, diluted with distilled water (final pH 7.4) and added to the transport assay at a final concentration of 4 nM and 40 nM to determine if haloperidol had any direct affect on glutamate transport.

The experimental data were analysed by non-linear regression using Graphpad 'Prism' software (San Diego, CA, USA). Differences between control and treated rats were evaluated with the two tailed Student's *t*-test ($P < 0.05$ was considered significant).

3. Results

The rate of transport of D-[^{3}H]aspartate into rat striatal synaptosomes was determined over a concentration range of 0.16–40 μM in both control and haloperidol-treated animals (Fig. 1). Calculated values for V_{max} decreased significantly from 0.73 ± 0.08 nmol mg protein^{-1} min^{-1} for controls to 0.27 ± 0.03 nmol mg protein^{-1} min^{-1} in chronic haloperidol treated rats ($P < 0.01$). At a substrate concentration of 40 μM D-aspartate, the rate of transport was reduced to 42% of the control level. There was no significant change in the K_m value (3.21 ± 1.41 for control vs. 2.29 ± 0.92 for haloperidol-treated rats).

The inclusion of haloperidol, at concentrations as high as 40 nM in the transport assay, caused no significant change in the rate of transport of D-[^{3}H]aspartate. The V_{max} for transport in the controls was 1.10 ± 0.11 nmol mg protein^{-1} min^{-1}, whereas with 40 nM haloperidol it was 1.06 ± 0.40 nmol mg protein^{-1} min^{-1} (results not shown). In addition, there were no significant differences in the body weight, health or striatal protein content between treated and control animals.

4. Discussion

These findings demonstrate that chronic haloperidol treatment significantly impairs the activity of the high-affinity, sodium-dependent glutamate transporters in the striatum. Although D-aspartate was used in place of L-glutamate in these experiments, one can assume that L-glutamate transport would be similarly affected. Thus, at physiologically relevant (low micromolar) concentrations of L-glutamate, the ability of the transporters to take up glutamate would be significantly reduced following long-term neuroleptic treatment. See and Lynch (1995) have previously shown that extracellular glutamate is elevated following chronic neuroleptic drug treatment. In their work they treated rats for 24 weeks with haloperidol. Following treatment, glutamate was released by high K^+ infusion and the extracellular glutamate concentration was significantly elevated in the haloperidol-treated rats following infusion compared to controls. Our results suggest that this increase in extracellular glutamate may be due to reduced activity of glutamate transporters.

The fact that the V_{max} is significantly reduced with no change in the K_m implies that non-competitive inhibition is taking place. This suggests a reduced number of transport sites rather than a change in the ability of individual transporters to take up glutamate. This implies that 27 weeks of haloperidol treatment reduces the number of glutamate transport sites and therefore impairs transport. These results are reinforced by the results of Schneider et al. (1998) who observed a decrease in the expression of mRNA for GLT-1 glutamate transporters following 28 days of haloperidol treatment in the rat.

While a reduced number of glutamate transporters may be the most likely explanation for impaired transport, a number of other mechanisms are possible. Since neuroleptic drug treatment increases dopamine efflux in the striatum (Santiago and Westerink, 1991), dopamine oxidation products could inhibit glutamate transport in synaptosomes (Berman and Hastings, 1997). Another mechanism could involve metabolites of haloperidol. One of these metabolites, haloperidol pyridinium, has recently been shown to impair the dopamine transporter and lead to high levels of dopamine in the synapse (Wright et al., 1998) that could then block high-affinity glutamate uptake (Kerkerian et al., 1987). However, in light of our own results that show non-competitive inhibition and no change in glutamate transport with the addition of haloperidol to the transport assay, these possibilities are unlikely to occur.

Chronic blockade of dopamine D_2 receptors increases synaptic release of glutamate in the striatum (Perry et al., 1979; Bardgett et al., 1993). In addition, reduced glutamate transport activity, as demonstrated in this study, would maintain a high concentration of glutamate in the synaptic cleft for longer than in controls. Therefore, impaired glutamate transport coupled with increased glutamate release could result in increased glutamate neurotransmission and

this may be the mechanism involved in the therapeutic action of haloperidol. Moreover, increased glutamatergic transmission may also lead to excitotoxic effects due to prolonged activation especially at NMDA receptors (Olney, 1990), and this may play a role in side effects of chronic neuroleptic drug treatment such as tardive dyskinesia (De Keyser, 1991; Andreassen and Jørgensen, 1994; Meshul et al., 1996).

In conclusion, our results indicate that chronic haloperidol treatment inhibits glutamate transport in the rat striatum and this is most likely due to a reduced number of glutamate transport sites. Further studies are required to establish where and how this takes place.

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