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Short communication

Normethylclozapine potentiates the action of quinpirole in the 6-hydroxydopamine lesioned rat

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Abstract

Systemic administration of the 5-HT_{2c} receptor antagonist, normethylclozapine, to previously untreated unilaterally 6-hydroxydopamine-lesioned rats elicits rotations contraversive to the lesion when given with a subthreshold dose of the dopamine D_2 receptor agonist, quinpirole. Normethylclozapine alone does not possess any anti-parkinsonian action. In animals which have previously received dopamine replacement therapy, i.e. primed, normethylclozapine potentiates the contraversive rotations induced by quinpirole. We speculate that these actions may result from reduced excitation of the output regions of the basal ganglia. 5-HT_{2c} receptor antagonists may have potential as treatment for Parkinson's disease in combination with dopamine receptor agonists.

Keywords: 5-HT_{2c} receptor; Quinpirole; Normethylclozapine; Parkinson's disease

1. Introduction

The neural mechanisms underlying parkinsonian symptoms are thought to involve increased activity of neurons in the output regions of the basal ganglia, i.e. substantia nigra pars reticulata and medial globus pallidus. This excessive excitation is driven, in part at least, by glutamate transmission at the *N*-methyl-D-aspartate (NMDA) receptor (Brotchie et al., 1993). However, to date, NMDA receptor antagonists have shown limited efficacy in reducing parkinsonian symptoms in clinical trials (e.g. Rabey et al., 1992). The neural mechanisms controlling the excitability of basal ganglia outputs are therefore of great interest in developing novel treatments for Parkinson's disease.

5-HT_{2c} receptors (previously known as 5-HT_{1c}) are concentrated in the substantia nigra pars reticulata and medial globus pallidus in human (Pazos et al., 1987) and in rat brain (e.g. Mengod et al., 1990). 5-Hydroxytryptamine (5-HT), acting via 5-HT_{2c} receptors, is excitatory in the rat substantia nigra pars reticulata (Rick and Lacey, 1993). In the 6-hydroxydopamine-lesioned rat model of parkinsonism, sprouting of 5-HT-utilising terminals has been observed in the substantia nigra pars reticulata (Zhou et al., 1991) and the levels of 5-HT_{2c}-like receptor binding are increased by 160% (Radja et al., 1993).

We therefore hypothesised that excessive 5-HT_{2c} stimulation may contribute to the increased activity of the substantia nigra pars reticulata and medial globus pallidus and hence the symptoms of parkinsonism. This study investigates the behavioural effects of administration of normethylclozapine (*N*-desmethylclozapine), a highly potent and selective 5-HT_{2c} receptor antagonist (Kuoppamaki et al., 1993), in the 6-hydroxydopamine lesioned rat model of Parkinson's disease.

2. Materials and methods

2.1. 6-Hydroxydopamine lesion

Male Sprague-Dawley rats (280–300 g, Manchester University) were anaesthetised with pentobarbitone (60 mg/kg i.p.) 30 min following pre-medication with pargyline (5 mg/kg i.p.) and desiprimine (25 mg/kg i.p.). Using routine stereotaxic procedures, 6-hydroxydopamine (2.5 μl, 5 mg/ml, 0.02% ascorbic acid) was infused via a 26-gauge Hamilton syringe over 5 min into the right medial forebrain bundle (co-ordinates: −2.8 mm AP, +2 mm R, −9 mm vertical in relation to bregma, according to

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Paxinos and Watson, 1986). The needle was left in place for 1 min post-infusion. Animals were housed in groups of 4 and maintained under temperature-controlled conditions with alternating 12 h light-dark cycles. Food and water were provided ad libitum.

2.2. Behavioural testing

3 weeks post-operation behavioural testing was carried out between 11 a.m. and 3 p.m. Quinpirole (0.1 mg/kg), normethylclozapine (1 mg/kg) and/or appropriate vehicle were injected at a volume of 1 ml/kg i.p. The animals were placed into hemispherical, stainless-steel bowls (diameter 50 cm) immediately post-injection and the behaviour videotaped. The net number of rotations contraversive to the 6-hydroxydopamine lesion in each 5 min time bin was recorded over 90 min. 3 days later animals were injected with L-3,4-dihydroxyphenylalanine methyl ester (L-DOPA-methyl ester) (50 mg/kg i.p.) and benserazide (25 mg/kg i.p.) and their behaviour assessed in an identical manner.

The effect of this 'priming' with L-DOPA-methyl ester was assessed by repeating the quinpirole/normethylclozapine treatments 3 days following the injection of L-DOPA-methyl ester and benserazide.

Rats were assigned to treatment groups at random. The mean rotational rate following L-DOPA-methyl ester treatment was not significantly different between any group.

2.3. Drugs

6-Hydroxydopamine-hydrochloride, pargyline, desiprimine, L-DOPA-methyl ester and benserazide (Sigma, UK) and quinpirole (Research Biochemicals, USA) were dissolved in sterile water.

Normethylclozapine (Research Biochemicals) was dissolved in a minimal amount of dilute acetic acid, 0.9% saline and brought to pH 6.5 with NaOH. Vehicle injections consisted of dilute acetic acid and 0.9% saline at pH 6.5. All drugs were administered at a volume of 1 ml/kg i.p.

2.4. Data analysis

Statistical analysis was carried out using a one-way analysis of variance (ANOVA) and a post-hoc Student-Newman-Keuls multiple comparison test. Only animals showing greater than 200 rotations per hour following L-DOPA-methyl ester administration were included in the analysis. Significance was assigned when $P < 0.05$.

3. Results

Injection of normethylclozapine alone produced no net contraversive rotations compared to vehicle, -20.4 ± 2.7 rotations per 90 min ($n = 8$) and -18.2 ± 2.8 rotations per 90 min ($n = 6$) respectively (Fig. 1a). Administration of quinpirole to previously untreated 6-hydroxydopamine lesioned rats elicited total net contraversive turning of 25 ± 11.9 rotations per 90 min ($n = 8$). This was not significantly different to that observed following injection of vehicle alone ($P > 0.05$). Concurrent administration of normethylclozapine and quinpirole elicited 126.4 ± 27.5 rotations per 90 min ($n = 8$) (Fig. 1a, $P < 0.05$, compared to vehicle and quinpirole alone).

Following treatment with L-DOPA-methyl ester, all animals included in the analysis exhibited more than 200 contraversive rotations per hour (mean 364 ± 67). Subsequent to priming with L-DOPA-methyl ester, normethylclozapine alone produced no significant rotational response

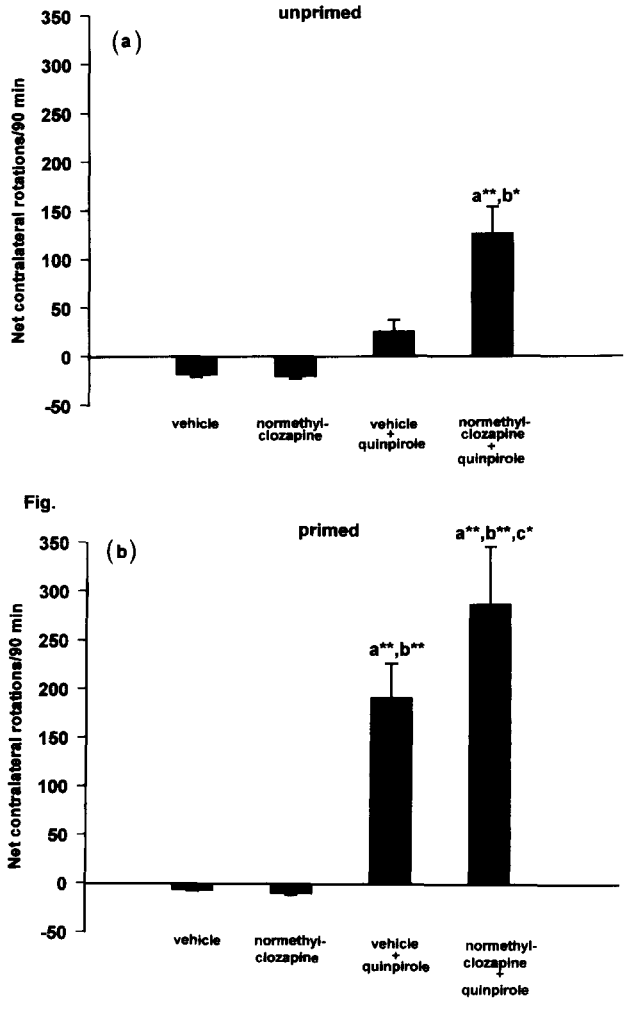

Fig. 1. Rotational behaviour following injection of vehicle ($n = 6$–8), normethylclozapine (1 mg/kg, $n = 8$), vehicle + quinpirole (0.1 mg/kg, $n = 8$) or normethylclozapine + quinpirole ($n = 8$) in the unilateral 6-hydroxydopamine lesioned rat model of Parkinson's disease. Data represent total contraversive rotations over 90 min ± S.E.M. Fig. 1a represents data in previously untreated rats (unprimed), Fig. 1b following priming with L-DOPA-methyl ester. [a] Compared to vehicle; [b] compared to quinpirole in untreated animals; [c] compared to quinpirole in primed animals, * $P < 0.05$, ** $P < 0.01$.

compared to vehicle, -9.8 ± 2 rotations per 90 min ($n = 8$) and -6.4 ± 0.8 rotations per 90 min ($n = 8$) respectively (Fig. 1b). Quinpirole induced 191.8 ± 35.5 contraversive rotations per 90 min ($n = 8$) which was significantly higher than that elicited by either vehicle following priming or quinpirole in untreated animals (Fig. 1b, $P < 0.01$). The rotational response following concurrent administration of quinpirole and normethylclozapine was significantly higher than that following quinpirole alone, 287.8 ± 59.6 contraversive rotations per 90 min ($n = 8$, Fig. 1b, $P < 0.05$).

4. Discussion

This study demonstrates that the 5-HT_{2c} receptor antagonist, normethylclozapine, enhances the rotational behaviour induced by quinpirole in the 6-hydroxydopamine lesioned rat. Furthermore, when administered with an ineffective dose of quinpirole, normethylclozapine elicits contraversive rotations.

All animals included in this study showed more than 200 contraversive rotations per hour following treatment with L-DOPA-methyl ester. Previous studies have shown that this degree of rotation is comparable to greater than 90% striatal dopamine depletion (Papa et al., 1994).

At the dose used in this study (0.1 mg/kg), quinpirole did not elicit significant rotational behaviour, compared to vehicle treatment, in untreated animals. However, following prior treatment with L-DOPA-methyl ester, i.e. priming, 0.1 mg/kg quinpirole elicited significant rotational behaviour. The mechanism of priming has been investigated with regard to potentiation of dopamine D_1 receptor agonist rotational behaviour by prior treatment with D_2 receptor or mixed D_1/D_2 receptor agonists in 6-hydroxydopamine lesioned rats. This effect is thought to involve changes in the transduction mechanisms in the dopamine-denervated striatum rather than any change in the receptor recognition site (Morelli et al., 1990). The mechanism underlying potentiation of dopamine D_2 receptor agonist mediated locomotor activity may reflect similar changes in the manner in which dopamine D_2 receptors are coupled to their signal transduction mechanisms. However, this study demonstrates the importance of timing of L-DOPA treatment to assess lesion efficacy in an experimental paradigm.

Quinpirole has been shown to have anti-parkinsonian effects in animal models of parkinsonism. In the 6-hydroxydopamine lesioned rat the actions of quinpirole result from stimulating upregulated dopamine D_2 receptors in the striatum ipsilateral to the 6-hydroxydopamine lesion. This D_2 receptor stimulation preferentially reduces activity in striatal outputs to the lateral pallidal segment (globus pallidus) and indirectly reduces the activity of excitatory amino acid utilising projections from the subthalamic nucleus to the substantia nigra pars reticulata and medial pallidal segment (entopeduncular nucleus). This excitatory amino acid mediated excitation of the substantia nigra pars reticulata and entopeduncular nucleus is mediated in part at least by NMDA receptors and is thought to be a key component of the mechanisms underlying parkinsonian symptoms (e.g. Brotchie et al., 1991). The mechanism by which 5-HT_{2c} receptor antagonists increase dopamine D_2 effects is unclear but given their concentration in the output regions of the basal ganglia we speculate that they might reduce excitability in these regions. Whilst there is little evidence to suggest that 5-HT_{2c} and NMDA receptors interact directly there is potential for a functional interaction downstream from the initial second messenger signals, for instance at the inositol 1,4,5-trisphosphate (IP_3) receptor. The 5-HT_{2c} receptor is coupled to the phospholipase C/IP_3 second messenger system (Conn et al., 1986). The levels of IP_3 receptors in the substantia nigra pars reticulata are amongst the highest in the brain (Sharp et al., 1993). Co-activation of the IP_3 receptor by 5-HT_{2c}-induced IP_3 and NMDA-evoked increases in calcium concentration, to release calcium from intracellular stores may cause longer term change in neural function. Blockade of such interactions may underlie the anti-parkinsonian effects of normethylclozapine.

Interestingly, normethylclozapine is a principal metabolite of the atypical neuroleptic clozapine, both compounds being 5-HT_{2c} receptor antagonists. Clozapine has been shown to be useful in the treatment of schizophrenia because of the lack of extrapyramidal side effects. Parkinsonian patients treated with clozapine for psychotic symptoms often show improvement in bradykinesia and tremor scores (Jansen, 1994).

In conclusion, 5-HT_{2c} receptor antagonists potentiate the anti-parkinsonian action of dopamine D_2 receptor agonists in the 6-hydroxydopamine lesioned rat model of Parkinson's disease, both in drug naive animals and following treatment with L-DOPA. As such, 5-HT_{2c} receptor antagonists may have potential in the development of novel therapeutic agents for Parkinson's disease both in terms of previously untreated patients and in patients who have received dopamine replacement therapy.

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