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Research report

Behavioural effects of trishomocubanes in rats with unilateral 6-hydroxydopamine lesions

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Abstract

Whilst dopamine replacement improves cardinal features of Parkinson's disease, chronic levodopa administration is associated with dose-related side effects and not all symptoms are ameliorated, necessitating the development of new treatments. Studies of trishomocubanes, a novel group of sigma ligands, have shown enhanced amphetamine-stimulated striatal release of dopamine and a potentially neuroprotective action *in vitro* and reversal of reserpine-induced catalepsy *in vivo*. Such effects warrant investigation in animal models of parkinsonism. Our study therefore examines two novel trishomocubane compounds, *N*-(3′-fluorophenyl)methyl-4-azahexacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$]dodecan-3-ol (**1**) and, *N*-(3′-fluorophenyl)ethyl-4-azahexacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$]dodecan-3-ol (**2**) in the 6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease. A variety of motor behaviours were studied in rats given 6-OHDA lesions. Groups of lesioned rats were given either (**1**) or (**2**) or vehicle solution i.p. Acute administration of 3 mg/kg (**1**) resulted in a decrease in locomotor activity. Twenty-five milligrams per kilogram (**2**) caused a decrease in locomotor activity at $t = 10$ and $t = 20$ min of the locomotor test but this was not found when (**2**) was co-administered with either apomorphine or amphetamine. The decreased locomotor activity indicates that (**1**) and (**2**) may have sedative/anxiolytic effect(s). However, elevated plus maze data failed to demonstrate anxiolysis with (**2**). Quantification of dopaminergic neurons did not demonstrate any significant difference in the magnitude of cell loss between drug-treated vs. vehicle treated rats so no neuroprotective effect was demonstrated in this model at the doses utilised.

Keywords: Rat; Medial forebrain bundle; Sigma receptors; Locomotor behaviour; Tyrosine hydroxylase; Anxiety; Sedation

1. Introduction

The dopamine precursor, levodopa has been the mainstay of therapy for Parkinson's disease (PD) since the 1960s [5]. Whilst it is effective in controlling the cardinal symptoms of PD (tremor, rigidity and bradykinesia), it does not control all symptoms (i.e. postural instability) or prevent disease progression. As well as a progressive loss of the drugs' effectiveness, many patients suffer debilitating side effects, such as dose-limiting dyskinesias and/or psychosis. Most other drug treatments are only effective at reducing the levodopa dosage required or prolonging the effect of the levodopa by enzyme inhibition, or are effective in controlling only individual symptoms [1]. There is clearly a need for development of new drug therapies for PD.

Trishomocubanes are a group of polycyclic hydrocarbon molecules which can be functionalised to include a variety of substituents. Interest first grew in the compounds when a subgroup of them, belonging to the D_3-trishomocubyl-4-amines type was shown to have anticataleptic activity in mice treated with reserpine [19]. Furthermore, these compounds also reduced oxotremorine-induced tremor and salivation in mice, indicating mild anticholinergic properties [19]. A subsequent investigation of trishomocubanes by Kassiou et al. [12] showed that

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the compounds had high affinity for sigma (σ) binding sites. Sigma receptors have been found to exist throughout the CNS and periphery. Notably, sigma receptors have been found in high concentrations in the striatum and nucleus accumbens and appear to be localised within the striatal dopaminergic system [6,7,11,25].

The σ_1 and σ_2 receptor subtypes were first described in the early 90s [8]. A study involving administration of (+)-pentazocine (σ ligand which has relative selectivity for the σ_1 receptor [21]) to rats found a dose-dependent increase in dopamine metabolism and release in the striatum and olfactory tubercle [11]. This could be reversed by pre-treatment with the *N*-methyl-D-aspartate (NMDA) antagonist CPP. Given that autoradiography showed little or no affinity for either dopamine or NMDA receptors, it was concluded that σ ligands can modulate dopaminergic function in both the dopaminergic A9 and A10 regions; an effect which may be mediated in part through NMDA receptors [11]. Another study found that unilateral intranigral injection of pentazocine produced circling behaviour which was potentiated by amphetamine and attenuated by 6-OHDA lesions [6]. These studies suggest that σ ligands could provide interesting candidates for study in animal models of parkinsonism.

Exploration of the trishomocubanes as sigma ligands produced agonists that were relatively selective for the σ_1 and σ_2 subtype receptors and are hence used in the present study [18]. Specificity for σ_1 in the compounds studied occurred with a secondary amine and a ketal function group, as well as at least a two-carbon chain separating the trishomocubane moiety and the aromatic ring [18,14]. A one-carbon alkyl chain and *meta* substitution on the aromatic ring were found to be essential features for σ_2 specificity [13,15].

One study of several trishomocubane compounds found that some increased amphetamine-stimulated dopamine release in striatal slices *in vitro*. The magnitude of dopamine release corresponded to the compounds' affinity for σ_2 receptors [15]. A similar result was reported in PC12 cells after pentazocine which could be blocked with a σ_2 antagonist [26]. It appears that σ_2 receptor is linked to the dopamine transporter via a Ca^{2+}/calmodulin-dependant protein kinase II transduction system [26]. The increase in dopamine concentrations may therefore be due to an outward flow of dopamine via the dopamine transporter.

A second action attributed to sigma receptor activation is that of neuroprotection. Studies of dopaminergic cell cultures have demonstrated that compounds with σ_1 activity have the ability to attenuate NMDA mediated excitotoxicity, most likely by directly modulating the activity of NMDA receptors [22]. Since excitotoxicity contributes to the pathogenesis of PD, the ability to reduce this action may have potential as a neuroprotective strategy [2].

The aim of the present study was to compare and contrast σ_1 and σ_2 specific trishomocubane compounds as potential symptomatic and/or neuroprotective therapies for parkinsonism. Compound (**2**), owing to a two-carbon chain separating the trishomocubane moiety and the aromatic ring, is a σ_1 specific compound with some affinity for σ_2 with a corresponding Ki of 10 nM vs. 370 nM, respectively [15]. Compound (**1**) showed high potency in modulating amphetamine-stimulated dopamine release in rat striatal slices [15]. The compound contains a *meta* substituted fluorine on the benzene ring, and a one carbon chain attaching the polycyclic moiety. These features give the compound relative selectivity for σ_2 with a corresponding Ki of 20 nM for σ_2 vs. 152 nM for σ_1, respectively [15].

2. Methods

2.1. Animals

Forty female Sprague-Dawley rats aged 11 weeks and weighing 250–300 g on arrival were used for the study. Animals were housed five per cage in an animal house with a 12 h light:12 h dark cycle. All rats were given standard rat chow and water available as required. Animal care was provided in accordance with the Australian National Health and Medical Research Council Guidelines on the Use and Care of Animals in Research (1997) and ethical approval was obtained from the University of Sydney Animal Ethics Committee.

2.2. Experimental design

The rats were divided into four groups called 1, 2, 3 and 4. Group 1 received vehicle and group 2 received compound (**1**). The rats that received compound (**2**) were in group 3 and group 4 was their vehicle control. All rats received a unilateral 6-OHDA lesion.

2.3. Surgery

Surgery was carried out either under i.p. injectable anaesthesia, consisting of 75 mg/kg ketamine hydrochloride (Ketavet®, Delvery, NSW, Australia) and 10 mg/kg xylazine hydrochloride (Ilium Xylazil-20®, Troy Laboratories, NSW, Australia). Corneal and gross motor reflexes were observed to confirm sufficient anaesthesia depth. The animals were then secured into a stereotaxic frame (model 51600 Stoelting Co., IL, USA) using 45° non-puncture earbars with the nosebar position 2.3 mm below the interaural line. A solution of 4 μg/μl of 6-hydroxydopamine (6-OHDA) as 6-OHDA·HBr in 0.1% ascorbate saline was injected via a 26 gauge Hamilton microsyringe mounted vertically on the stereotaxic frame into the medial forebrain bundle (coordinates: $A = -4.4$ mm posterior to the bregma, $L = \pm 1.1$mm lateral to the midline, $V = -8.0$ mm vertical to the dura) at a rate of 1 μl/min for 4 min [9]. The side lesioned was randomised so that 1/2 received lesions to the left and 1/2 to the right side in each group. Following infusion the syringe was left in place for 5 min to allow the toxin to diffuse away from the lesion site and the syringe was slowly withdrawn. The wound was then cleaned and sutured.

2.4. Preparation of drug solutions

Drug solutions of 1 mg/ml, 3 mg/ml (**1**) and 12.5 mg/ml (**2**) were prepared in a 5% dimethyl sulfoxide (DMSO) solution in corn oil. Incomplete dissolution of higher doses of the compounds prevented doses greater than 3 mg/kg (**1**) and 25 mg/kg (**2**) from being investigated. For comparison with the literature, these doses also corresponded to those used in a study of effects on trishomocubanes on locomotor activity in normal mice [16]. Vehicle for the control rats therefore consisted of 5% DMSO in corn oil. Drug and control solutions were freshly prepared shortly before each testing session and remnants were discarded after each test. Amphetamine was prepared as a 2.5 mg/ml solution in sterile saline. Apomorphine was prepared as a 0.2 mg/ml solution in 0.1% ascorbate saline.

2.5. Neuroprotection

Group 3 and group 4 received either a dose of 25 mg/kg (**2**) or vehicle solution, i.p., respectively, from 3 days before until 4 days after the 6-OHDA surgery to evaluate if the σ_1 ligand (**2**) had any neuroprotective effect.