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.
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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

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.

2.6. Behavioural testing

The behavioural tests of head position, curling, sensorimotor neglect and the disengage test, were conducted in triplicate, starting 1 week before and repeated at both 2 weeks and 1 month after surgery to establish the full extent of the 6-OHDA toxin actions [9]. All behavioural testing was conducted during the light phase of a 12:12 h light:dark cycle. Behavioural testing was done in an identical matter for all groups.

2.6.1. Locomotion

The animals were placed in 40 cm × 20 cm × 10 cm rectangular box which recorded a count every time an infrared beam was broken by the animal moving around inside. Beam breaks were recorded every 10 min for a 1 h period. The results found for this test were used to establish a time course relationship for the drugs to define the optimal period between drug administration and behavioural testing.

2.7. Acute dose–response studies

These studies were performed 2 weeks after completion of the chronic drug-free behavioural studies (approximately 6 weeks after lesioning).

2.7.1. Trishomocubane mediated locomotion

The two drugs were found to have a slightly different onset and duration of action influencing the timing of injections and placement into the locomotor box for subsequent 1 h recordings. This was based on a previous study of these compounds on locomotor activity in mice [16] and also based on initial time course analysis of data over a 2 h period from injection to completion of locomotor recording in our rats. Therefore, for compound (**1**) the animals were administered either drug or vehicle and were immediately placed in the locomotor box, whereas for (**2**) the animals were administered either drug or vehicle and were placed in the locomotor box 15 min after injection. The differential timing of the onset of the locomotor response found for the two trishomocubane drugs governed the timing of administration in relation to either amphetamine or apomorphine in subsequent drug challenges.

2.7.2. Amphetamine and trishomocubane mediated locomotion

The animals were administered amphetamine and either drug solution or vehicle in two separate (due to different carrier solutions), but successive i.p. injections. Following administration of (**1**) they were immediately placed in the box, whereas for (**2**) the rats were placed in the locomotor box 20 min after the injections.

2.7.3. Apomorphine and trishomocubane mediated locomotion

Animals treated with (**1**) were administered either trishomocubane or vehicle injection (i.p.) and were placed in a holding cage. Twenty minutes later the animals received an injection of apomorphine (s.c.) and were then placed in the locomotor box. For compound (**2**) the animals were given two separate, successive injections and put in the locomotor box after 20 min.

2.7.4. Rotation

Rotational asymmetry was measured in the animals by placing them into a white, circular rotation bowl (42 cm wide at the top and 22 cm deep). A video camera was mounted above the bowl. The animals were filmed for 30 min. The total number of ipsilateral and contralateral rotations (360° turns) was scored from the video footage. Net ipsilateral–contralateral rotation was then calculated [9].

2.7.5. Amphetamine and trishomocubane mediated rotation

The animals received amphetamine and either (**1**), (**2**) or vehicle in separate, successive injections (since the trishomocubanes were lipophilic and amphetamine was hydrophilic). They were placed in a holding cage for 30 min, and then transferred into the bowl for recording.

2.7.6. Apomorphine and trishomocubane mediated rotation

The animals received i.p. (**1**), (**2**) or vehicle injections, and were transferred to a holding cage. Twenty minutes later they received an apomorphine injection s.c. They were placed back in the holding cage for a further 10 min, and then transferred to the bowl for recording.

2.8. Chronic behavioural studies

2.8.1. Head position

This was measured as previously described [9]. The net head position bias represented the amount of time the head was turned ipsilaterally minus the total time the head was turned contralaterally during three, 60 s test periods, recorded in succession on each test week.

2.8.2. Body axis bias

The animal was gently lifted by the base of the tail and any bias in body axis ("curling") was recorded in triplicate on each test week as previously described [9].

2.8.3. Sensorimotor neglect and disengage testing

The amount of time for the animal to react to a tactile stimulation of the vibrissae of the rat whilst eating a 2.5 g piece of chocolate (disengage task) and during its' absence (simple sensorimotor task) was recorded on both the ipsilateral and contralateral sides. The test was repeated three times per test week and a mean latency calculated as previously described [9].

2.8.4. Elevated plus maze

The animals were each filmed for a 5 min period using a Sony DVD video camera. The DVD was then scored for the number of arm entries and time spent in each arm as a measure of anxiety and other parameters (below) [20,24]. An arm entry was counted when a rat entered an arm of the maze (open or closed) with all four feet. If all four feet were not within an arm it was counted as "in between". Other parameters measured included the number of stretch-attend postures (where the rat's body was stretched forwards), head dips (where the rat bent it's head down towards the floor), rearing (where the rat stood up on both hindlimbs) grooming frequency and time spent grooming [20,24].

2.9. Histology

On the completion of behavioural testing the animals were deeply anaesthetised with halothane. The animals were perfused with 0.1 M phosphate-buffered saline solution followed by 4% buffered paraformaldehyde solution, then decapitated. The brains were stored in 4% phosphate-buffered saline solution (PBS). Following cytoprotection in a 30% sucrose solution for 24 h, the brains were cut into 40 μm slices using a freezing microtome. Every fifth section was collected and stained with cresyl violet tyrosine hydroxylase and (TH) immunohistochemistry was carried on a parallel series as previously described [9]. The sections were mounted on slides and coverslipped with DPX/Histoclear.

2.9.1. Cellular quantification

Each of the stained nigral TH sections was quantified under the binocular microscope using stereology (fractionator technique). The total numbers of cells on the lesioned and unlesioned sides were counted in one complete series of parallel sections spaced 200 μm apart using a grid eyepiece, and the % cell loss on the lesioned side calculated, relative to the unlesioned side [9].

2.10. Statistical analysis

Results were analysed using the Statview statistics package. For the comparison of lesion sizes, a one-way analysis of variance (ANOVA) was used. To analyse locomotion over time or chronic variables such as curling and head position, a two-way repeated measures ANOVA was used, with a *post hoc* Fisher's Protected Least Significant Differences test used to evaluate any significant differences between groups. ANOVA was also used to investigate related elevated plus maze variables (i.e. time spent in open and closed arms or between them,

time spent grooming in open vs. closed arms etc.) with *post hoc* Fisher's Protected Least Significant Differences [24]. Unpaired *t*-tests were used to compare total locomotion between groups. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Confirmation of lesions

There was no significant difference in nigral dopaminergic lesion size between the four groups (Table 1) The mean tyrosine hydroxylase immunoreactive cell loss ranged from 79% to 89% in the four groups and was not statistically different. Several rats were excluded from analysis. The resulting group sizes were group 1 (9), group 2 (8), group 3 (7) and group 4 (8).

3.2. Chronic behavioural studies

Prior to 6-OHDA lesions the rats did not exhibit any postural biases and were quick to respond to perioral stimulation of the vibrissae both in the presence and absence of chocolate (approx 0.3 s latency). After surgery there were significant ipsilateral biases in head position (ANOVA $F = 21.654$, $P < 0.0001$) and body axis (ANOVA, $F = 14.570$, $P < 0.0001$) and there was a significant bilateral delay in response to tactile stimulation in the disengage sensorimotor test (ipsilateral ANOVA, $F = 11.415$, $P < 0.0001$; contralateral ANOVA, $F = 15.011$, $P < 0.0001$). Treatment with either (**1**) or (**2**) did not produce any improvement of these parameters relative to their controls (Fig. 1).

3.3. Elevated plus maze

The elevated plus maze was carried out following the unexpected observation that parkinsonian animals in the 1st part of the study which received (**1**) exhibited less locomotor activity. Since this may have represented sedation or anxiety, all animals in the second part of the study (involving group 3 and group 4 given vehicle or (**2**), respectively) underwent this test. Overall both groups of animals entered less frequently and performed less line crossings (Fig. 2A and B) as well as spend-
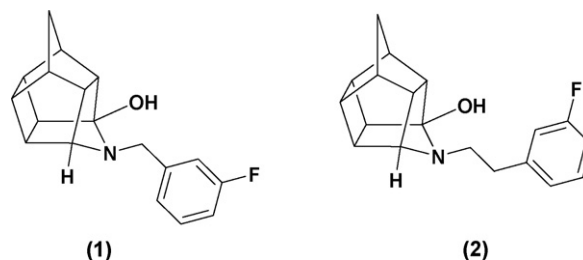

Fig. 1. The structures of the two trishomocubane compounds, *N*-(3′-fluorophenyl)methyl-4-azahexacyclo[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecan-3-ol (**1**) and, *N*-(3′-fluorophenyl)ethyl-4-azahexacyclo[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecan-3-ol (**2**) evaluated.

ing less time in the open arms of the maze, compared to the closed arms of the elevated plus maze (for proportion of time spent in closed vs. open arms ANOVA $F = 24.186$, $P < 0.0001$; $P > 0.05$ for *post hoc* group comparisons). The only significant difference in performance between groups was that there was less stretch-attend behaviour in the closed arms in drug-treated rats (Fig. 2C.). No other significant differences in behaviours measured in the elevated plus maze were found between groups.

3.4. Acute dose–response studies

3.4.1. Trishomocubane mediated locomotion

There was no significant difference in locomotion in controls given vehicle on two successive occasions. Unlike 1 mg/kg of compound (**1**), 3 mg/kg injection of (**1**) caused a significant decrease in locomotion when compared to the respective controls (Figs. 3 and 4.). Rats which received 25 mg/kg (**2**) had a significantly reduced number of beam breaks at both $t = 10$ and $t = 20$ min and total counts for the 1 h period compared to those which received vehicle (Figs. 3 and 4).

3.4.2. Amphetamine or apomorphine and trishomocubane mediated locomotion

There was no significant alteration by either of the trishomocubanes studied of either amphetamine- or apomorphine-induced locomotion in the 6-OHDA lesioned rats at the doses studied.

Table 1
% Cell loss in dopaminergic substantia nigra in the four treatment groups

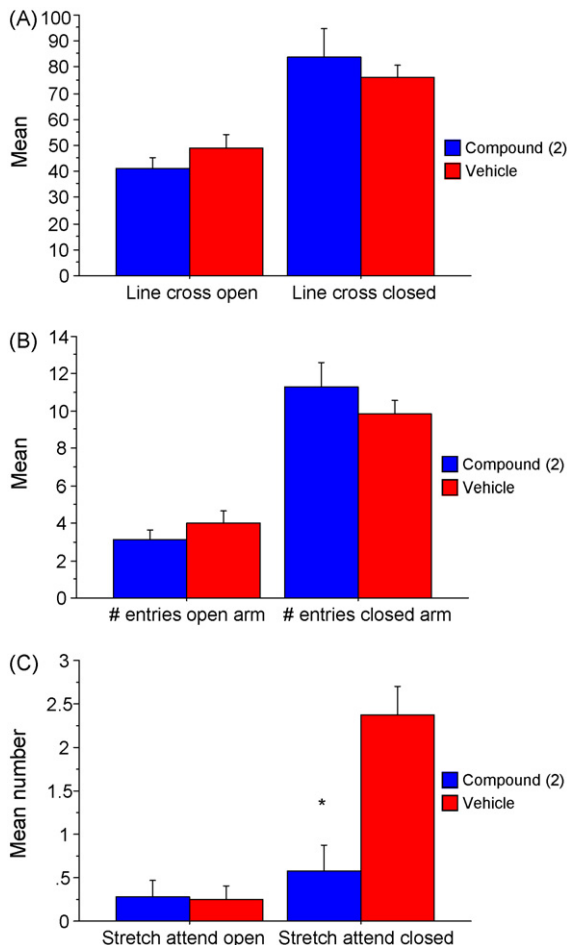| Rat number	Group 1 ($n = 9$)	Group 2 ($n = 8$)	Group 3 ($n = 7$)	Group 4 ($n = 8$)
Treatment	Vehicle	Compound (**1**)	Vehicle	Compound (**2**)
1	74	96	87	75
2	59	92	86	92
3	75	82	89	63
4	87	97	76	100
5	99	92	97	96
6	82	76	91	99
7	73	100	100	86
8	97	78	–	74
9	97	–	–	–
Mean ± S.E.M.	78.7 ± 7.3	81.8 ± 9.4	89.4 ± 2.9	85.6 ± 4.8

Fig. 2. Elevated plus maze. Data obtained for line-crossing (A), arm entries (B) and stretch-attend movements (C). Vertical columns indicate mean ± standard error of mean for each group. *$P < 0.05$ for group comparison. (A) Line crossing. Whilst both crossed the lines more in the closed arms of the maze than in the open arms of the maze, there was no difference in the mean number of line crosses in either the open or closed arms when comparing between rats treated with (**2**) and those treated with vehicle (ANOVA $F = 37.278$, $P < 0.0001$, *post hoc* group comparisons $P > 0.05$). (B) Arm entries. Mean number of times that the open and closed arms were entered by the two groups of rats. Whilst both groups entered into the closed arms of the maze more than into the open arms of the maze, there was no difference in the mean number of entries made into either the open or closed arms when comparing between rats treated with (**2**) and those treated with vehicle (ANOVA $F = 88.523$, $P < 0.0001$; $P > 0.05$ for *post hoc* group comparisons). (C) Stretch-attend movements. Mean number of stretch-attend movements made by the two groups in the open and closed arms of the elevated plus maze. Note that trishomocubane-treated rats exhibited significantly less stretch-attend movements than vehicle-treated rats near the entrance of the closed arms of the elevated plus maze (ANOVA $F = 19.719$, $P = 0.0007$; relevant *post hoc* group comparison $P = 0.0291$).

3.5. Drug-induced rotational asymmetry

Trishomocubanes when administered alone did not induce rotational asymmetry. All unilateral 6-OHDA lesioned rats showed a marked, net ipsilateral turning bias under amphetamine and net contralateral bias after apomorphine. No significant difference was found between the trishomocubane 3 mg/kg of (**1**) or 25 mg/kg (**2**) treated rats vs. respective controls after either amphetamine or apomorphine.

4. Discussion

This study of trishomocubanes (**1**) and (**2**) has found that there is no antiparkinsonian effect but a decrement in locomotion after either 3 mg/kg (**1**) or 25 mg/kg (**2**) in hemiparkinsonian rats. In contrast, both locomotion and rotational asymmetry measured after administration of either amphetamine or apomorphine were unaffected by either compound (**1**) or (**2**).

4.1. The 6-OHDA model of Parkinson's disease

6-OHDA lesions successfully produced marked parkinsonian features in our animals which was evidenced by significant deficits in a variety of behavioural tests measuring rotational asymmetries, postural biases, sensorimotor "disengage" reaction latencies, as well as detailed quantitative analysis of nigral dopaminergic cell loss. These results were consistent with those previously described in well-lesioned rats [9]. However, no antiparkinsonian effects were exerted by either of compounds (**1**) or (**2**) at the doses used in the model studied.

4.2. Acute locomotion and rotation studies

During trishomocubane mediated locomotion testing the animals that received (**1**) alone showed a significant decrease in locomotion at 3 mg/kg, relative to vehicle which was maximal approximately 30–50 min post-administration.

Increases in locomotor activity have been linked with increases in dopaminergic activity caused by drugs [3]. There was a significant reduction of locomotion (by at least 40% in hourly counts) after either 3 mg/kg (**1**) or 25 mg/kg (**2**), but no effect (relative to vehicle) in the presence of amphetamine. The latter contrasts with the previously described *in vitro* phenomenon where trishomocubanes increased the amount of amphetamine-stimulated dopamine release in striatal slices [15]. Nor was there a difference in magnitude of locomotion between the apomorphine and trishomocubane-treated vs. the apomorphine plus vehicle treated controls. Instead of an effect involving dopamine release or modulation of post-synaptic dopamine receptors, it appears that trishomocubane monotherapy exerted a sedative effect at the doses studied in this model. In support of our behavioural observations, a prior study found a dose-related decrease in locomotion in normal mice given (**2**) and doses above 3.6 mg/kg of (**1**) [16]. Also, an early study cited sedation and depressed locomotion in guinea pigs given either pentazocine or the putative sigma agonist NANM [4]. Since the effects were similar to those seen after the non competitive NMDA antagonist, dizocilpine and could be reversed by the dopamine D2 receptor agonist quinpirole, those authors suggested that the behavioural depression could be mediated via sigma receptor interactions involving both DA D2 and NMDA receptors [4]. Also, the regional distribution of sigma receptors, with a preponderance of σ_2 in brainstem motor areas [7], may provide an underlying anatomical substrate for the decreased locomotion observed in our trishomocubane-treated animals. Further studies of sigma ligands are required to determine the exact mechanisms by which such compounds exert their actions.
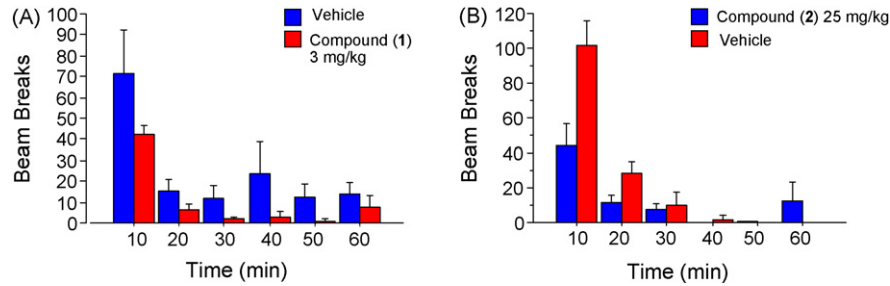
Fig. 3. Trishomocubane mediated locomotion (A). The number of beam breaks in a light box were recorded every 10 min for 1 h. The 3 mg/kg injections of (**1**) caused a decrease in locomotor activity compared to the vehicle injections (two way repeated measures ANOVA $F = 13.933$, $P < 0.0001$, *post hoc* group comparison $P = 0.0094$). The drug appears to have it peak activity at the 30–50 min mark. Trishomocubane mediated locomotion (B). The number of beam breaks in a light box were recorded every 10 min for 1 h. Whilst there was no significant difference over the whole 60 min period (ANOVA $F = 29.863$, $P < 0.0001$ *post hoc* $P = 0.1294$), the compound (**2**) treated animals (group a) showed a significant decrease in locomotor activity at both $t = 10$ ($t = -3.006$, $P = 0.0101$) and $t = 20$ min ($t = -2.230$, $P = 0.0440$). Vertical columns indicate mean ± standard error of mean of each group.

The effects of trishomocubanes on locomotion appeared to be outweighed by the dopaminergic effects of amphetamine and apomorphine. This is also not surprising given that amphetamine is a potent stimulant and is anxiogenic and so could counter either sedative/anxiolytic effects of the trishomocubanes at
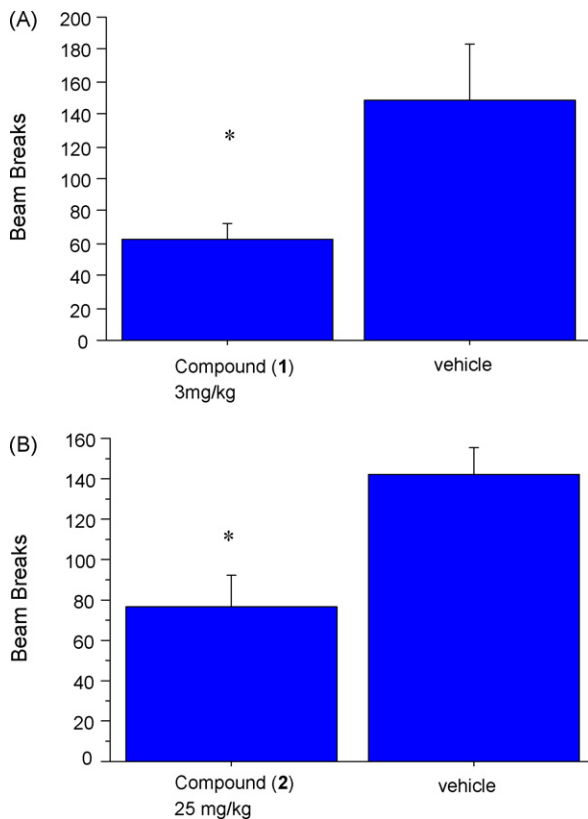

Fig. 4. Total trishomocubane mediated locomotion (A). The total number of beam breaks in a 1 h period. There is decreased total locomotor activity for 3 mg/kg of (**1**) (group 1) compared to vehicle treated animals (group 2, $t = 2.675$ *$P = 0.0166$).Vertical columns indicate mean ± standard error of mean for each group. Total trishomocubane mediated locomotion (B). The cumulative total number of beam breaks in a 1 h period. There is a significantly decreased locomotor activity after injection of (**2**) (group 4) compared to the control group (group 3; $t = -3.281$, *$P = 0.0060$). Vertical columns indicate mean ± standard error of mean of each group.

the doses administered. Of relevance, a previous study of trishomocubanes did not find significantly altered cocaine-induced locomotor activity in mice given similar doses of either (**1**) or (**2**) to those administered in the present study [16].

Based on our observations from the first phase of the study (comparison of (**1**) vs. controls) we introduced the elevated plus maze in the second phase of the study in which (**2**) was tested against controls to establish whether there was evidence for an anxiolytic effect. It is well-established that when rats are stressed they prefer to stay in a "safe", dark environment [20,24]. The absence of a significant difference between groups in time spent in the open, light arm of the elevated plus maze fails to support an anxiolytic effect (at least for (**2**)). The only effects were a reduction in stretch-attend movements which are considered a "risk assessment" behaviour [24]. However, this effect only achieved significance in the closed, not open arms of the maze.

The structural attributes of compound (**2**) confer greater selectivity for the σ_1 receptor (37-fold greater affinity for $\sigma_1 > \sigma_2$). Anxiolysis has been reported in compounds with σ_2 activity such as opipramol and LU 28–179 [21,10]. The former has an eight-fold higher affinity for σ_1 over σ_2 receptors [10] and is a potent atypical anxiolytic used clinically. In rats it has been shown to decrease immobility in the forced swim test and reduced the density of σ_2 (but not σ_1 receptors) in the mid/hindbrain after daily administration for 2 weeks [10]. On the contrary, LU 28–179 has a 90-fold greater affinity for σ_2 over σ_1. In rats LU 28–179 improved exploration in the black and white two-compartment test, increased social interaction and decreased shock-induced suppression of drinking [21]. Overall, the data from various studies suggest that the absence of an anxiolytic effect observed in our rats after (**2**) may be due to the relative lack of affinity for σ_2. Therefore, this does not preclude that (**1**) – a potent σ_2 ligand, may have potential anxiolytic effects, but this would need to be tested in future studies.

The (**2**)- and (**1**)-treated groups showed no evidence of sedative effects with the drug-induced rotation tests. Nor was there evidence of sedation in the elevated plus maze as arm entries and line-crossing behaviour was not significantly decreased. The latter measures are considered to be crude indices of locomo-

tor behaviour [20,24]. It must be noted however, that the tests of locomotion in the presence of either (**1**), (**2**) or vehicle alone, were conducted over a 1 h period in a darkened box in which one would expect the rats to feel "safer" [20,24]. In contrast the elevated plus maze data was collected over a 5 min period without habituation, representing a "novel" environment. It is possible that the differences in environment and length of testing as well as mode of testing (objective automated counts vs. more "subjective" investigator observation) may have enabled mild sedation to be detected in the locomotor box but not under other testing conditions. Of relevance, a clinical study of opipramol reported 9% of patients experienced tiredness vs. only 2% of those treated with placebo [23]. As expected after unilateral 6-OHDA lesions, rats showed significant turning biases corresponding to the drug administered (strong ipsilateral turns after amphetamine and strong contralateral turns after apomorphine) but there were no significant differences between the trishomocubane vs. vehicle treated groups in these tests.

4.3. Neuroprotection

The behavioural tests and post-mortem histology established that the 6-OHDA lesions were in general large and of similar magnitude. Earlier studies involving sigma ligands concluded that activation of the σ receptors, particularly the σ_1 receptor, produced neuroprotection [17]. Both σ_1 agonists, pentazocine and SA4503 significantly decreased neurotoxicity induced by both hypoglycaemia and hypoxia, but not after NMDA, when exogenously applied in rat primary neuronal cultures [17]. In contrast, another study using the σ_1 agonist SKF 10047 found neuroprotection in a model of NMDA mediated cytotoxicity in dopaminergic neurons in rat organotypic mesencephalic slice cultures [22]. However, compound (**1**), has little activity on the σ_1 receptor so would not be expected to reduce cell loss in the model used [13]. Compound (**2**) has a high affinity for the σ_1 receptor and some affinity for the σ_2 receptor [14]. However, after administering the drug 3 days before and 4 days after the lesion there was no significant difference in the magnitude of cell loss in the (**2**) group vs. the vehicle group. Whilst it is possible that neuroprotection in the 6-OHDA model may require earlier administration, no evidence was found that either (**1**) or (**2**) had any neuroprotective effects at the doses administered in our study. Whether such compounds exhibit neuroprotective properties may depend on factors as diverse as the relative selectivity for σ_1 receptors of the compound used, dose administered and the mechanism(s) responsible for cell death, and the type of model used (i.e. animal or tissue culture) [22].

5. Conclusion

No antiparkinsonian properties of (**1**) or (**2**) were demonstrated in the extensive battery of behavioural tests conducted in unilateral 6-OHDA lesioned rats. Nor was there evidence of neuroprotection in this model at the doses administered. However, decreased locomotion was observed in 6-OHDA lesioned rats for both (**1**) at 3 mg/kg and (**2**) at 25 mg/kg.

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