Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 458 (2003) 73–79
www.elsevier.com/locate/ejphar

Screening of novel pentacyclo-undecylamines for neuroprotective activity

Werner J. Geldenhuys[a], Gisella Terre'Blanche[a], Cornelis J. Van der Schyf[a,b], Sarel F. Malan[a,*]

[a] *Department of Pharmaceutical Chemistry, Potchefstroom University for Christian Higher Education, Private Bag X6001, Potchefstroom 2531, South Africa*
[b] *Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center, School of Pharmacy, Amarillo, TX, USA*

Received 27 June 2002; received in revised form 29 October 2002; accepted 5 November 2002

Abstract

A novel series of pentacyclo-undecylamines with 8-benzylamino-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (NGP1-01) as the lead compound was synthesised and screened for neuroprotective activity in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) parkinsonian mouse model. We hypothesise that these compounds may attenuate excitotoxic neuronal cell death mediated through the NMDA receptor (similar to memantine), and through calcium channel block. The pentacyclo-undecylamines (300 mg/kg) were administered to C57BL/6 mice 30 min before intraperitoneal (i.p.) MPTP administration (35 mg/kg). Striatal dopamine, 3,4-hydroxyphenylacetic acid (DOPAC), and homovanillic acid levels were analysed 10 days later by means of HPLC with electrochemical detection. Increased levels of DOPAC and homovanillic acid were observed when some of the test compounds were administered together with MPTP (compared to animals receiving only MPTP). One compound in the series, 8-phenylethylamino-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane, attenuated MPTP-induced striatal dopamine depletion when compared to animals treated with MPTP only ($p < 0.05$).

Keywords: Excitotoxicity; MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine); Neuroprotection; Pentacyclo-undecylamine

1. Introduction

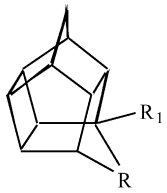
Excitotoxicity has been implicated in processes that underlie neuronal cell death associated with acute neurodegeneration such as ischemia and trauma, and in chronic neurodegenative diseases such as Parkinson's and Huntington's disease (Alexi et al., 2000). Excitotoxicity is mediated through intraneuronal Ca^{2+} overload during excessive stimulation of the NMDA receptor (Greene and Greenamyre, 1996). Modulation of Ca^{2+} influx through these receptors may therefore attenuate neurotoxicity by reducing or preventing Ca^{2+} overload in neuronal cells. The development of neuroprotective agents for the prevention of neuronal cell loss has focused on drugs that inhibit excitatory amino acid neurotransmission or exhibit antioxidant properties (Kornhuber and Weller, 1997). Noncompetitive *N*-methyl-D-aspartate (NMDA) receptor channel blockers that have specific affinity for the phencyclidine (PCP) binding site in the NMDA receptor—such as MK-801 (dizocilpine) or the dimethyl derivative of amantadine, memantine—also show promise as neuroprotective agents (Bigge, 1993; Kornhuber and Weller, 1997). Calcium channel blockers have also been shown to protect against glutamate-induced damage (Kobayashi and Mori, 1998).

This study is part of an ongoing investigation into the therapeutic potential of novel synthetic pentacyclo-undecylamines. The synthesis and pharmacological properties of some trishomocubylamines and pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecylamines were reported by Oliver et al. (1991a,b). Amongst their other activities, these compounds displayed antagonism of reserpine-induced catatonia which compared favourably to that of amantadine, as well as reduction of oxotremorine-induced tremor and salivation in rats (Oliver et al., 1991a,b). These authors suggested that polycyclic amines may have potential as a new class of anti-parkinsonian agents due to their anticataleptic and mild to weak anticholinergic activities. L-type calcium channel antagonism has also been described for pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecylamines, in particular for the prototypical

* Corresponding author. Tel.: +27-18-2992266; fax: +27-18-2994243.
E-mail address: fchsfm@puknet.puk.ac.za (S.F. Malan).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02701-2

Table 1
Structural formulas of compounds **1**–**7**



Compound	R	R_1
1	O	$NHCH_2Ph$
2	O	$NHCH_2CH_2Ph$
3	O	$NHCH_2C_6H_{10}$
4	O	$NHCH_2PhNH_2$[a]
5	O	$NHCH_2PhNO_2$[a]
6	O	NH_2
7	NCH_2Ph	OH

[a] Substituents on the aromatic ring are in *para*-position.

compound 8-benzylamino-8,11-oxapentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane (NGP1-01, **1**) which was extensively studied (Malan et al., 2000; Van der Schyf et al., 1986).

The structural similarity between the polycyclic cage structures of memantine and NGP1-01 (**1**) prompted the evaluation of these compounds and their derivatives (Table 1) for possible neuroprotective activity. Such putative protective activity can be hypothesized to be initiated by a dual mechanism of action including attenuation of the NMDA receptor, thereby preventing excessive influx of Ca^{2+} into neuronal cells, as well as direct blockade of L-type Ca^{2+} channels. Protection against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced striatal dopamine depletion was used as a model to assess neuroprotective activity. The MPTP parkinsonian mouse model is a useful animal model for studying neurodegenerative and neuroprotective processes (Castagnoli et al., 2001) and was therefore chosen for the current screening study.

MPTP is a neurotoxin, the administration of which leads to a decrease in dopamine content in the striatum and a reduction in the number of nigrostriatal dopaminergic neurons in some experimental animals. The pathology described in these animals closely mimics the neuropathological changes observed in idiopathic Parkinson's disease (Araki et al., 2001; Speciale, 2002). The neurotoxic effects of MPTP are mediated through its oxidation to the neurotoxic species 1-methyl-4-phenylpyridinium (MPP^+) that accumulates in dopaminergic neurons by actively being transported through the dopamine transporter. The ultimate neurotoxicity of MPP^+ is the result of inhibition of electron transport at mitochondrial complex 1, leading to reduced ATP formation and neuronal cell death (Venero et al., 1996; Speciale, 2002). There is some controversy in the literature regarding the role of the NMDA receptor and the protective effects of NMDA antagonists on the neurotoxicity of MPTP (Venero et al., 1996; Araki et al., 2001). Some laboratories have reported that blockade of the NMDA receptor attenuates MPTP and MPP^+ neurotoxicity (Turski et al., 1991; Chan et al., 1993; Brouillet and Beal, 1993; Venero et al., 1996; Zuddas et al., 1992). In contrast, others have not confirmed these observations (Araki et al., 2001) or even disputed the findings of neuroprotection (Sonsalla et al., 1992; Kupsch et al., 1992). Further studies to assess the importance of such mechanisms are thus warranted.

As a first approach to compare structural similarity between known NMDA receptor channel modulators or blockers and the pentacyclo-undecylamines described in this report, we used a simple computer modelling method (Marshall et al., 1979). The cage compounds were fitted onto the ligand PCP, and two known antagonists (memantine and MK-801) of the PCP binding site in the NMDA receptor. Favourable root mean square (RMS) fits suggested similar interaction with the PCP binding site by the pentacyclo-undecylamines than those found for PCP, memantine and MK-801. In short, a minimum energy conformation of each structure was obtained by optimising the structures with the Chemplus™ extension of Hyperchem® modelling software (Release 4.5 for Windows 1994 Hypercube, Ontario, Canada) using MM^+ and AM1 with the Polak–Ribiere minimisation procedure (Polak, 1971). The lowest energy conformer of each of the test compounds as well as the template compounds (PCP, memantine and MK-801) was used for the fitting. The candidate pentacyclo-undecylamines were then fitted onto PCP, MK-801 and memantine, and the RMS calculated for each fit. The fitting was done by means of atom-based alignment. A common nitrogen atom and the six carbon atoms in a common phenyl ring were used. In the absence of an aromatic ring, the carbon adjacent to the common nitrogen was used as well as three carbon atoms attached to the latter carbon. Favourable fitting (RMS <1.0 Å) was found for all test compounds on PCP, memantine and MK-801 (example, Fig. 1), prompting us to proceed with the in vivo neuroprotection studies using the full series of polycyclic compounds.

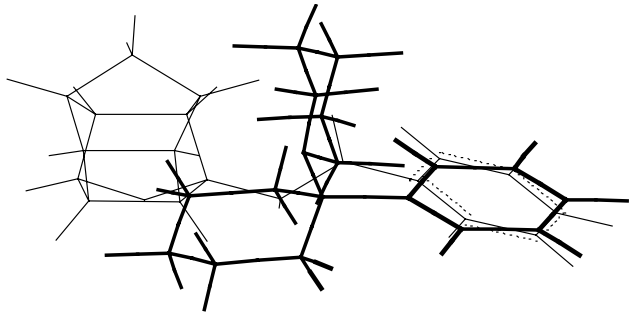


Fig. 1. RMS fit of **1** (thin line) on PCP (thick line).

2. Materials and methods

2.1. Materials

Pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dione was prepared according to the method of Cookson et al. (1985). Compounds **1**–**7**, with the exception of **4** (Table 1), were prepared according to the methods of Malan et al. (2000) and Marchand et al. (1988). Compound **4** was prepared from **5** by reduction of the nitro moiety using mossy tin and hydrochloric acid (Harwood et al., 1999). Memantine (hydrochloride salt) and (*R*)-deprenyl (hydrochloride salt) were purchased form Sigma (South Africa). (+)-MK-801 (maleate) was obtained from Research Biochemicals (Natick, MA, USA). Buffer components were obtained from commercial sources.

2.2. Biological evaluation

Approval for the performance of this study was obtained from the Ethics Committee (Medical), and the Animal Care and Use Committee of the Potchefstroom University for Christian Higher Education.

2.2.1. Animals

Three to four months old male C57BL/6 mice, weighing approximately 25 g each, were used. The animals were maintained under a constant 12-h light–dark cycle and housed with free access to food and water in a room with the temperature controlled ca. 23 °C and relative humidity ca. 45–55%.

2.2.2. Neuroprotection studies

To investigate the possible neuroprotective activity of this novel series of pentacyclo-undecylamines, we used the MPTP parkinsonian mouse model utilising C57BL/6 mice. An i.p. dose of MPTP·HCl at 35 mg/kg was used, since this dose of MPTP has been shown to be sublethal but elicits substantial striatal dopamine depletion (~ 50%, Heikkila et al., 1989). Dosages used for the other reference compounds were derived from their effective doses as reported in the literature (Wenk et al., 1995; Yahr, 1987).

Injection solutions of the test compounds in sweet oil B.P. were prepared by stirring and heating at 50 °C until clear solutions were obtained. In the event of insolubility, homogeneous suspensions were prepared. MK-801-maleate and the HCl-salts of MPTP, memantine and (*R*)-deprenyl were dissolved in normal sterile saline (0.9%). Injection volumes of 0.2 ml/30 g body weight were administered. Mice in the "compound only" group ($n = 10$/compound) received 1 × 300 mg/kg s.c. injection of compounds **1**–**7**, 1 × 5 mg/kg of memantine, 1 × 0.25 mg/kg of MK-801, and 1 × 4 mg/kg of (*R*)-deprenyl. This was followed at $t = 30$ min by a vehicle only (normal saline, i.p.) injection. Animals in the "MPTP only" group ($n = 10$) received s.c. vehicle only (sweet oil B.P.), followed at $t = 30$ min with a 35 mg/kg i.p. injection of MPTP·HCl. Animals in the "MPTP plus compound" group ($n = 10$/compound) received the test compound followed at $t = 30$ min with a 35 mg/kg i.p. injection of MPTP·HCl. Control animals ($n = 10$) received the same injection schedule with vehicle only (sweet oil B.P. s.c. and saline i.p.).

2.2.3. Tissue preparation

Ten days after treatment, the mice were sacrificed by cervical dislocation and the striata were rapidly dissected on ice, placed into polypropylene tubes, frozen with liquid nitrogen and kept at − 77 °C until analysed. On the day of analyses, samples were thawed and 1 ml of a 0.1 M perchloric acid solution (also containing 0.5 mM sodium metabisulfite and 0.3 mM Na_2EDTA) was added to each tube. The tissue was then disrupted by sonication (2 × 12 s). The tubes were allowed to stand on ice for 20 min. Following this period, samples were vortexed for 10 s and centrifuged at 4 °C for 15 min at 16 000 × *g*. The pH of the sample was adjusted to ca. 5 with the addition of one drop of 10 M potassium acetate. An aliquot of 200 μl of the tissue extract was removed, to which 5 μl of ascorbic acid oxidase (22.2 ng/ml) and 15 μl of an internal standard solution (1511 ng/ml isoprenaline in perchloric acid) was added. The total volume of the sample was 220 μl of which a volume of 50 μl was injected onto the column.

2.2.4. Analysis of dopamine and metabolites

Dopamine, DOPAC, and homovanillic acid determinations were performed using a Waters M460 electrochemical detector, an Ultrasphere C18 150 × 4.6 mm column, a Beckman (model 110B) pump and a Spectra-Physics (SP4400) integrator. The mobile phase consisted of 0.1 M sodium formate buffer, 0.5 M ethylenediaminetetraacetic acid (EDTA), 5 mM sodium heptane sulfonic acid, 4% (v/v) methanol and 4% (v/v) acetonitrile. The pH of the buffer was adjusted to 3.85 by addition of concentrated formic acid. Stock solutions of dopamine and its metabolites were made in the same solution as was used for tissue homogenisation as described above. The HPLC setup was calibrated on a daily basis.

2.2.5. Statistical analyses

Statistical analysis was performed by using InStat 2.03 (GraphPad Software, San Diego, CA, USA). Two-sample *t*-tests were carried out to evaluate differences and p values ≤ 0.05 were considered to be statistically significant. Where mean values were calculated, the standard deviation (S.D.) are indicated by error bars on the graphs.

3. Results

3.1. Neuroprotection study

Using HPLC with electrochemical detection, the striatal dopamine content in the vehicle control mice were found to

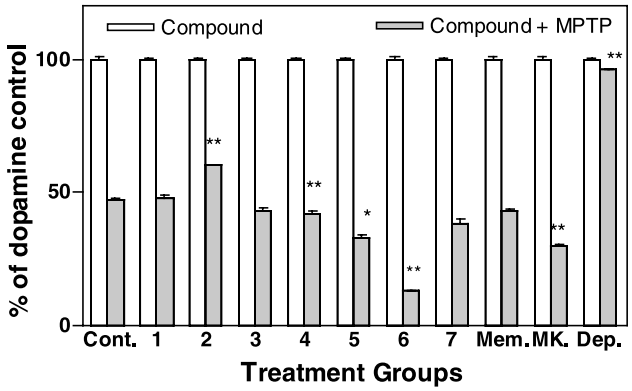


Fig. 2. Striatal dopamine levels ± S.D. (pmol/mg of wet tissue) in C57BL/6 mice expressed as percentage of the (100%) control values. Treatments are abbreviated as follows: control (Cont.), compounds **1**–**7** (300 mg/kg), memantine (Mem., 0.5 mg/kg), MK-801 (MK., 0.25 mg/kg) and (*R*)-deprenyl (Dep., 4 mg/kg). Error bars (S.D.) are shown and where not visible are contained in the horizontal bar. $*p<0.05$, $**p<0.001$, compared to animals treated with only MPTP.

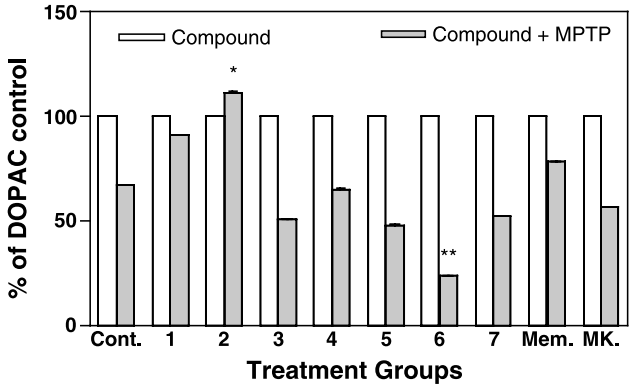


Fig. 3. Striatal DOPAC levels ± S.D. (pmol/mg of wet tissue) in C57BL/6 mice expressed as percentage of the (100%) control values. Treatments are abbreviated as follows: control (Cont.), compounds **1**–**7** (300 mg/kg), memantine (Mem., 0.5 mg/kg), MK-801 (MK., 0.25 mg/kg) and (*R*)-deprenyl (Dep., 4 mg/kg). Error bars (S.D.) are shown and where not visible are contained in the horizontal bar. $*p<0.05$, $**p<0.001$, compared to animals treated with only MPTP.

be 49.64 ± 1.10 (S.D.) pmol/mg wet tissue (100%). The striatal dopamine in the MPTP only treated mice were 23.50 ± 0.82 pmol/mg wet tissue or 47.33% of vehicle control levels, a decrease in striatal dopamine which is consistent with data reported in the literature (Gerlach and Riederer, 1996; Van der Schyf et al., 2000). The two NMDA receptor antagonists used as reference compounds—MK-801 (0.25 mg/kg) and memantine (5 mg/kg)—did not exhibit any protection against MPTP-induced striatal dopamine depletion (Fig. 2). The striatal dopamine content in mice treated with **2** plus MPTP, and with (*R*)-deprenyl plus MPTP, were elevated compared to animals treated with MPTP only (Fig. 2; $p<0.05$ for **2**; $p<0.001$ for (*R*)-deprenyl). (*R*)-deprenyl treated mice in particular exhibited almost total protection against MPTP-induced dopamine depletion. In contrast, the striatal dopamine content was significantly lower in mice treated with **4**, **5**

Table 2
Effects of cage and reference compounds on murine striatal dopamine, DOPAC, and homovanillic acid contents 10 days after MPTP treatment

Treatment	Dopamine (pmol/mg wet tissue)	DOPAC (pmol/mg wet tissue)	Homovanillic acid (pmol/mg wet tissue)
Vehicle (oil)	49.64 ± 1.10	3.82 ± 0.12	14.06 ± 1.79
MPTP + oil	23.50 ± 0.82**	2.56 ± 0.12	9.12 ± 0.34
1	49.80 ± 0.63	3.53 ± 0.19	11.73 ± 1.06^{+}
1 + MPTP	25.06 ± 0.72	3.21 ± 0.15	8.28 ± 0.26
2	43.44 ± 0.62^{++}	3.62 ± 0.11	10.09 ± 1.39^{+}
2 + MPTP	26.11 ± 1.90**	4.02 ± 0.75*	8.96 ± 2.06**
3	49.10 ± 0.78	3.95 ± 0.11	12.21 ± 1.05^{++}
3 + MPTP	20.97 ± 1.11	2.03 ± 0.10	10.25 ± 0.81
4	59.33 ± 0.71^{++}	4.30 ± 0.14	8.27 ± 0.94^{++}
4 + MPTP	25.08 ± 0.71**	2.81 ± 0.10	4.02 ± 1.05**
5	55.38 ± 0.77^{+}	6.10 ± 0.14^{++}	7.66 ± 1.57^{++}
5 + MPTP	18.77 ± 1.12*	2.93 ± 0.15	8.35 ± 1.88**
6	48.94 ± 0.68	4.50 ± 0.15	12.14 ± 0.44
6 + MPTP	6.32 ± 1.83**	1.07 ± 0.01**	10.08 ± 0.57
7	55.36 ± 1.83	5.48 ± 0.17^{++}	11.10 ± 1.40^{+}
7 + MPTP	21.16 ± 0.88	2.85 ± 0.25	3.84 ± 0.13*
Memantine	49.10 ± 1.06	4.03 ± 0.09	1.87 ± 0.03^{++}
Memantine + MPTP	21.01 ± 1.83	3.14 ± 0.15	5.56 ± 1.14**
MK-801	59.11 ± 1.13^{+}	4.14 ± 0.20	4.39 ± 0.14^{++}
MK-801 + MPTP	18.02 ± 0.42**	2.36 ± 0.09	5.50 ± 1.05**
(*R*)-deprenyl	48.14 ± 0.69	–	–
(*R*)-deprenyl + MPTP	46.06 ± 0.50**	–	–

Values are expressed as mean ± S.D. $*p<0.05$, $**p<0.01$, compared with MPTP + oil group. $^{+}p<0.05$, $^{++}p<0.01$, compared with vehicle control + saline group. $n=10$ mice. Drug treatment schedules are described in the text.

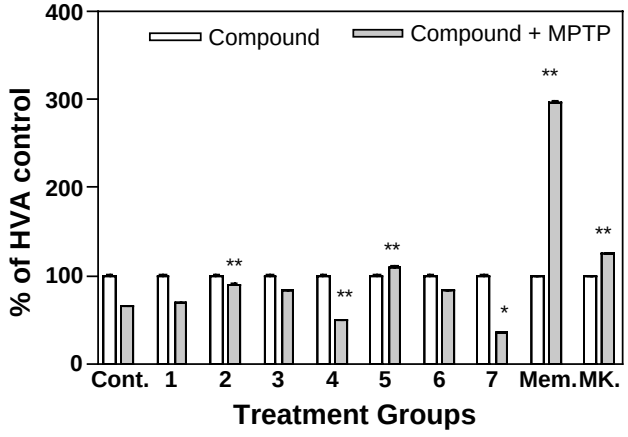


Fig. 4. Striatal homovanillic acid (HVA) levels ± S.D. (pmol/mg of wet tissue) in C57BL/6 mice expressed as percentage of the (100%) control values. Treatments are abbreviated as follows: control (Cont.), compounds **1**–**7** (300 mg/kg), memantine (Mem., 0.5 mg/kg), MK-801 (MK., 0.25 mg/kg) and (*R*)-deprenyl (Dep., 4 mg/kg). Error bars (S.D.) are shown and where not visible are contained in the horizontal bar. $*p<0.05$, $**p<0.001$, compared to animals treated with only MPTP.

and MK-801 plus MPTP, respectively, compared to MPTP only treated animals ($p < 0.05$). Mice treated with **6** plus MPTP exhibited a near total depletion of striatal dopamine ($p < 0.001$).

Interestingly, animals treated with **4**, **5**, **7** and MK-801 exhibited an increase in striatal dopamine content (Table 2) compared to vehicle treated animals, and a comparison of these values showed significant difference ($p < 0.05$) except for **7**.

In all "test compound plus MPTP treated mice" groups, striatal DOPAC levels showed substantially more variation than striatal dopamine levels (Fig. 3). A significant increase in striatal DOPAC was only seen in mice treated with **2** plus MPTP ($p < 0.05$) while decreased levels were seen in mice treated with **6** plus MPTP ($p < 0.001$), both groups compared to animals treated with MPTP only.

Striatal homovanillic acid content were significantly increased in mice treated with **2**, **5**, memantine and MK-801 plus MPTP, respectively, with the memantine plus MPTP group showing a ~ 300% increase in striatal homovanillic acid content, compared to MPTP treated animals only (Fig. 4). Decreased levels of striatal homovanillic acid were found in mice treated with **4** and **7** plus MPTP, respectively.

The ratios of DOPAC/dopamine and homovanillic acid/dopamine were assigned as an index of dopamine turnover (Bennazzouz et al., 1995). For the DOPAC/dopamine ratio (Table 3), all animals treated with the test compounds and MPTP exhibited an increase in turnover, compared to animals treated with the compounds only, with **6** showing the largest increase in DOPAC/dopamine turnover. A similar increase was seen with homovanillic acid/dopamine ratios. This ratio was increased in all animals in the "test compounds plus MPTP" group. Animals treated with compound **6** plus MPTP showed the highest homovanillic acid/dopamine ratio increase of all.

Table 3
Effects of cage and reference compounds on murine striatal dopamine turnover 10 days after MPTP treatment

Treatment	DOPAC/dopamine	HVA[a]/dopamine
Vehicle (oil)	0.08	0.34
MPTP + oil	0.11	0.39
1	0.07	0.24
1 + MPTP	0.13	0.34
2	0.08	0.23
2 + MPTP	0.23	0.34
3	0.08	0.25
3 + MPTP	0.10	0.49
4	0.07	0.14
4 + MPTP	0.11	0.16
5	0.11	0.14
5 + MPTP	0.16	0.44
6	0.09	0.25
6 + MPTP	0.17	1.59
7	0.10	0.20
7 + MPTP	0.13	0.18
Memantine	0.08	0.04
Memantine + MPTP	0.15	0.26
MK-801	0.07	0.07
MK-801 + MPTP	0.13	0.30

[a] HVA = homovanillic acid.

4. Discussion

Within this group of pentacyclo-undecylamines screened in the MPTP parkinsonian mouse model, only the phenylethyl derivative (**2**) attenuated MPTP-induced striatal dopamine depletion. The degeneration of dopaminergic neurons by MPTP is initiated by the accumulation of the oxidised metabolite MPP^+. MPP^+ is actively accumulated into dopaminergic neurons by means of the dopamine transporter and is then accumulated into the mitochondria where it inhibits respiration (Speciale, 2002). Compounds that block this uptake of MPP^+ may therefore prevent the degeneration of dopaminergic neurons. Besides possible NMDA receptor channel modulation and L-type calcium channel block, such an action may offer one possible explanation for the increased extraneuronal striatal dopamine content of mice treated with **2** and MPTP. The activity of **2** and other related polycyclic cage compounds on the dopamine reuptake system is currently under investigation in our laboratory and will be reported soon.

In sharp contrast, mice treated with compound **6** plus MPTP had a pronounced reduction in striatal dopamine concentration. This reduced striatal dopamine concentration came as a surprise because of the favourable RMS-fit of **6** on memantine ($R^2 = 0.084$), suggesting similar interactions at pharmacologically relevant sites. The reduced striatal dopamine content may suggest increased dopamine turnover as indicated by the increased DOPAC/dopamine and homovanillic acid/dopamine ratios when compared with MPTP only treated animals (Table 3). This effect might be due to an initial increase in dopamine release in the striatum, which could explain the increase in striatal homovanillic acid levels, with homovanillic acid being a product of both intra- and extraneuronal metabolism of released dopamine (Yehuda, 2002). Alternatively, up-regulation of the dopamine transporter could provide arguments for a mechanism that suggests a synergistic interaction between the toxin MPP^+ and **6** that may result in dopamine levels lower than those found in MPTP only treated animals (Kirby et al., 1999).

The striatal dopamine content in mice treated with **4**, **5**, **7** and MK-801 were increased compared to animals treated with vehicle only, although the DOPAC/dopamine was similar and homovanillic acid/dopamine levels for these compounds were less than for vehicle control. This effect might also be due to dopamine release.

In mice treated with only the polycyclic compounds, striatal DOPAC/dopamine ratios were found to be similar (Table 3) to vehicle-treated control. In contrast, the striatal DOPAC/dopamine ratios found in animals treated with the compounds plus MPTP were higher compared to animals

treated with MPTP only. The homovanillic acid/dopamine ratios of animals treated with compound only and those treated with compound and MPTP were lower in both groups compared to vehicle control. It would seem that the DOPAC/dopamine ratio tends to elevate in animals treated with the cage compounds in the presence of MPTP. Similar tendencies were seen for DOPAC/dopamine and homovanillic acid/dopamine ratios in striata of animals treated with MK-801 and memantine.

MK-801 is one of the most potent and selective NMDA receptor antagonists available and has been used as a neuroprotectant to decrease MPTP/MPP^+ toxicity in animals (Turski et al., 1991; Wenk et al., 1995). In contrast, other reports suggest MK-801 failed to protect against the striatal toxicity caused by systemic administration of MPTP in mice (Sonsalla et al., 1992; Araki et al., 2001). Much of the evidence for the efficacy of NMDA receptor antagonists against MPTP-induced striatal dopamine depletion is thus contradictory. In the current study, the NMDA receptor antagonists (memantine, MK-801) showed no significant attenuation of MPTP-induced dopamine depletion in the mouse striatum. However, increased striatal DOPAC (for memantine) and homovanillic acid (for memantine and MK-801) concentrations were observed. The same effect on striatal dopamine metabolite concentration without any change in the striatal dopamine concentration was also observed by Araki et al. (2001) in mice co-treated with MK-801 and MPTP.

We were surprised to note the ~300% increase in striatal homovanillic acid content in mice treated with memantine plus MPTP. The striatal DOPAC content also increased in this cohort, although it was not statistically significant. In the same group, the striatal dopamine content did not differ significantly from those seen in the MPTP-treated control animals. This effect was still present 10 days after MPTP treatment. Such an increase in homovanillic acid might arguably be the result either of an increase in intra- and extraneuronal metabolism of released dopamine or of a larger population of dopaminergic neurons spared. Currently, we cannot offer any explanation for this effect by memantine.

Striatal DOPAC and homovanillic acid levels in mice treated with some of the pentacyclo-undecylamines and MPTP were similar to those seen with memantine and MK-801. This may indicate similar mechanisms of action for the pentacyclo-undecylamines and these NMDA antagonists. Further studies are, however, necessary to confirm this and to determine the activity profile of the pentacyclo-undecylamines in the central nervous system. Why the NMDA receptor antagonists attenuated striatal homovanillic acid depletion but not dopamine and DOPAC also remains unclear and needs to be further investigated.

The protection that (*R*)-deprenyl, a selective monoamine oxidase B (MAO-B) inhibitor afforded to mice co-exposed to MPTP, confirms the important role of MAO-B in MPTP-induced striatal dopamine depletion. This finding is supported by other reports that have shown inhibitors of MAO-B to protect C57BL/6 mice against the neurotoxicity of MPTP (Di Monte et al., 1997). In the current study, only (*R*)-deprenyl displayed any significant protection against MPTP-induced striatal dopamine depletion under the experimental conditions used. It still needs to be determined whether compound **2**, with marginal protection against striatal dopamine depletion, possesses MAO-B inhibitory activity.

The failure of the two known NMDA receptor antagonists to protect against MPTP induced neurotoxicity in mice during the current study suggests that the standard MPTP parkinsonian mouse model may be inadequate to screen mechanistically for neuroprotection based on NMDA receptor antagonism. The pentacyclo-undecanes screened in this study can therefore not be dismissed as being totally devoid of neuroprotective activity, and the similarity in the activity profiles of these compounds and the known NMDA receptor antagonists as well as the effects these compounds on dopamine turnover and release needs to be further investigated in other parkinsonian models.

Acknowledgements

The financial assistance of the National Research Foundation (NRF) of South Africa towards this research is hereby acknowledged.

References

Alexi, T., Borlongan, C.V., Faull, R.L.M., Williams, C.E., Clark, R.G., Gluckman, P.D., Huges, P.E., 2000. Neuroprotective strategies for basal ganglia degeneration: Parkinson's disease and Huntington's disease. Prog. Neurobiol. 60, 409–470.

Araki, T., Kumagai, T., Tanaka, K., Matsubara, M., Kato, H., Itoyama, Y., Imai, Y., 2001. Neuroprotective effect of riluzole in MPTP-treated mice. Brain Res. 918, 176–181.

Bennazzouz, A., Boraud, T., Dubédat, P., Boireau, A., Stutzman, J., Gross, C., 1995. Riluzole prevents MPTP-induced parkinsonism in the rhesus monkey: a pilot study. Eur. J. Pharmacol. 284, 299–307.

Bigge, C.F., 1993. Structural requirements for the development of potent *N*-methyl-aspartic acid (NMDA) receptor antagonists. Biochem. Pharmacol. 45, 1547–1561.

Brouillet, E., Beal, M.F., 1993. NMDA antagonists partially protect against MPTP induced neurotoxicity in mice. NeuroReport 4, 387–390.

Castagnoli, K.P., Steyn, S.J., Petzer, J.P., Van der Schyf, C.J., Castagnoli Jr., N., 2001. Neuroprotection in the MPTP parkinsonian C57BL/6 mouse model by a compound isolated from tobacco. Chem. Res. Toxicol. 14, 523–527.

Chan, P., Langston, J.W., Di Monte, D.A., 1993. MK-801 temporarily prevents MPTP-induced acute dopamine depletion and MPP^+ elimination in the mouse striatum. J. Pharmacol. Exp. Ther. 267, 1515–1520.

Cookson, R.C., Grundwell, E., Hudec, J., 1985. Synthesis of cage-like molecules by irradiation of Diels–Alder adducts. Chem. Ind. (Lond.), 1003–1004.

Di Monte, D.A., Royland, J.E., Anderson, A., Castagnoli, K., Castagnoli Jr., N., Langston, J.W., 1997. Inhibition of monoamine oxidase contributes to the protective effects of 7-nitroindazole against MPTP neurotoxicity. J. Neurochem. 69, 1771–1773.

Gerlach, M., Riederer, P., 1996. Animal model of Parkinson's disease: an empirical comparison with phenomenology of the disease in man. J. Neural Transm. 103, 987–1041.

Greene, J.G., Greenamyre, J.T., 1996. Bioenergetics and glutamate excitotoxicity. Progr. Neurochem. 48, 613–634.

Harwood, L.M., Moody, C.J., Percy, J.M., 1999. Experimental Organic Chemistry, 2nd ed. Blackwell, Oxford.

Heikkila, R.E., Siber, B.A., Mazino, L., Sonsalla, P.K., 1989. Some features of the nigrostriatal dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the mouse. Mol. Chem. Neuropathol. 10, 171–183.

Kirby, M.L., Castagnoli, K., Bloomquist, J.R., 1999. In vivo effects of deltamethrin on dopamine neurochemistry and the role of augmented neurotransmitter release. Pestic. Biochem. Physiol. 65, 160–168.

Kobayashi, T., Mori, Y., 1998. Ca^{2+} channel antagonists and neuroprotection from cerebral ischemia. Eur. J. Pharmacol. 363, 1–15.

Kornhuber, J., Weller, M., 1997. Psychotogenicity and *N*-methyl-D-aspartate receptor antagonism: implications for neuroprotective pharmacotherapy. Biol. Psychiatry 41, 135–144.

Kupsch, A., Löschmann, P.A., Sauer, H., Arnold, G., Renner, P., Pufal, D., Burg, M., Watchel, H., Ten Bruggencate, G., Oertel, W.H., 1992. Do NMDA receptor antagonists protect against MPTP-toxicity? Biochemical and immunocytochemical analysis in black mice. Brain Res. 592, 74–83.

Malan, S.F., Van der Walt, J.J., Van der Schyf, C.J., 2000. Structure–activity relationships of polycyclic amines with calcium channel blocking activity. Arch. Pharm. 333, 10–16.

Marchand, A.P., Arney, B.E., Dave, P.R., 1988. Transannular cyclizations in the pentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane-8,11-dione system: a reinvestigation. J. Org. Chem. 53, 2644–2647.

Marshall, G.B., Barry, C.D., Bosshard, H.E., Dammkoehler, R.A., Dunn, D.A., 1979. The conformational parameter in drug design: the active analog approach. In: Olson, E.C., Christofferson, R.E. (Eds.), Computer-Assisted Drug Design. Am. Chem. Soc., Washington, DC, pp. 205–226.

Oliver, D.W., Dekker, T.G., Snyckers, F.O., 1991a. Pentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecylamines. Synthesis and pharmacology. Eur. J. Med. Chem. 26, 375–379.

Oliver, D.W., Dekker, T.G., Snyckers, F.O., Fourie, T.G., 1991b. Synthesis and biological activity of D_3-trishomocubyl-4-amines. J. Med. Chem. 34, 851–854.

Polak, E., 1971. Computational Methods in Optimisation, A Unified Approach. Academic Press, New York.

Sonsalla, P.K., Zeevalk, G.D., Mazino, L., Giovanni, A., Nicklas, W.J., 1992. MK-801 fails to protect against the dopaminergic neuropathology produced by systemic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice or intranigral 1-methyl-4-phenylpyridium in rats. J. Neurochem. 58, 1979–1982.

Speciale, S.G., 2002. MPTP. Insights into parkinsonian neurodegeneration. Neurotoxicol. Teratol. 24, 607–620.

Turski, L., Bressler, K., Rettig, K.J., Löschmann, P.A., Wachtel, H., 1991. Protection of substantia nigra from MPP^+ neurotoxicity by *N*-methyl-D-aspartate antagonists. Nature 349, 414–418.

Van der Schyf, C.J., Squier, G.J., Coetzee, W.A., 1986. Characterisation of NGP1-01, an aromatic polycyclic amine, as a calcium antagonist. Pharmacol. Res. 18, 407–417.

Van der Schyf, C.J., Castagnoli, K., Palmer, S., Hazelwood, L., Castagnoli Jr., N., 2000. Melatonin fails to protect against long-term MPTP-induced dopamine depletion in mouse striatum. Neurotox. Res. 1, 261–269.

Venero, J.L., Santiago, M., Machado, A., Cano, J., 1996. MK-801 partially protects against the acute MPP^+ depleting effects on dopamine levels in rat striatal slices. Neurochem. Int. 29, 411–416.

Wenk, G.L., Danysz, W., Mobley, S.L., 1995. MK-801, memantine and amantadine show neuroprotective activity in the nucleus basalis magnocellularis. Eur. J. Pharm., Environ. 293, 267–270.

Yahr, M.D., 1987. *R*-(−)-deprenyl and parkinsonism. J. Neural Transm., Suppl. 25, 5–12.

Yehuda, S., 2002. Possible anti-Parkinson properties of *N*-(α-linolenoyl) tyrosine, a new molecule. Pharmacol. Biochem. Behav. 72, 7–11.

Zuddas, A., Oberto, G., Vaglini, F., Fascetti, F., Fornai, F., Corsini, G.U., 1992. MK-801 prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in primates. J. Neurochem. 59, 733–739.