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Inhibition by memantine of the development of persistent oral dyskinesias induced by long-term haloperidol treatment of rats

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1 Tardive dyskinesia (TD) is a serious side-effect of long-term treatment with neuroleptics. To investigate if neuroleptic-induced excessive stimulation of striatal glutamate receptors may underlie TD development, the effect of the NMDA antagonist, memantine (1-amino-3,5-dimethyladamantane), was studied in a rat model of TD.

2 In an acute experiment, six groups of rats were treated daily for 1 week with either vehicle or memantine 20 or 40 mg kg^{-1} day^{-1}, and on the seventh day they received one injection of either haloperidol 1.0 mg kg^{-1} i.p. or saline i.p. In a subsequent long-term experiment lasting 20 weeks, the same treatment was continued, except that haloperidol was injected i.m. as decanoate (38 mg kg^{-1} every 4 weeks) and control rats received sesame oil. The behaviour was videotaped and scored at intervals during both experiments, and for 16 weeks after cessation of the long-term treatment.

3 In the acute experiment, haloperidol decreased motor activity and memantine increased moving and tended to attenuate the immobility induced by haloperidol. Memantine also enhanced the haloperidol-induced increase in the putative TD-analogue vacuous chewing movements (VCM).

4 In the long-term experiment, the most marked effect of haloperidol was a gradual increase in VCM and the increase persisted significantly for 12 weeks after cessation of treatment. Memantine dose-dependently increased VCM and moving during long-term treatment. However, only one week after stopping treatment, both these effects of memantine disappeared. In contrast to rats previously treated with haloperidol alone, rats co-treated with memantine (both doses) and haloperidol had VCM at the level of controls two weeks after stopping treatment. The blood levels of drugs were within the therapeutic range achieved in human subjects.

5 These results suggest that long-lasting changes induced by haloperidol are prevented by memantine, which supports the theory that excessive NMDA receptor stimulation may be a mechanism underlying the development of persistent VCM in rats and maybe also TD in human subjects.

Keywords: Neuroleptics; memantine (1-amino-3,5-dimethyladamantane); vacuous chewing movements; motor activity; tardive dyskinesia; glutamate; NMDA antagonist

Introduction

Tardive dyskinesia (TD) is a serious motor side-effect of long-term treatment with neuroleptics. The hyperkinetic movement disorder is characterized by involuntary choreoathetotic movements predominantly of the face, mouth and tongue, but a variety of less frequent motor abnormalities of the rest of the body may occur (Kane, 1992). The syndrome has a delayed onset, and in most patients the movements subside after drug-withdrawal, but it may also persist. Because of its occurrence of approximately 20% in neuroleptic-treated subjects and its potential irreversibility, TD is a major clinical and ethical issue in psychiatry (APA, 1992).

The pathophysiology of TD is still unknown, but brain damage and aging are prominent risk-factors (Waddington, 1989; Jeste & Caligiuri, 1993). This, together with the potential persistent character of the disorder, might indicate that long-lasting cerebral changes, probably located in the basal ganglia (Miller & Chouinard, 1993), are involved in the pathogenesis of TD. It has been suggested that TD might be a result of neuroleptic-induced excitotoxic changes in striatal neurones (McGeer & McGeer, 1976; DeKeyser, 1991; Andreassen & Jørgensen, 1994). This is supported by findings in rats of histopathological changes in striatum after long-term neuroleptic treatment (Jeste *et al.*, 1992), haloperidol (Hal)-induced increase in striatal glutamate release (Moghaddam & Bunney, 1993; Yamamoto & Cooperman, 1994), and Hal-induced morphological changes in striatum consistent with enhanced glutamatergic activity (Meshul *et al.*, 1994). In addition, clinical and *in vitro* studies have shown that classical neuroleptics inhibit mitochondrial function (Gallagher *et al.*, 1965; Byczkowski & Borysewicz, 1979; Burckhardt *et al.*, 1993), which may lead to increased susceptibility to excitotoxic damage (Beal *et al.*, 1993).

In a rat model of TD, in which the putative TD analogue vacuous chewing movements (VCM) were induced with long-term Hal treatment, it was shown that GM1 ganglioside, an inhibitor of excitotoxicity, attenuated the development of VCM (Andreassen & Jørgensen, 1994). These results suggest that excitotoxic mechanisms may be involved in the development of Hal-induced VCM, possibly by means of an excessive activation of the NMDA receptor. This led us to investigate the effect of NMDA receptor blockade on the development of Hal-induced VCM.

The major problems with long-term treatment with many NMDA antagonists are the serious behavioural side-effects (Willets *et al.*, 1990). However, the NMDA antagonist, memantine (Mem), is used clinically in the treatment of Parkinson's disease and spasticity and is well tolerated (Weseman *et al.*, 1983; Schneider *et al.*, 1984). It is a non-competitive, open channel blocker of the NMDA-receptor cation channel (Bormann, 1989; Chen *et al.*, 1992; Parsons *et al.*, 1993; Mistry *et al.*, 1995), which protects against excitotoxic neurodegeneration (reviewed in Kornhuber *et al.*, 1994). Mem is proposed as a candidate for long-term neuroprotection in chronic neurodegenerative diseases (Lipton, 1993; Rogawski, 1993).

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To study the effect of Mem in the rat model of TD, Mem and Hal were administered in a 20 week long-term experiment where VCM and the general behaviour were observed in unrestrained rats. In addition, the acute behavioural effects of Mem and Hal were studied.

Methods

Animals

Female Sprague-Dawley rats (Mol:SPRD, Møllegaard, Denmark) weighing 225–260 g at the start and 270–310 g at the end of the experiment were used. The rats were housed 3 in each cage with free access to water. In order to limit weight gain, the food was restricted to 15 g of pellets per animal per day. The light phase lasted from 08 h 00 min to 20 h 00 min. Ambient temperature was 22–24°C. The animals were treated according to the guidelines of the Norwegian Committee for Experiments on Animals.

Drugs

In the acute experiment the animals were injected with commercially available haloperidol (Hal; Janssen) 1.0 mg kg^{-1} i.p., control rats were injected with an equal volume of saline (sal) i.p.. In the long-term experiment the animals were injected with Hal decanoate (Janssen) 38 mg kg^{-1} i.m. every fourth week, control rats were injected i.m. with equal volumes of the vehicle sesame oil (Oil; Nycomed Pharma, Norway). The rats were hand-held with minimal restraint during the injections. Memantine (1-amino-3,5-dimethyladamantane; Mem; Merz, Germany) was prepared in the rat chow (Altromin, Germany) in two concentrations and administered to the rats twice daily (1/3 of the food at 07 h 00 min and 2/3 of the food at 16 h 00 min). The consumption of the food lasted 2–3 h and 3–5 h for the two meals, respectively. The rats were weighed every 14 days, and the concentration of Mem in the diet was adjusted to keep the two doses of Mem close to 20 mg kg^{-1} day^{-1} (Mem20) and 40 mg kg^{-1} day^{-1} (Mem40) throughout the treatment period. The half-life of Mem in rats is approximately 12 h during oral administration (Barnes *et al.*, 1996).

Behavioural observations

Before the experiment started, the animals were handled and habituated to the behaviour observation situation. The behaviour of the rats was videotaped in the animal room. During videotaping, the rats were kept in a clear perspex cage (25 × 11 × 13 cm) placed in an observation chamber equipped with mirrors allowing videotaping of the rat from all sides simultaneously. The behaviour of the animals was videotaped for 3 min after 1 min of adaptation of the cage.

A trained observer, unaware of the treatment of the rats, scored the behaviour while watching the videotapes. Using a computer with specially designed recording software, the observer scored and analysed the following behavioural categories: VCM (single, purposeless mouth openings in the vertical plane, with or without tongue protrusion, not scored during grooming), jerking (sudden muscular movements, which may involve different parts of the body), jaw tremor (high frequency fasciculations of the mouth or jaw), immobility, sitting/standing (no movement of the trunk), moving (horizontal movement of the whole body), rearing and grooming. For the first two behavioural categories, the numbers were counted. For the other categories the durations were recorded.

Protocol

Before the acute experiment took place, baseline behaviour was recorded. Then the animals were randomly assigned to six groups ($n = 15$ per group), each being treated with one of the following combinations: Mem20 + Hal, Mem40 + Hal, vehicle + Hal (Hal alone), Mem20 + Sal, Mem40 + Sal, or vehicle + Sal (Sal alone). Mem was administered in the food for 7 days before Hal or Sal were injected i.p., and the behaviour of the rats was videotaped 1 h after the i.p. injections. The rats were videotaped in a systematic order, thereby the time from the morning feeding of the Mem diet was the same in each treatment group (mean = 5 h, range = 3.5–6.5 h).

After the acute experiment the animals entered the long-term experiment in which they were treated for 20 weeks. Each of the six groups was treated as in the acute experiment, with the exception that Hal was administered i.m. as decanoate every four weeks (last injection week 16) and the controls (Oil alone) received Oil i.m. every four weeks. The Mem-containing diet was administered twice daily in the whole 20 weeks treatment period. Videorecording of the behaviour was performed with intervals of 2–4 weeks during both the long-term treatment period and during the 16 weeks post-treatment period.

Drug analysis

An additional group of rats, not included in the behaviour analysis, was used for pharmacokinetic analysis. Five h after the morning feeding on the seventh day of Mem administration, rats receiving Mem20 and Mem40 ($n = 3$ per group) were anaesthetized with halothane gas. Then, after transcardial puncture, blood samples (5–10 ml) were collected and the brains were removed and frozen at −20°C until analysis. The same procedure was used when blood was collected 1 h after i.p. injection of Hal 1.0 mg kg^{-1} ($n = 3$) and Hal 0.1 mg kg^{-1} ($n = 2$). Blood was also collected after 3 weeks of Hal alone treatment ($n = 2$), and after 7 weeks of treatment with Mem20 + Oil, Hal alone and Mem20 + Hal ($n = 6$ per group). The blood samples for Mem analysis were collected in EDTA containing tubes, and after 30 min at room temperature the samples were centrifuged, the plasma (2–5 ml) was transferred to plastic tubes and frozen at −20°C until analysis. The same procedure was used for Hal analysis, except that tubes without EDTA were used, and serum was separated. After extraction, Mem was analysed with a gas-chromatography system coupled to a mass selective detector (Misztal *et al.*, 1996). For the analysis of Hal, trimipramine was used as internal standard. Serum (2.0 ml) was transferred to a polypropylene tube and mixed with 400 μl of 1 M Na_2CO_3 and 100 μl internal standard. Extraction was performed by adding 8 ml n-hexane:acetonitrile (98.2, v/v) and then rotating the samples for 10 min. Thereafter the samples were centrifuged at 3000 r.p.m. for 5 min followed by shaking and then centrifuged for another 10 min. The tubes were then placed in liquid nitrogen, after which the organic layer was transferred to a new tube and evaporated at 37°C to dryness in a heat block. The residue was reconstituted into 200 μl of the eluent mixture, and an aliquot of 100 μl was injected. Analytical grade solvents and reagents were used. The h.p.l.c. system consisted of a pump (Shimadzu LC-6A), an autoinjector (Shimadzu SIL-9A), an integrator (Shimadzu C-R5A) and a u.v. detector (Shimadzu SPD-6A) operated at a wavelength of 225 nm. The column (15 cm × 4.6 mm i.d.) was a Supelcosil LC-PCN with a particle size of 5 μm. The eluent consisted of 1 mM phosphate buffer at pH 7.0: acetonitrile (25 : 75 v/v). Flow was 2.0 ml min^{-1}. The lower limit of detection of Hal was under these conditions 3–4 nmol l^{-1}.

Statistics

The overall effects of Hal and Mem were analysed by two-way analysis of variance (ANOVA; Mem, Hal) in the acute experiment, and with repeated measures ANOVA (three-way: Mem, Hal, time) in the long term experiment. Differences between treatment groups were analysed with Newman-Keuls test subsequent to ANOVA. Group differences were analysed either at single time-points, or in the periods from week 4 to week 20 during long-term treatment and from week 1 to week

12 after cessation of treatment. Due to the non-homogeneity of variance between the treatment groups, the behavioural data were log-transformed ($X' = \log(X+1)$) before statistical calculations. Student's t test was used in the analysis of the serum level measurements.

Results

Before treatment was started no differences in behaviour were observed between the treatment groups (Figure 2 and 3, other results not shown).

The acute experiment

One h after the i.p. injection, Hal strongly affected the behaviour (Figure 1). The main effect was a marked reduction in motor activity, shown by the significant overall increase in sitting/standing (Hal: $F(1,84)=34.6$, $P<0.001$) and immobility (Hal: $F(1,84)=24.7$, $P<0.001$), and an overall decrease in moving (Hal: $F(1,84)=23.1$, $P<0.001$) and rearing (Hal: $F(1,84)=60.7$, $P<0.001$). Together with the decrease in motor activity, Hal significantly increased both VCM (Hal: $F(1,84)=176,7$; $P<0.001$) and jaw tremor (Hal: $F(1,84)=7.4$, $P<0.01$). Hal also significantly increased jerking (Hal: $F(1,84)=19.9$, $P<0.001$), but did not significantly influence grooming. The effect of 1 week of Mem pretreatment was less than and contrary to the effect of Hal (Figure 1). Mem tended to increase the general motor activity, and the main effect was a significant increase in moving (Mem: $F(2,84)=4.2$, $P<0.05$). Mem tended to attenuate the Hal-induced increase in immobility, but this did not reach significance. No significant effects of Mem were found on sitting/standing, rearing, jaw tremor, jerking or grooming. There was a tendency for Mem to increase VCM in Hal-treated rats, but the overall Mem effect did not reach significance (Mem: $F(2,84)=2.9$, $P=0.06$).

The long-term experiment

The number of VCM during long-term treatment are shown in Figure 2. Hal significantly increased VCM (Hal: $F(1,83)=17.9$, $P<0.001$), and the effect became stronger during treatment (Hal × time: $F(7,581)=2.3$, $P<0.05$). The Hal alone group did not show significantly more VCM than the control group until weeks 16 and 20 (both time-points: $P<0.05$, Newman-Keuls test). Mem also significantly increased VCM (Mem: $F(2,83)=3.8$, $P<0.05$), but this effect was mostly seen when Mem was co-administered with Hal, and the interaction was nearly significant (Mem × Hal: $F(2,83)=2.4$, $P=0.098$). The VCM scores of the Mem20 + Oil and Mem40 + Oil groups were not significantly different from the control group at any time point, or in the whole week 4–20 period (Newman-Keuls test). The other type of oral movements, jaw tremor, was significantly increased by Hal (Hal: $F(1,83)=5.7$, $P<0.05$, data not shown), and Mem decreased the amount of jaw tremor when it was co-administered with Hal (Mem × Hal: $F(2,83)=3.9$, $P<0.05$, data not shown). The Hal alone group showed significantly more jaw tremor than the control group in the whole week 4–20 period ($P<0.01$, Newman-Keuls test, data not shown).

The general motor activity was less affected in the long-term experiment than in the acute experiment. However, the effects on moving were relatively strong, which is illustrated in Figure 3. Hal induced a significant increase in moving (Hal: $F(1,83)=11.9$, $P<0.001$), but this effect was only present when Hal was co-administered with Mem (Hal × Mem, $F(2,83)=3.7$, $P<0.05$). The Hal alone group did not show a significantly different amount of moving compared to the control group at any time-point, or in the whole week 4–20 period (Newman-Keuls test). Mem dose-dependently and highly significantly increased moving (Mem: $F(2,83)=26.9$, $P<0.001$), and the Mem effect increased with duration of treatment (Mem × time: $F(14,581)=3.0$, $P<0.001$). Rearing was significantly decreased by Mem (Mem: $F(2,83)=5.6$, $P<0.05$, data not shown), but

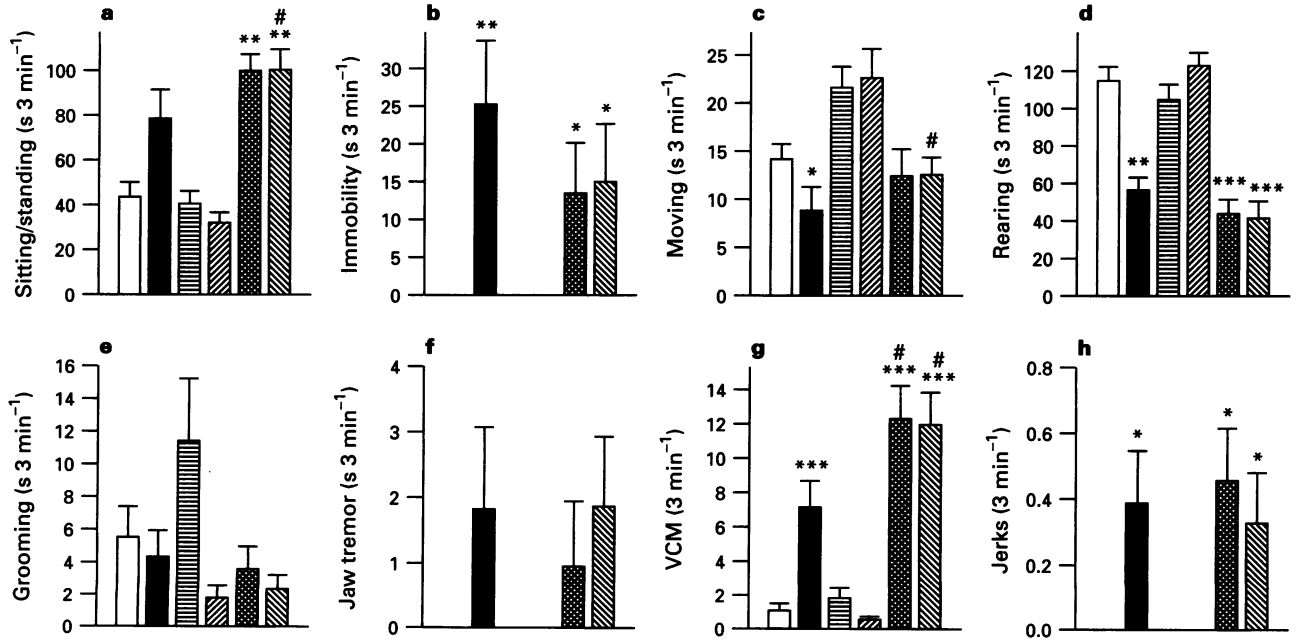

Figure 1 Acute experiment. Duration ($s\,3\,min^{-1}$) of sitting or standing (a), immobility (b), moving (c), rearing (d), grooming (e) and jaw tremor (f) and number per 3 min ($3\,min^{-1}$) of vacuous chewing movements (VCM; g) and jerks (h), 1 h after the i.p. injections of haloperidol or saline at the seventh day of pretreatment with memantine diet. Values are presented as means ± s.e.mean. Vehicle diet + saline i.p. (open columns), vehicle diet + haloperidol $1.0\,mg\,kg^{-1}$ i.p. (solid columns), memantine $20\,mg\,kg^{-1}\,day^{-1}$ diet + saline i.p. (horizontally lined columns), memantine $40\,mg\,kg^{-1}\,day^{-1}$ diet + saline i.p. (left diagonally hatched columns), memantine $20\,mg\,kg^{-1}\,day^{-1}$ diet + haloperidol $1.0\,mg\,kg^{-1}$ i.p. (cross-hatched columns), memantine $40\,mg\,kg^{-1}\,day^{-1}$ diet + haloperidol $1.0\,mg\,kg^{-1}$ i.p. (right diagonally hatched columns). $n=15$ per group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. control group; $\#P<0.05$ vs. haloperidol group (Newman-Keuls test).

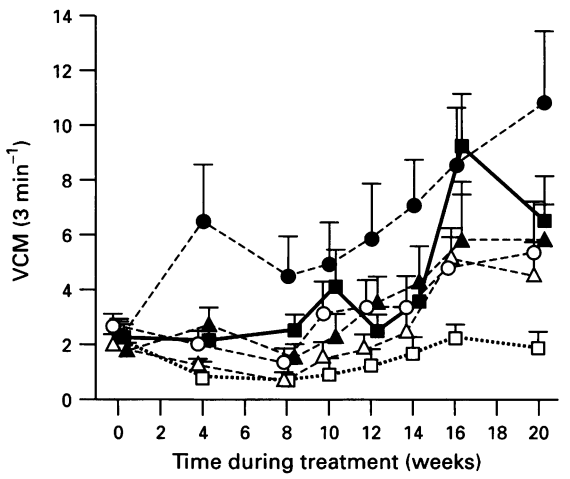

Figure 2 Number of vacuous chewing movements (VCM) for 3 min during treatment in the long-term experiment (last i.m. injection of haloperidol/sesame oil in week 16). Values are presented as means ± s.e.mean. Vehicle diet + sesame oil i.m. (□, dotted line), vehicle diet + haloperidol 38 mg kg^{-1} every 4 weeks i.m. (■, continuous line), memantine 20 mg kg^{-1} day^{-1} diet + sesame oil i.m. (△, broken line), memantine 40 mg kg^{-1} day^{-1} diet + sesame oil i.m. (○, broken line), memantine 20 mg kg^{-1} day^{-1} diet + haloperidol 38 mg kg^{-1} every 4 weeks i.m. (▲, broken line), memantine 40 mg kg^{-1} day^{-1} diet + haloperidol 38 mg kg^{-1} every 4 weeks i.m. (●, broken line). $n = 15$ per group. Hal effect: $F(1,83) = 17.9$, $P < 0.001$; Mem effect: $F(2,83) = 3.8$, $P < 0.05$; Mem × Hal effect: $F(2,83) = 2.4$, $P < 0.10$ (repeated measures ANOVA).

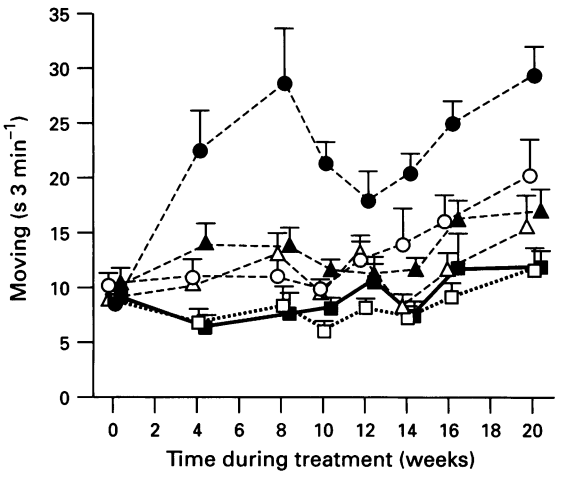

Figure 3 Duration of moving (s 3 min^{-1}) during treatment in the long-term experiment (last i.m. injection of haloperidol/sesame oil in week 16). Values are presented as means ± s.e.mean. Vehicle diet + sesame oil i.m. (□, dotted line), vehicle diet + haloperidol 38 mg kg^{-1} every 4 weeks i.m. (■, continuous line), memantine 20 mg kg^{-1} day^{-1} diet + sesame oil i.m. (△, broken line), memantine 40 mg kg^{-1} day^{-1} diet + sesame oil i.m. (○, broken line), memantine 20 mg kg^{-1} day^{-1} diet + haloperidol 38 mg kg^{-1} every 4 weeks i.m. (▲, broken line), memantine 40 mg kg^{-1} day^{-1} diet + haloperidol 38 mg kg^{-1} every 4 weeks i.m. (●, broken line). $n = 15$ per group. Hal effect: $F(1,83) = 11.9$, $P < 0.001$; Mem effect: $F(2,83) = 26.9$, $P < 0.001$; Hal × Mem effect, $F(2,83) = 3.7$, $P < 0.05$ (repeated measures ANOVA).

this dose-dependent reduction was probably secondary to the strong increase in moving. No other types of behaviour was significantly affected by Mem (data not shown). Hal tended to decrease the general motor activity, shown by a significant increase in sitting/standing (Hal: $F(1,83) = 6.2$, $P < 0.05$; data not shown). Hal also significantly increased grooming (Hal: $F(1,83) = 12.8$, $P < 0.001$; data not shown), but no other overall-effects of Hal were found for the other categories of behaviour.

The long-term experiment after cessation of treatment

The VCM recordings after cessation of treatment are shown in Figure 4. Similar to the effect of Hal during treatment, a significant Hal-induced increase in VCM was present in the 12 first weeks after stopping treatment (Hal: $F(1,85) = 19.1$, $P < 0.001$). This effect, however, was only present in rats not previously co-treated with Mem. The post-treatment effect of Mem was a significant reduction in VCM in week 1–12 after drug withdrawal (Mem: $F(1,85) = 7.5$, $P < 0.01$), which was contrary to the effect during treatment. The interaction between Hal and Mem was significant (Hal × Mem: $F(1,85) = 4.3$, $P < 0.05$), showing that Mem inhibited the development of Hal-induced persistent VCM. There were no differences in the effects of the two doses of Mem after termination of treatment, and these groups were pooled in the statistical anslysis. The Hal alone group demonstrated significantly more VCM compared to each of the other groups in the whole week 1–12 post-treatment period and at post-treatment week 12 ($P < 0.05$–0.001, Newman-Keuls test). There was a significant persistent increase in jaw tremor in rats that had previously received Hal treatment (Hal: $F(1,83) = 6.3$, $P < 0.05$, data not shown). No significant effects of Mem were present for jaw tremor.

The drug effects on the general motor activity were much weaker after stopping treatment than during treatment. The strong Mem-induced increase in moving observed during treatment disappeared completely during the first week after cessation of treatment (Figure 5), and except for the decrease

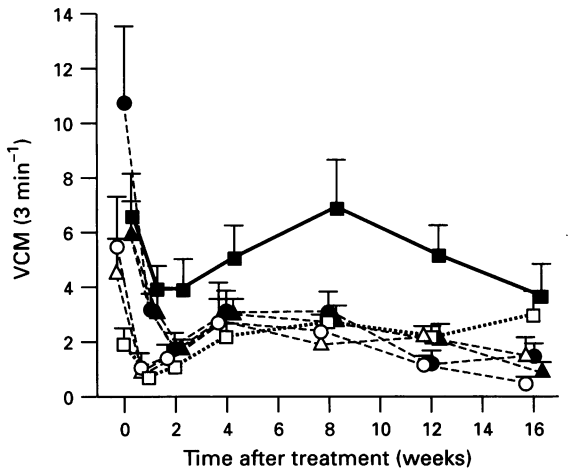

Figure 4 Number of vacuous chewing movements (VCM) during 3 min after cessation of treatment in the long-term experiment (week 0 = week 20 during treatment). Values are presented as means ± s.e.mean. Rats previously treated with vehicle diet + sesame oil i.m. (□, dotted line), vehicle diet + haloperidol 38 mg kg^{-1} every 4 weeks i.m. (■, continuous line), memantine 20 mg kg^{-1} day^{-1} diet + sesame oil i.m. (△, broken line), memantine 40 mg kg^{-1} day^{-1} diet + sesame oil i.m. (○, broken line), memantine 20 mg kg^{-1} day^{-1} diet + haloperidol 38 mg kg^{-1} every 4 weeks i.m. ▲, (broken line), memantine 40 mg kg^{-1} day^{-1} diet + haloperidol 38 mg kg^{-1} every 4 weeks i.m. (●, broken line). $n = 15$ per group. Hal effect: $F(1,85) = 19.1$, $P < 0.001$; Mem effect: $F(1,85) = 7.5$, $P < 0.01$; Hal × Mem effect: $F(1,85) = 4,3$ $P < 0.05$ (post-treatment weeks 1–12, repeated measures ANOVA).

in VCM, no significant behavioural effects of Mem was present after stopping treatment. In the 12 weeks post-treatment period, there was a small but significant effect of previous Hal treatment on rearing, which was reduced (Hal: $F(1,83) = 4.0$, $P < 0.05$, data not shown), and grooming, which was increased

(Hal: $F(1,83)=12.7$, $P<0.001$, data not shown). For the other categories of behaviour, no significant effects of previous Hal treatment were found.

Drug levels

The results of the drug analysis are shown in Table 1. After 7 weeks of treatment, no significant difference in Hal serum level was found in the Hal alone compared to the Hal + Mem20 group (Student's *t* test).

Discussion

The main findings of the present study were that 20 weeks of Hal treatment induced an increase in VCM which persisted for 12 weeks after stopping treatment, and that co-treatment with Mem clearly inhibited the development of persistent VCM.

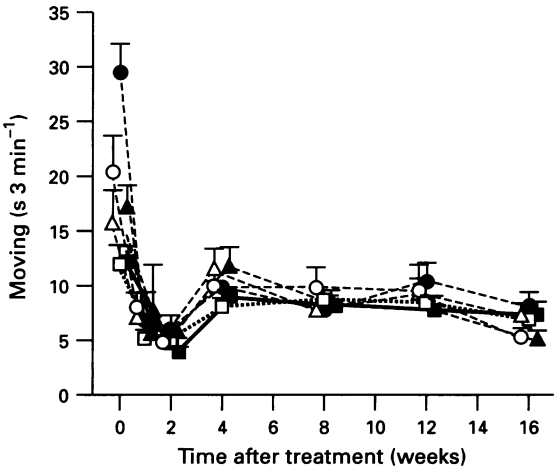

Figure 5 Duration of moving (s 3 min^{-1}) after cessation of treatment in the long-term experiment (week 0 = week 20 during treatment). Values are presented as means ± s.e.mean. Rats previously treated with vehicle diet + sesame oil i.m. (□, dotted line), vehicle diet + haloperidol 38 mg kg^{-1} every 4 weeks i.m. (■, continuous line), memantine 20 mg kg^{-1} day^{-1} diet + sesame oil i.m. (△, broken line), memantine 40 mg kg^{-1} day^{-1} diet + sesame oil i.m. (○, broken line), memantine 20 mg kg^{-1} day^{-1} diet + haloperidol 38 mg kg^{-1} every 4 weeks i.m. (▲, broken line), memantine 40 mg kg^{-1} day^{-1} diet + haloperidol 38 mg kg^{-1} every 4 weeks i.m. (●, broken line). $n=15$ per group. Hal effect: NS.; Mem effect: NS.; Hal × Mem effect: NS (post-treatment weeks 1–12, repeated measures ANOVA).

The Hal-induced increase in VCM and a persistent high level of VCM after cessation of neuroleptic treatment has been found earlier (Waddington *et al.*, 1983; Gunne *et al.*, 1986; Andreassen & Jørgensen, 1994; Egan *et al.*, 1994), and the accompanying increase in jaw tremor is also in accordance with earlier results (Andreassen & Jørgensen, 1995b). The locomotor effects of Mem are consistent with earlier findings, both regarding the acute increase in motor activity (Bubser *et al.*, 1992; Danysz *et al.*, 1994), and the attenuating effects on Hal-induced hypokinesia (reviewed in Ossowska, 1994). An increase in oral movements in rats has been reported after intrastriatal injections of NMDA antagonists (Pisa & Bosiljevac, 1994), and during ketamine anaesthesia (Marco & Joshi, 1992). This is compatible with the present Mem-induced increase in VCM, although Mem mainly increased VCM during co-treatment with Hal. Stimulation of striatal dopamine D_1 receptors induces oral movements in rats (Rosengarten *et al.*, 1983; Levin *et al.*, 1989), and this probably also contributes to the increase in VCM seen during Hal treatment. Hal blocks striatal dopamine D_2 receptors with a subsequent increase in striatal dopamine release (Imperato & Di Chiara, 1985; Westerink & de Vries, 1989), giving a net dopamine D_1 receptor stimulation. Such stimulation could be enhanced by Mem, which increases striatal dopamine release (Spanagel *et al.*, 1994). During the long-term treatment, the Mem-induced increase in moving was potentiated by Hal. This effect may be due to increased dopaminergic activity in the forebrain (Ahlenius & Hillegart, 1986). The results indicate that long-term Mem treatment is not without behavioural side-effects, especially when higher doses are used.

The behavioural effects of Mem disappeared shortly after discontinuation of treatment, indicating that no long-lasting neuronal changes were induced. Contrary to this, the Hal-induced increase in VCM persisted for 12 weeks after stopping treatment. However, previous Mem co-treatment clearly inhibited development of persistent VCM. This indicates that Mem had a protective effect against Hal-induced long-lasting changes in the brain. The lack of inhibitory effect of Mem on VCM development during treatment supports the assumption that different mechanisms may underlie transient and persisting VCM (Andreassen & Jørgensen, 1994; 1995b). Mem is a non-competitive, open channel blocker of the NMDA-receptor cation channel (Bormann, 1989; Chen *et al.*, 1992; Parsons *et al.*, 1993; Mistry *et al.*, 1995), and the protective effect of Mem demonstrated in the present study is in all probability due to its NMDA antagonism. In other studies a protective effect of Mem against NMDA receptor-mediated neurotoxicity has been found both *in vitro* and *in vivo* (reviewed by Kornhuber *et al.*, 1994). The present results indicate that excitotoxicity through stimulation of NMDA receptors is involved in Hal-induced persistent VCM. This is in accordance with a previous study; where GM1 ganglioside, which protects against ex-

Table 1 Drug levels in rats during acute and long-term experiments

	Haloperidol (Hal)	*Serum level* (nmol l^{-1})	*Memantine* (Mem)	*Plasma level* (μmol l^{-1})	*Brain level* (μmol l^{-1})
Acute expt	Hal i.p.		Mem diet		
	1.0 mg kg^{-1} ($n=3$)	143.7 ± 7.1	20 mg kg^{-1} day^{-1} ($n=3$)	0.9 ± 0.23	47.5 ± 10.0
	0.1 mg kg^{-1} ($n=2$)	8.4 ± 0.0	40 mg kg^{-1} day^{-1} ($n=3$)	1.3 ± 0.29	60.1 ± 7.2
Long-term expt	Hal decanoate i.m.		Mem diet		
	38 mg kg^{-1} every 4 weeks		20 mg kg^{-1} day^{-1}		
3 weeks	Hal alone ($n=2$)	20.5 ± 0.5			
7 weeks	Hal alone ($n=6$)	16.5 ± 1.5	Mem20 alone ($n=6$)	1.1 ± 0.14	
	Hal + Mem20 ($n=6$)	20.8 ± 3.2			

In the acute experiment, blood for haloperidol analysis was collected 1 h after i.p. injections of haloperidol and blood for memantine analysis was collected 5 h after morning feeding at the seventh day of memantine administration. In the long-term experiment, 'Hal alone' received haloperidol decanoate, 'Hal + Mem20' received haloperidol deconoate and memantine 20 mg kg^{-1} day^{-1}, and 'Mem20 alone' received memantine 20 mg kg^{-1} day^{-1}. The blood samples were collected 3 weeks after the last i.m. injection of haloperidol decanoate or vehicle, 5 h after the morning feeding of memantine. Data are presented as means ± s.e.mean.

citotoxicity without blocking glutamate receptors (Favaron *et al.*, 1988; Manev *et al.*, 1990), attenuated the development of Hal-induced persistent VCM (Andreassen & Jørgensen, 1994). These results support the hypothesis that TD might be a result of neuroleptic-induced excitotoxic changes in the striatum (McGeer & McGeer, 1976; DeKeyser, 1991; Andreassen & Jørgensen, 1994). Other recent studies, where long-term Hal treatment of rats increased the striatal glutamate release (Moghaddam & Bunney, 1993; Yamamoto & Cooperman, 1994) and induced morphological striatal changes indicating increased glutamate activity (Meshul *et al.*, 1994), may also support this position. Interestingly, histological signs of striatal degeneration have been found in rats receiving long-term neuroleptic treatment and in TD patients (reviewed in Miller & Chouinard, 1993). Classical neuroleptics inhibit the energy metabolism (Burkhardt *et al.*, 1993), and recently signs of impaired energy metabolism were found in CSF of TD-patients (Goff *et al.*, 1995). It might therefore be speculated that impaired energy metabolism, which increases the susceptibility to excitotoxicity (Beal *et al.*, 1993), may enhance the risk for neurotoxic effects of the increased striatal glutamate release during long-term neuroleptic treatment (Andreassen & Jørgensen, 1995a). This is consistent with the fact that aging, which is related to decay of mitochondrial function (Shigenaga *et al.*, 1994), is the most prominent risk-factor for TD.

During long-term treatment, the present blood levels of Mem and Hal were within the therapeutic range achieved in human subjects, and the serum level of Hal was unaffected by Mem co-treatment. This indicates that the protective effect of Mem on development of persistent VCM was not due to pharmacokinetic interactions.

To conclude, the present study showed that long-term Hal treatment induced an increase in VCM which persisted after treatment, and that co-treatment with Mem clearly inhibited the development of these Hal-induced persistent VCM. This is in accordance with earlier results (Andreassen & Jørgensen, 1994), and indicates that excessive activation of striatal NMDA receptors is involved in the development of persistent VCM in rats, and maybe in TD development in human subjects.

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References

AHLENIUS, S. & HILLEGAART, V. (1986). Involvement of extrapyramidal motor mechanisms in the suppression of locomotor activity by antipsychotic drugs: a comparison between the effects produced by pre- and post-synaptic inhibition of dopaminergic neurotransmission. *Pharmacol. Biochem. Behav.*, **24**, 1409–1415.

AMERICAN PSYCHIATRIC ASSOCIATION. (1992). Tardive dyskinesia: a Task Force Report of the American Psychiatric Association. Washington DC: APA.

ANDREASSEN, O.A. & JØRGENSEN, H.A. (1994). GM1 ganglioside attenuates the development of vacuous chewing movements induced by long-term haloperidol treatment of rats. *Psychopharmacology*, **116**, 517–522.

ANDREASSEN, O.A. & JØRGENSEN, H.A. (1995a). The mitochondrial toxin 3-nitropropionic acid induces vacuous chewing movements in rats. Implications for tardive dyskinesia? *Psychopharmacology*, **119**, 474–476.

ANDREASSEN, O.A. & JØRGENSEN, H.A. (1995b). The rat model of tardive dyskinesia: Relationship between vacuous chewing movements and gross motor activity during acute and long-term haloperidol treatment. *Life Sci.*, **57**, 2263–2272.

BARNES, C.A., DANYSZ, W. & PARSONS, C.G. (1996). Effects of uncompetitive NMDA receptor antagonist memantine on hippocampal long-term potentiation, short-term exploratory modulation and spatial memory in awake freely moving rats. *Eur. J. Neurosci.*, **8**, 565–571.

BEAL, M.F., HYMAN, B.T. & KOROSHETZ, W. (1993). Do defects in mitochondrial energy metabolism underlie the pathology of neurodegenerative diseases? *Trends Neurosci.*, **16**, 125–131.

BORMANN, J. (1989). Memantine is a potent blocker of N-methyl-D-aspartate (NMDA) receptor channels. *Eur. J. Pharmacol.*, **166**, 591–592.

BUBSER, M., KESEBERG, U., NOTZ, P.K. & SCHMIDT, W.J. (1992). Differential behavioural and neurochemical effects of competitive and non-competitive NMDA receptor antagonists. *Eur. J. Pharmacol.*, **229**, 75–82.

BURCKHARDT, C., KELLY, J.P., LIM, Y.H., FILLEY, C.M. & PARKER, W.D.JR. (1993). Neuroleptic medications inhibit complex I of the electron transport chain. *Ann. Neurol.*, **33**, 512–517.

BYCZKOWSKI, J.Z. & BORYSEWICZ, R. (1979). The action of chlorpromazine and imipramine on rat brain mitochondria. *Gen. Pharmacol.*, **10**, 369–372.

CHEN, H.S.V., PELLEGRINI, J.W., AGGARWAL, S.K., LEI, S.Z., WARACH, S., JENSEN, F.E. & LIPTON, S.A. (1992). Open channel block of N-Methyl-D-Aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. *J. Neurosci.*, **12**, 4427–4436.

DANYSZ, W., ESSMANN, U., BRESINK, I. & WILKE, R. (1994). Glutamate antagonists have different effects on spontaneous locomotor activity in rats. *Pharmacol. Biochem. Behav.*, **48**, 111–118.

DEKEYSER, J. (1991). Excitotoxic mechanisms may be involved in the pathophysiology of tardive dyskinesia. *Clin. Neuropharmacol.*, **14**, 562–565.

EGAN, M.F., HURD, Y., HYDE, T.M., WEINBERGER, D.R., WYATT, R.J. & KLEINMAN, J.E. (1994). Alterations in mRNA levels of D2 receptors and neuropeptides in striatonigral and striatopallidal neurons of rats with neuroleptic-induced dyskinesias. *Synapse*, **18**, 178–189.

FAVARON, M., MANEV, H., ALHO, H., BERTOLINO, M., FERRET, B., GUIDOTTI, A. & COSTA, E. (1988). Gangliosides prevent glutamate and kainate neurotoxicity in primary neuronal cultures of neonatal rat cerebellum and cortex. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 7351–7355.

GALLAGHER, C.H., KOCH, J.H. & MANN, D.M. (1965). The effect of phenothiazine on the metabolism of liver mitochondria. *Biochem. Pharmacol.*, **14**, 789–797.

GOFF, D.C., TSAI G., BEAL, M.F. & COYLE, J.T. (1995). Tardive dyskinesia and substrates of energy metabolism. *Am. J. Psychiatry*, **152**, 1730–1736.

GUNNE, L.M., ANDERSSON, U., BONDESSON, U. & JOHANSSON, P. (1986). Spontaneous chewing movements in rats during acute and chronic antipsychotic drug administration. *Pharmacol. Biochem. Behav.*, **25**, 897–901.

IMPERATO, A. & DI CHIARA, G. (1985). Dopamine release and metabolism in awake rats after systemic neuroleptics as studied by trans-striatal dialysis. *J. Neurosci.*, **5**, 297–306.

JESTE, D.V. & CALIGIURI, M.P. (1993). Tardive dyskinesia. *Schizophr. Bull.*, **19**, 303–315.

JESTE, D.V., LOHR, J.B. & MANLEY, M. (1992). Study of neuropathological changes in the striatum following 4, 8 and 12 months of treatment with fluphenazine in rats. *Psychopharmacology*, **106**, 154–160.

KANE, J.M. (1992). Tardive dyskinesia. In *Movement Disorders in Neurology and Neuropsychiatry*, ed. Joseph, A.B. & Young, R.R. pp. 33–39. Oxford: Blackwell.

KORNHUBER, J., WELLER, M., SCHOPPMEYER, K. & RIEDERER, P. (1994). Amantadine and memantine are NMDA receptor antagonists with neuroprotective properties. *J. Neural Transm.*, (Suppl), **43**, 91–104.

LEVIN, E.D., SEE, R.E. & SOUTH, D. (1989). Effects of dopamine D1 and D2 receptor antagonists on oral activity in rats. *Pharmacol. Biochem. Behav.*, **34**, 43–48.

LIPTON, S.A. (1993). Prospects for clinically tolerated NMDA antagonists: open channel blockers and alternative redox states of nitric oxide. *Trends. Neurosci.*, **16,** 527–532.

MANEV, H., COSTA, E., WROBLEWSKI, J.T. & GUIDOTTI, A. (1990). Abusive stimulation of excitatory amino acid receptors: a strategy to limit neurotoxicity. *FASEB J.*, **4,** 2789–2797.

MARCO, L.A. & JOSHI, R.S. (1992). A ketamine-induced rat model of tardive dyskinesia. *Prog. Neurobiol.*, **38,** 571–600.

MCGEER, E.G. & MCGEER, P.L. (1976). Duplication of biochemical changes of Huntington's chorea by intrastriatal injections of glutamic and kainic acids. *Nature*, **263,** 517–519.

MESHUL, C.K., STALLBAUMER, R.K., TAYLOR, B. & JANOWSKY, A. (1994). Haloperidol-induced morphological changes in striatum are associated with glutamate synapses. *Brain Res.*, **648,** 181–195.

MILLER, R. & CHOUINARD, G. (1993). Loss of striatal cholinergic neurons as a basis for tardive dyskinesia and L-dopa-induced dyskinesias, neuroleptic-induced supersensitivity psychosis and refractory schizophrenia. *Biol. Psychiatry*, **34,** 713–738.

MISTRY, R., WILKE, R. & CHALLISS, R.A.J. (1995). Modulation of NMDA effects on agonist-stimulated phosphoinositide turnover by memantine in neonatal rat cerebral cortex. *Br. J. Pharmacol.*, **114,** 797–804.

MISZTAL, M., FRANKIEWICZ, T., PARSONS, C.G. & DANYSZ, W. (1996). Learning deficits induced by chronic intraventricular infusion of quinolinic acid - protection by MK-801 and memantine. *Eur. J. Pharmacol.*, **296,** 1–8.

MOGHADDAM, B. & BUNNEY, B.S. (1993). Depolarization inactivation of dopamine neurons: terminal release characteristics. *Synapse*, **14,** 195–200.

OSSOWSKA, K. (1994). The role of excitatory amino acids in experimental models of Parkinson's disease. *J. Neural Transm.*, (P-D Sect) **8,** 39–71.

PARSONS, C.G., GRUNER, R., ROZENTAL, J., MILLAR, J. & LODGE, D. (1993). Patch clamp studies of the kinetics and selectivity of N-methyl-D-aspartate receptor antagonism by memantine (1-amino-3,5-dimethyladamantan). *Neuropharmacology*, **32,** 1337–1350.

PISA, M. & BOSILJEVAC, D. (1994). Heterogeneous motor roles of glutamate receptor subtypes in the rat's striatum. *Soc. Neurosci. Abstr.*, **20,** 1.

ROGAWSKI, M.A. (1993). Therapeutic potential of excitatory amino acid antagonists: channel blockers and 2,3-benzodiazepines. *Trends. Pharmacol. Sci.*, **14,** 325–331.

ROSENGARTEN, H., SCHWEITZER, J.W. & FRIEDHOFF, A.J. (1983). Induction of oral dyskinesias in naive rats by D1 stimulation. *Life Sci.*, **33,** 2479–2482.

SCHNEIDER, E., FISCHER, P.A., CLEMENS, R., BALZEREIT, F., FÜNFGELD, E.W. & HAASE, H.J. (1984). Effects of oral memantine on symptoms of Parkinson's disease. *Deutsche Med. Wochenzeitschr.*, **109,** 987–990.

SHIGENAGA, M.K., HAGEN, T.M. & AMES, B.N. (1994). Oxidative damage and mitochondrial decay in aging. *Proc. Natl. Acad. Sci. U.S.A.*, **91,** 10771–10778.

SPANAGEL, R., EILBACHER, B. & WILKE, R. (1994). Memantine-induced dopamine release in the prefrontal cortex and striatum of the rat - a pharmacokinetic microdialysis study. *Eur. J. Pharmacol.*, **262,** 21–26.

WADDINGTON, J.L. (1989). Schizophrenia, affective psychoses, and other disorders treated with neuroleptic drugs: The enigma of tardive dyskinesia, its neurobiological determinants, and the conflicts of paradigms. *Int. Rev. Neurobiol.*, **31,** 297–353.

WADDINGTON, J.L., CROSS, A.J., GAMBLE, S.J. & BOURNE, R.C. (1983). Spontaneous orofacial dyskinesia and dopaminergic function in rats after 6 months of neuroleptic treatment. *Science*, **220,** 530–532.

WESEMAN, W., SONTAG, K.H. & MAJ, J. (1983). On the pharmacodynamics and pharmacokinetics of memantine. *Arzneim Forsch./Drug Res.*, **33,** 1122–1134.

WESTERINK, B.H.C. & DE VRIES, J.B. (1989). On the mechanism of neuroleptic induced increase in striatal dopamine release: brain dialysis provides direct evidence for mediation by autoreceptors localized on nerve terminals. *Neurosci. Lett.*, **99,** 197–202.

WILLETS, J., BALSTER, R.L. & LEANDER, J.D. (1990). The behavioral pharmacology of NMDA receptor antagonists. *Trends Pharmacol. Sci.*, **11,** 423–428.

YAMAMOTO, B.K. & COOPERMAN, M.A. (1994). Differential effects of chronic antipsychotic drug treatment on extracellular glutamate and dopamine concentrations. *J. Neurosci.*, **14,** 4159–4166.

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