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Acute and Chronic Behavioural Sequelae Resulting From Intrastriatal Injection of an NMDA Agonist

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BLACK, M. D., A. R. CROSSMAN, A. G. HAYES AND P. J. ELLIOTT. *Acute and chronic behavioural sequelae resulting from intrastriatal injection of an NMDA agonist.* PHARMACOL BIOCHEM BEHAV **48**(2) 441–446, 1994.– Unilateral and bilateral injections of *cis*-2,4-methanoglutamate, a potent and selective NMDA agonist, were made into the striatum of rats. Unilateral injections elicited MK-801-sensitive dose-related increases in contralateral turning, beginning 10–15 min after injection. Bilateral injections elicited typical seizure-like behaviours commencing approximately 80 min postinjection. Forty-eight hours after unilateral injection, and presumably after lesion development, no spontaneous preference for turning was seen. Upon challenge with apomorphine (1 mg/kg SC), ipsilateral turning lasting approximately 60 min was seen. Correlates are drawn between this model and some of the features of Huntington's disease.

NMDA Huntington's disease Basal ganglia Rotation Open-field behaviours Lesions

HUNTINGTON'S DISEASE (HD) is an autosomal dominant inherited disorder characterised by progressive cognitive decline and involuntary movements starting in midlife (13, 14,20). Pathologically, HD is characterised by extensive neuronal loss in the striatum (caudate nucleus and putamen) and relative sparing of neurones in the rest of the brain (30). Genetic linkage studies have localised the HD gene to the short arm of chromosome 4, although the actual gene has not been identified (11).

Over the past two decades considerable attention has been focused on the role of excitatory amino acids in the pathophysiology of HD. Excitotoxins such as kainic and quinolinic acid have been injected into the striatum and found to reproduce many of the biochemical characteristics of HD (2,6, 7,21,23). Excitatory amino acid receptors can be split into four main subtypes: the NMDA, AMPA, kainate, and metabotropic receptors. Of these subtypes, the NMDA receptor in particular has been linked with the pathophysiology and the sequential degeneration of striatal efferents in HD (1,31).

Thus, there is accumulating evidence to suggest that the NMDA receptor plays a part in the neurodegenerative aspects of HD [for review see (8)]. It has also been shown that patients diagnosed with early HD often have no pathologically discernible damage (19,29) but show abnormalities in striatal receptor levels, particularly NMDA (1). Therefore evidence seems to suggest that NMDA receptor overactivity may be involved with the predegenerative symptoms as well as the later degenerative events.

Animal models, both rodent and primate, have concerned themselves with excitotoxic lesions of the striatum using kainic, quinolinic, and ibotenic acids (2,6,7,12,15,21). All the above produced extensive lesions of the striatum and are models of the later stages of HD, which are characterised by rigidity and akinesia. The chorea also associated with HD appears much earlier and often not as a result of extensive striatal degeneration.

From the above information one could propose that a compound with high selectivity for the NMDA receptor injected into the striatum would acutely mimic some of the early stage events of HD. Furthermore, in time, such a compound would cause lesions indicative of the later stages of HD. The most potent and selective NMDA agonist to date is *cis*-2,4-meth-

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anoglutamate (MG) (17). As well as having NMDA-like binding ability, this compound is known to produce MK-801-sensitive lesions of the striatum (3).

We therefore chose to investigate the acute (2 h postinjection) and longer term (48 h postinjection) behavioural consequences resulting from injection of this compound into the striatum.

METHODS

Animals

Male random-bred hooded rats (200–300 g; Glaxo, Herts, UK) housed in groups of four to six were used. Animals were kept in a 24-h light- and temperature-controlled environment with both food and water present ad lib.

Surgical Procedure

Rats were cannulated as described previously (9). Anaesthesia was induced and maintained by injection of sodium pentobarbitone (60 mg/kg IP; Sagatal, M&B, UK). The animals were placed in a stereotaxic frame and stainless steel guide cannulae (23 gauge) were implanted above the striatum. Coordinates were 0.7 mm anterior to bregma, 2.8 mm lateral to the midline, and 4 mm below the skull surface in accordance with Paxinos and Watson (24). The guides were fixed in position with screws and acrylic cement. Animals were allowed at least five days to recover before behavioural studies were started. Stylets (30-gauge stainless steel wire) were placed in the guide cannulae to keep them patent during the recovery period.

Drug Infusion

Rats were hand-held while 30-gauge injection needles were inserted down the guide cannulae into the striatum. Drug and vehicle infusions were made in freely moving animals using infusion pumps (Harvard) over a 1.0-min period. The injection needles remained in place for a further 1.0 min, after the infusion period, to allow for drug/vehicle diffusion from the needle tip. All central injections were made in 1-μl volumes. Unilateral injections were used for the rotational studies, whereas bilateral injections were performed prior to the open-field study.

Drugs

cis-2,4-Methanoglutamate (Tocris Neuramin), MK-801 (dizocilpine), and apomorphine were all dissolved in phosphate-buffered saline (pH 7.4). MK-801 (0.3 mg/kg^{-1}, IP) was given 30 min prior to the central injection, whereas apomorphine (1.0 mg/kg, SC) was injected just prior to behavioural testing.

Rotational Activity Monitoring

Subjects were placed in a close-fitting harness attached, via a vertical steel wire (0.4 mm diameter), to a transducer of a rotometer (33 cm diameter, 30 cm high). Movement of the animal/wire, in either direction, produced an electrical pulse which was recorded automatically by an on-line computer. A turn was defined as one complete 360° revolution. Studies in the rotometers were carried out between 0900 and 1700 in a quiet environment.

Open-Field Monitoring

Subsequent to a central infusion, rats were placed immediately in a novel white box (60 × 60 × 60 cm) in a quiet room for 5 min and videotaped. Subjects were then removed and returned to cages. At 40, 80, 120, and 160 min postinjection the animals were placed in the box and videotaped again. This procedure was also repeated 24 h after the initial drug/vehicle administration.

Videos were analysed at a later stage by an observer blind to the drug treatment. The behaviours analysed were locomotor activity (LMA), rearing, barrel-rolling, and tonic–clonic movements of the forelimbs. LMA was defined by the number of lines crossed as the animal moved into a different quadrant. Rearing was recorded as the total number of rears made during each test period. Barrel-rolling was recorded as the duration of time spent in this seizure-type state. Tonic–clonic activity was scored as the number of forelimb movements. Each behaviour was analysed by a computerised keypad system and summed over the 5-min epoch.

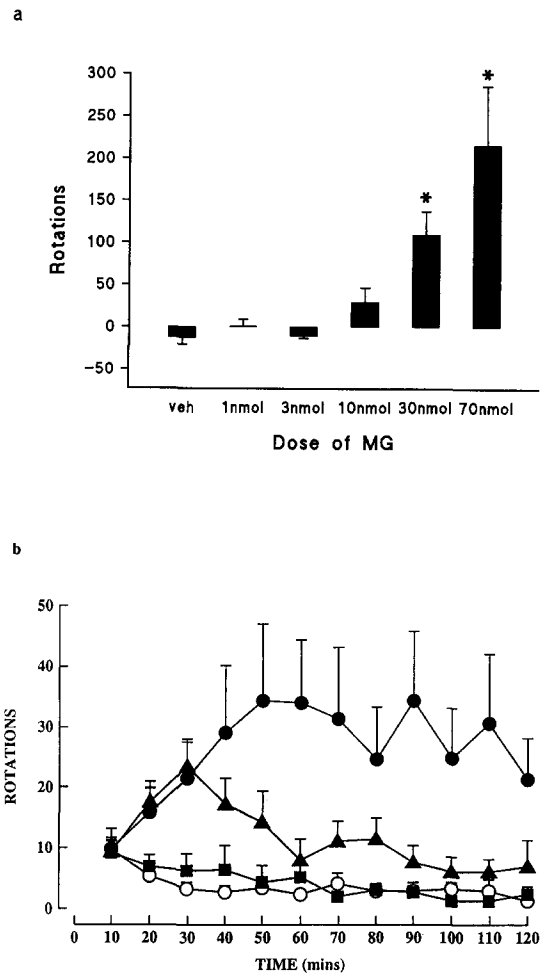


FIG. 1. (a) Dose–response relationship for contralateral rotational behaviour produced by intrastriatally injected MG. Rotations are defined as the sum of contralateral minus ipsilateral turns in 90 min post-central injection. N = 5–10. *$p < 0.05$ with respect to vehicle (see text for statisical analysis). (b) Time course of rotational behaviour produced by unilateral intrastriatal injection of MG. Rotations are defined as the number of contralateral turns every 10-min epoch. Zero denotes time of injection. ○, vehicle; ■, 10 nmol MG; ▲, 30 nmol MG; ●, 70 nmol MG. N = 7–10.

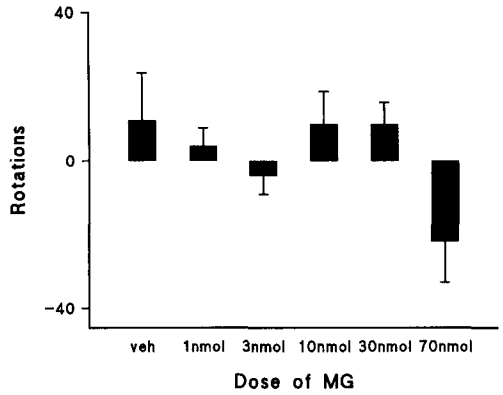


FIG. 2. Rotational behaviour of animals 48 h after injection of various doses of MG, unilaterally into the striatum. Rotations are defined as the sum of contralateral minus ipsilateral turns in 60 min. N = 4-10.

Histology and Statistics

Following each behavioural study the animals were sacrificed by decapitation and their brains dissected. Histological analysis was performed on the animals to establish correct guide cannulae placement. Data from the animals were analysed using analysis of variance (ANOVA) and a post hoc Dunnett's test. Data from the barrel-rolling and tonic-clonic experiments were analysed using a Wilcoxon-rank analysis.

RESULTS

Contralateral Turning in Response to Unilateral Intrastriatal Injection of MG

MG caused dose-related contralateral turning (see Fig. 1a). This effect began approximately 10-20 min after injection. The 30-nmol dose reached a maximum (approximately five times that of vehicle) at 30 min postinjection and declined to control levels by 90 min (Fig. 1b). The 70-nmol dose took longer to reach a maximum (approximately seven times that of vehicle) at 50-60 min, and the effect was reduced to five times that of vehicle after 2 h (Fig. 1b). None of the doses of MG had any effect on rotational activity 48 h after their initial injection (Fig. 2).

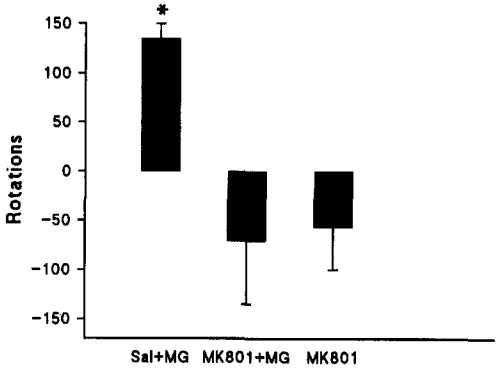
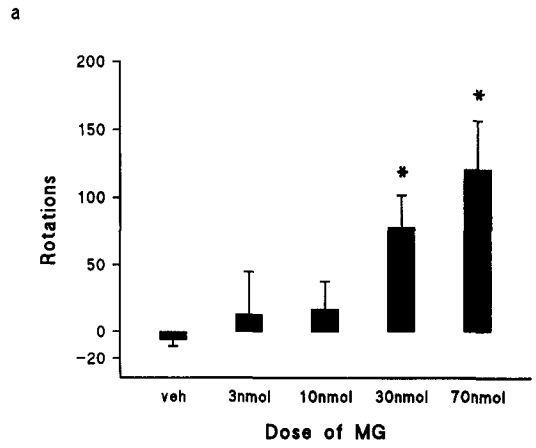


FIG. 3. Antagonism of rotational behaviour produced by unilateral intrastriatal MG by predosing with 0.3 mg/kg MK-801. MK-801 = IP injection of MK-801 only, 30 min prior to behavioural testing. MK-801 + MG = IP MK-801 30 min prior to unilateral intrastriatal injection of 30 nmol MG. SAL + MG = IP saline 30 min prior to unilateral intrastriatal injection of 30 nmol MG. N = 7. *p < 0.05 with respect to MK-801 group (see text for statistical analysis).

Effect of MK-801 on MG-Induced Turning Behaviour

In order to assess whether or not an NMDA receptor-mediated system was producing the turning behaviour, we decided to try and antagonise it with the selective NMDA channel-blocking agent MK-801. MK801 (0.3 mg/kg, IP) injected 30 min prior to the central injection caused hypermotility but no preference to contralateral or ipsilateral turning. IP saline had no effect on the turning behaviour produced by 30



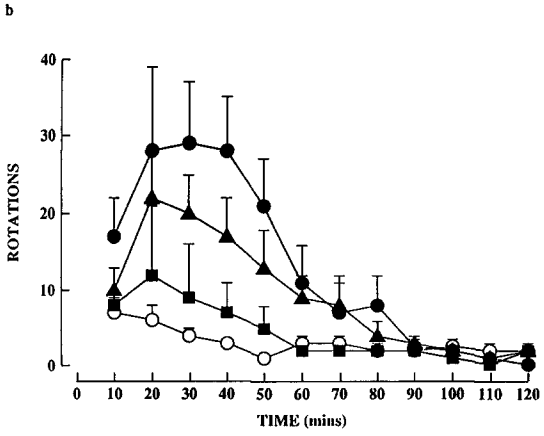


FIG. 4. Effect of apomorphine (1 mg/kg, SC) on rotational behaviour of animals previously injected unilaterally with various doses of MG, 48 h previously. (a) Graph showing that the dose of MG previously injected dictates size of rotational behaviour produced by apomorphine (see text for explanation). Rotations are defined as the sum of ipsilateral minus contralateral turns in 60 min post-central injection. (b) Time course of rotational behaviour produced by apomorphine. Rotations are defined as the number of ipsilateral turns every 10-min epoch. Zero denotes time of injection. ○, vehicle; ■, 10 nmol MG; ▲, 30 nmol MG; ●, 70 nmol MG.○, vehicle; ■, 10 nmol MG; ▲, 30 nmol MG; ●, 70 nmol MG. N = 7-9. *p < 0.05 with respect to vehicle (see text for statistical analysis).

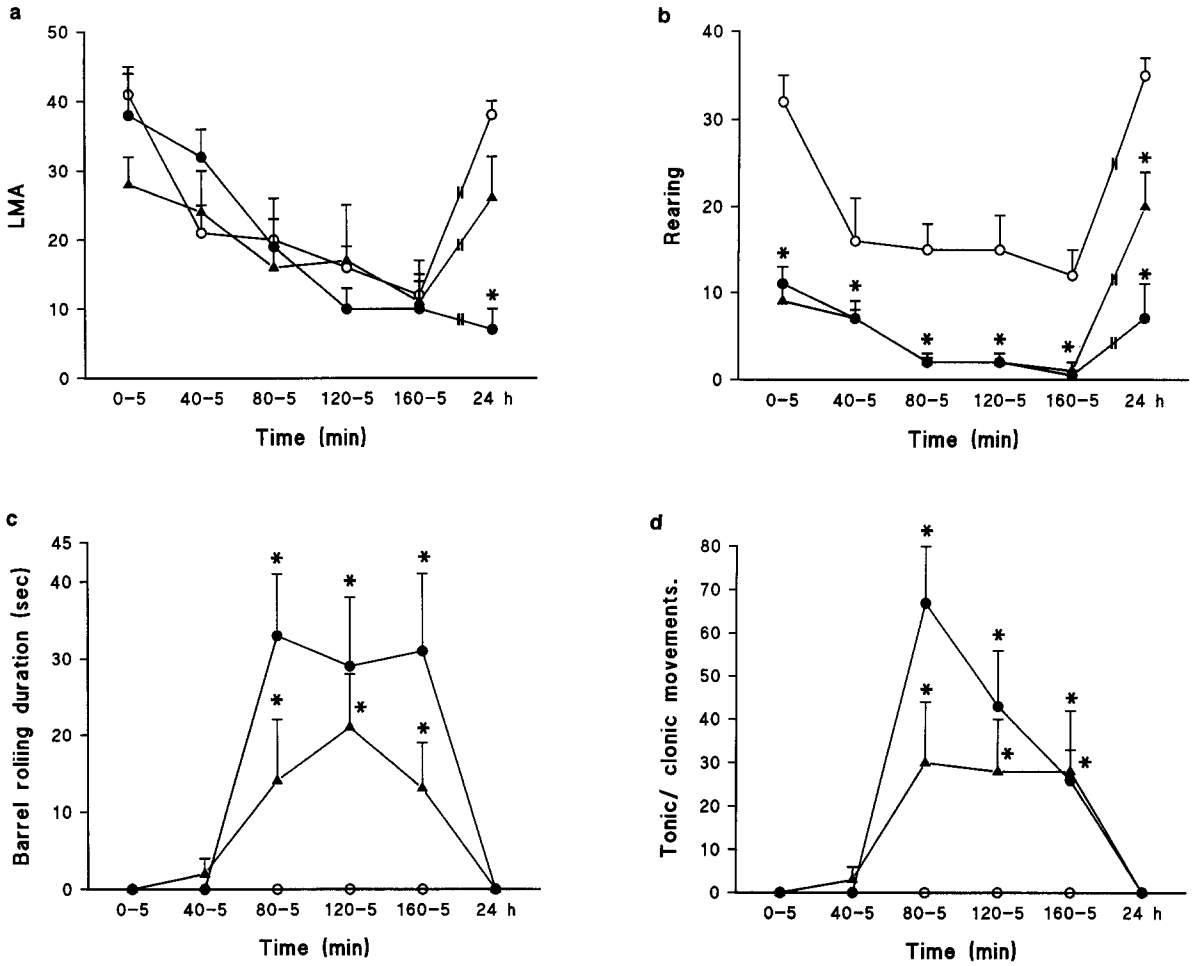


FIG. 5. Open-field behaviours produced by bilateral injection of various doses of MG into the striatum. Locomotor activity (LMA) is defined as number of line crossings, rearing as the number of rears, tonic/clonic as the number of rapid forelimb movements, and barrel-rolling as the duration of time (s) spent in that behaviour. All behaviours were made in 5-min sampling periods at set intervals. (a) Time course of LMA over the 165-min postinjection period and at 24 h. (b) Time course of rearing over the 165-min postinjection period and at 24 h. (c) Time course of barrel-rolling behaviour over the 165-min postinjection period and at 24 h. (d) Time course of tonic/clonic behaviour over the 165-min postinjection period and at 24 h. ○, vehicle; ■, 10 nmol MG; ▲, 30 nmol MG; ●, 70 nmol MG. $N = 6$. *$p < 0.05$ with respect to vehicle (see text for statistical analysis).

nmol MG. However, a predose of MK-801 decreased turning behaviour to control levels (Fig. 3).

Effect of Apomorphine Challenge, 48 h After Intrastriatal Injection of MG

It has previously been shown that MG can cause NMDA receptor-induced lesions 24 h after injection (3). To investigate whether a lesion had occurred under the present experimental conditions, we investigated the effect of apomorphine challenge on those animals injected 48 h previously with MG.

The apomorphine challenge (1 mg/kg, SC) induced a significant ipsilateral turning behaviour in those animals previously injected with 30 and 70 nmol MG (Fig. 4a). The effect took approximately 20–30 min to reach a maximum (six times that of vehicle) and lasted for up to 70 min (Fig. 4b).

Effect of a Bilateral Intrastriatal Injection of MG on Open-Field Behaviours

Only the 30- and 70-nmol doses were investigated in this study, since they produced a significant behavioural response in the rotational study.

Locomotor Activity (LMA)

MG did not have any noticeable effect on motility during the 160-min postinjection period at the doses used (Fig. 5a). The activity of all the animals decreased over the test period, as they became acclimatised to their surroundings. Twenty-four hours after the injection of MG (70 nmol) the animals exhibited a dose-related decrease in LMA, compared to the control vehicle-injected animals (Fig. 5a).

Rearing

Intrastriatal injection of both doses of MG reduced the number of rears over the 160-min test period (Fig. 5b). This effect was apparent in under 5 min. After 24 h the number of rears was still significantly decreased with respect to control values, for both groups.

Barrel-Rolling and Tonic-Clonic

Barrel-rolling and tonic-clonic movements of the forelimbs are typical responses to excessive glutamatergic stimulation.

Both behaviours appeared after 40 min postinjection and lasted for the rest of the 160-min test period (Figs. 5c and 5d). The effects were dose-dependent and significant for each dose. After 24 h both the behaviours had disappeared.

DISCUSSION

We have shown that the NMDA agonist *cis*-2,4-methanoglutamate, when infused unilaterally into the striatum of a conscious rat, induced dose-related contralateral rotations. These appear 10 min after infusion and last for a further 60–70 min. This behaviour is mediated by the NMDA receptor, since the effect was abolished with the selective NMDA antagonist MK-801.

A wealth of evidence suggests that glutamate agonists, in particular those that act at the NMDA receptor, can release dopamine from the nerve terminals in the striatum (4,5,25,26). Dopamine and basal ganglia-related movement have a long history. It is therefore plausible to allocate at least some role for the release of dopamine in the behaviours seen. Further studies using dopamine antagonists could evaluate this. Recently nitric oxide has been shown to release striatal dopamine (18,32). It is known that NMDA receptor activation can release nitric oxide [for review see (10)]; the rotational behaviour induced by MG may thus be sensitive to nitric oxide synthase inhibitors.

After 48 h the rotation induced by MG had disappeared. However, upon challenge with apomorphine, ipsilateral rotations were observed. It has been shown that MG can cause lesions at a similar dose (3). Neuronal death in just one striata would cause a receptor imbalance (including dopamine receptors). The different dopamine receptor populations between the striata probably account for the response to apomorphine. Correlates can be made between this phenomenon and the exacerbation of HD seen upon administration of dopamine agonists (16).

A series of behaviours were analysed in the open field following bilateral infusion of MG. These included seizure type effects, which are characteristic of excessive glutamatergic stimulation.

In contrast to a previous report using intrastriatal injection of NMDA (27), the doses used in the present study had no acute effect on LMA. However, after a 24-h period, and presumably after extensive lesion development (3), the group infused with 70 nmol exhibited a significant decrease in LMA. It is known that large-scale destruction of the striatum occurs in the later stages of HD and that this tends to result in rigidity and akinesia. Similar processes may be mimicked here.

Rearing was measured to give an index of the animals' exploratory behaviour and motor control. In accordance with previous reports (27,28) using excitotoxins, the number of rears was significantly decreased both acutely and at the 24-h period, indicating that the neural circuits required for the rearing behaviour are disrupted by striatal NMDA receptor-mediated activation, and subsequent striatal degeneration has a similar effect.

Barrel-rolling and tonic–clonic movements of the forelimbs were analysed to see if there was an association between the rotational behaviours seen and those typical of seizure. In this model the seizures started 40–80 min postinjection, long after the peak of the circling behaviour seen with the unilateral injections in the rotational study (approximately 20 min). It is therefore proposed that the circling behaviour and the seizure activities are distinct behaviours.

The behavioural consequences of intrastriatal injection of MG are concluded to be mediated by the NMDA receptor. The involvement of dopamine in the transmission of the resultant behaviours is implicated. Correlates between this model and some of the features of HD can be made.

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