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Effects of 7-nitroindazole, N^G-nitro-L-arginine, and D-CPPene on harmaline-induced postural tremor, *N*-methyl-D-aspartate-induced seizures, and lisuride-induced rotations in rats with nigral 6-hydroxydopamine lesions

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Abstract

The present behavioral study was undertaken to investigate whether neuronal nitric oxide (NO) synthase mediates the abnormal consequences of increased NMDA receptor-mediated synaptic transmission in models of postural tremor, Parkinson's disease and epilepsy. We used 7-nitroindazole, a selective inhibitor of neuronal NO synthase, and N^G-nitro-L-arginine (L-NAME), an unspecific NO synthase inhibitor, and compared their action with that of the competitive NMDA receptor antagonist 3-[(*R*)-2-carboxypiperazin-4-yl]-prop-2-enyl-1-phosphonic acid (D-CPPene). In both mice and rats, 7-nitroindazole, L-NAME and D-CPPene dose dependently reversed the harmaline-induced increase of cerebellar cyclic guanosine-5'-monophosphate (cGMP) levels. For subsequent behavioral experiments we used doses of 7-nitroindazole, L-NAME and D-CPPene which were equipotent in preventing harmaline-induced cGMP increase. Harmaline-induced tremor in mice and rats was suppressed by D-CPPene, but not by 7-nitroindazole or by L-NAME. This effect of D-CPPene was not due to unspecific suppression of motor activity, since D-CPPene did not affect locomotor activity at doses which reduced tremor. D-CPPene, but not 7-nitroindazole and L-NAME potentiated the antiparkinsonian action of the dopamine agonist lisuride in rats with unilateral 6-hydroxydopamine lesions of the substantia nigra. D-CPPene antagonized seizures induced by intracerebroventricular injection of NMDA in mice. In contrast, 7-nitroindazole and L-NAME had only a tendency to prevent seizures and to delay the latency to onset of seizures. We conclude from these results that neuronal NO synthase does not serve as a major mediator of increased NMDA receptor-mediated synaptic transmission in animal models of Parkinson's disease, postural tremor and epilepsy. The novel observation that D-CPPene suppresses harmaline-induced tremor leads us to suggest that NMDA receptor antagonists should be considered as novel therapeutics for postural tremor.

Keywords: Nitric oxide (NO); 7-Nitroindazole; Harmaline; NMDA (*N*-methyl-D-aspartate); Parkinsonism; Epilepsy

1. Introduction

Stimulation of the NMDA L-glutamate receptor subtype activates neuronal nitric oxide (NO) synthase leading to increased formation of NO (Garthwaite et al., 1988; Knowles et al., 1989). NO serves as a messenger molecule mediating the physiological actions of L-glutamate. However, NO may also have neurotoxic actions by inhibiting a number of enzymes including complex I of the mitochondrial respiration chain, and giving rise to the formation of highly reactive free radicals (Beckman et al., 1990; Stadler et al., 1991).

The principal intracellular action of NO is activation of soluble guanylate cyclase, which leads to the formation of cyclic guanosine-5'-monophosphate (cGMP) (Arnold et al., 1977). The discovery that NO activates guanylate cyclase prompted extensive studies in the cerebellum, as the cere-

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bellum contains the highest levels of cGMP in the central nervous system. NO is formed in cerebellar granule cell cultures following activation of NMDA receptors, leading to enhanced formation of cGMP (Garthwaite et al., 1988). Similarly, NO has been found to mediate the effects of NMDA receptor activation on cerebellar cGMP levels in brain slices and in vivo (Bredt and Snyder, 1989; Wood et al., 1990). In addition, both NMDA and NO antagonists prevent the rise of cerebellar cGMP induced by the tremorogenic compound harmaline (Wood et al., 1990). Harmaline is a β-carboline which causes inferior olive neurons to fire synchronously and to act as a pacemaker for the generation of tremor (Llinas and Yarom, 1986). The harmaline-induced increase of cerebellar cGMP levels is presumably due to activation of the glutamatergic climbing fiber pathway from the inferior olive to the cerebellar cortex.

The discovery that NO serves as a mediator of the actions of NMDA receptor agonists on cerebellar cGMP has led to the hypothesis that there is close association of NMDA receptor- and NO-mediated processes. Enhanced NMDA receptor-mediated synaptic transmission plays a crucial role in the pathophysiology of a number of neurological diseases associated with abnormal motor behavior, such as Parkinson's disease, epilepsy and possibly also postural tremor (Croucher et al., 1982; Meldrum, 1987; Schmidt and Bubser, 1989; Klockgether and Turski, 1990; Löschmann et al., 1991). It is not known whether the consequences of NMDA receptor activation under these pathological conditions are mediated by NO. Studies on the role of NO in the control of normal and abnormal behavior have been hampered by the lack of specific antagonists of neuronal NO synthase. Arginine analogues, such as N^G-nitro-L-arginine (L-NAME) are not suitable for behavioral experimental studies because they inhibit the activity of both endothelial and neuronal NO synthase and cause pronounced increases of arterial blood pressure (Dwyer et al., 1991). Recently, however, a novel NO synthase inhibitor, 7-nitroindazole, has been described and is a selective inhibitor of neuronal NO synthase in vivo (Babbedge et al., 1993; Moore et al., 1993a, b). We have undertaken the present study to investigate whether NMDA receptor-dependent behaviors are mediated by activation of neuronal NO synthase. In particular, we wished to study whether (1) NMDA receptor antagonists and 7-nitroindazole suppress harmaline-induced tremor, (2) whether 7-nitroindazole shares antiparkinsonian actions of NMDA receptor antagonists, and (3) whether 7-nitroindazole prevents NMDA-induced seizures. To this end we compared the action of 7-nitroindazole with that of the unspecific NO synthase inhibitor L-NAME and the competitive NMDA receptor antagonist 3-[(*R*)-2-carboxypiperazin-4-yl]-prop-2-enyl-1-phosphonic acid (D-CPPene). To select appropriate doses for the behavioral experiments we performed a series of ex vivo experiments in which we measured cerebellar cGMP levels.

2. Materials and methods

2.1. Animals

Experiments were performed in male NMRI mice (28–35 g; Interfauna, Tuttlingen, Germany) and male Wistar rats (biochemical and tremor experiments: 250–300 g; rotational experiments: 400–590 g; Interfauna). Animals were housed under standard conditions at a temperature of 22 ± 1°C and under a 12 h light-dark cycle (light on from 6.00–18.00 h). They had free access to food and water. Experiments were carried out between 9.00 and 17.00 h. All animal experiments were done in accordance with animal protection guidelines and laws of the Federal Republic of Germany and had been approved by a regional Committee on Animal Care.

2.2. Drug preparations

The following drugs were used: 7-nitroindazole (Lancaster, Mühlheim/Main, Germany), N^G-nitro-L-arginine (L-NAME; RBI, Natick, MA, USA), 3-[(*R*)-2-carboxypiperazin-4-yl]-prop-2-enyl-1-phosphonic acid (D-CPPene; Sandoz, Basel, Switzerland), apomorphine (Sandoz), amphetamine (Sigma, St. Louis, MO, USA), lisuride (Schering, Berlin, Germany), NMDA (Tocris, Buckhurst Hill, Essex, UK), harmaline (Fluka, Ulm, Germany), pargyline (Sigma) and 6-hydroxydopamine (Sigma). 7-Nitroindazole was dissolved in normal saline containing 10% (v/w) polyethoxylated castor oil (Cremophor EL, Fluka, Ulm, Germany). NMDA was brought into solution with a minimum quantity of 1 N NaOH, and the final volume was made up with saline solution. The pH was adjusted to 7.4. 6-Hydroxydopamine was dissolved in saline solution containing 0.02% ascorbic acid (Sigma). All other drugs were dissolved in normal saline. Dosages refer to the free form (base, acid). Drugs were administered intraperitoneally if not stated otherwise.

2.3. Measurement of cerebellar cGMP

Harmaline was given to mice and rats 15 min after treatment with either vehicle or active drug. Animals were killed by cervical dislocation 15 min after treatment with harmaline. The cerebella were quickly removed and homogenized in an ice-cold 6% (w/v) aqueous solution of trichloro-acetic acid using an ultrasonic cell disrupter. Extraction of cGMP followed the protocol from Steiner et al. (1972). Briefly, the homogenates were centrifuged at 2500 × *g* for 15 min, the supernatants were extracted three times with water-saturated ether, and the extracts were vacuum-dried at 60°C overnight. For cGMP detection, a commercial radioimmuneassay kit was used (DuPont, Bad Homburg, Germany). Probes were redissolved in 1.5 ml assay buffer and 100 μl aliquots were used in the assay. cGMP values were expressed as nmol/mg protein. Protein determinations were done using the method of Lowry et al. (1951) modified by Petersen (1977).

2.4. Harmaline-induced tremor

Harmaline was given to mice and rats 15 min after treatment with either vehicle or active drug. The occurrence of tremor was rated by an observer blinded to the treatment over a period of 60 min at 5-min intervals.

2.5. Locomotor activity

Mice were randomly assigned to treatment with vehicle and active drug. Animals were placed singly without prior acclimatisation onto the floor of circular cages (30 cm diamater) equipped with a second inner cylinder (10 cm diameter) which formed a circular runway (10 cm wide). Horizontal movements were registered automatically by six infrared sensitive photocells per cylinder (Elektro- und Elektronik Anlagen, Berlin, Germany). The number of interruptions giving a measure of locomotion (counts) was accumulated at 10-min intervals and stored for individual sensors on a personal computer system for subsequent analysis. The system allowed testing of 8 animals in parallel. Experimental groups consisted of 7–12 animals.

2.6. Rotational behavior in 6-hydroxydopamine-lesioned rats

For stereotaxic lesions of the substantia nigra rats were pretreated with pargyline (25 mg/kg subcutaneously). 30 min later 6-hydroxydopamine (16 μg/4 μl) was injected into the left nigra under pentobarbital anesthesia (50 mg/kg). The stereotaxic coordinates were: AP 1.9; L 1.8; AP −2.1 according to a stereotaxic atlas (König and Klippel, 1963). After the animals recovered from surgery they were screened with apomorphine and amphetamine (see below). Experiments started 2 months after the lesion. Animals were not used for longer than 6 months. Ipsiversive and contraversive rotations were registered by means of an automatic device consisting of 6 Perspex bowls (40 cm diameter) and electro-mechanical transducer systems. The latter registered a count each time the animal moved through 36 degrees in a clockwise or counterclockwise direction. In addition, full 360-degree rotations were registered for each direction. Animals were placed into the bowls and connected to the transducers following injection of test compounds. Counts and full circle rotations were accumulated at 10-min intervals and recorded for 60 or 120 min. Only rotations were analyzed since the number of rotation counts and full rotations show a robust correlation in animals exposed to apomorphine or amphetamine, respectively. Animals showing more than 30 contraversive rotations in 30 min when exposed to a standard dose of apomorphine (0.1 mg/kg s.c.) and more than 60 ipsiversive rotations in 60 min following treatment with amphetamine (1.56 mg/kg) at 1 and 2 weeks after the lesion were included in experimental groups. Rats were allocated to treatment groups of 6–8 animals in a quasi-random fashion with the restriction that no animal received active or non-active treatment more than twice consecutively. A wash-out period of 2 weeks was allowed between experiments.

2.7. NMDA-induced seizures

In mice, NMDA was injected intracerebroventricularly (0.7 nmol/3.5 μl) by freehand injection over a period of 20 s. Pretreatment with vehicle or active drug was performed 30 min before NMDA was given. The convulsive response to NMDA at the selected dose consists of wild running or jumping followed by clonic movements of the limbs with loss of the righting reflex. All animals were observed for 30 min for the presence and absence of seizures. In addition, the latencies to onset of wild running (a) and clonic seizures (b) were determined separately using a cut-off time of 120 s.

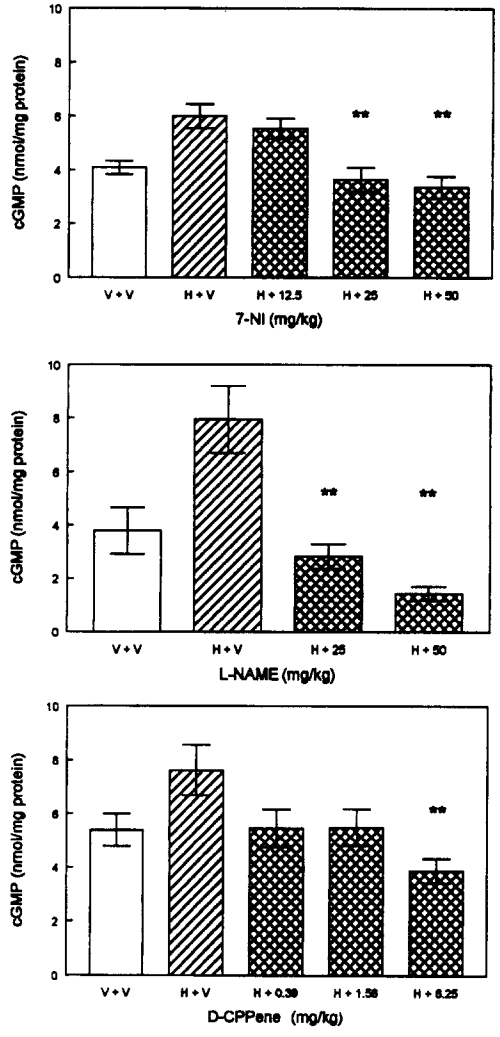

Fig. 1. Effect of 7-nitroindazole (7-NI, 12.5–50 mg/kg), L-NAME (25–50 mg/kg) and D-CPPene (0.39–6.25 mg/kg) on the increase of cerebellar cGMP in male mice treated with vehicle (V) or harmaline (H; 50 mg/kg). Experimental groups consisted of 6–8 animals. Data are presented as means ± S.E.M. Significances: ** $P < 0.01$ vs. harmaline, Tukey test.

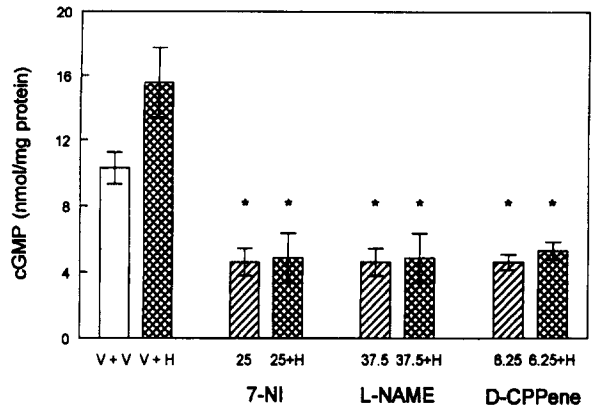

Fig. 2. Effect of 7-nitroindazole (7-NI, 25 mg/kg), L-NAME (37.5 mg/kg) and D-CPPene (6.25 mg/kg) on cerebellar cGMP content in male rats treated with vehicle (V) or harmaline (H; 40 mg/kg). Experimental groups consisted of 8 animals. Data are presented as means ± S.E.M. Significances: * $P < 0.05$ vs. vehicle or harmaline, Tukey test.

2.8. Statistical analysis

Statistical analysis of the tremor and seizure incidence data was performed using the Kruskal-Wallis test. In all other instances, statistical differences were calculated by analysis of variance (ANOVA) followed by a Tukey test.

3. Results

3.1. Cerebellar cGMP levels

In mice, harmaline (50 mg/kg) led to an increase of cerebellar cGMP of 40 to 100% 15 min after drug application. This effect was dose dependently reversed by pretreatment with 7-nitroindazole (12.5–50 mg/kg), L-NAME (25–50 mg/kg) and D-CPPene (0.39–6.25 mg/kg) (Fig. 1). In rats, treatment with 7-nitroindazole (25 mg/kg),

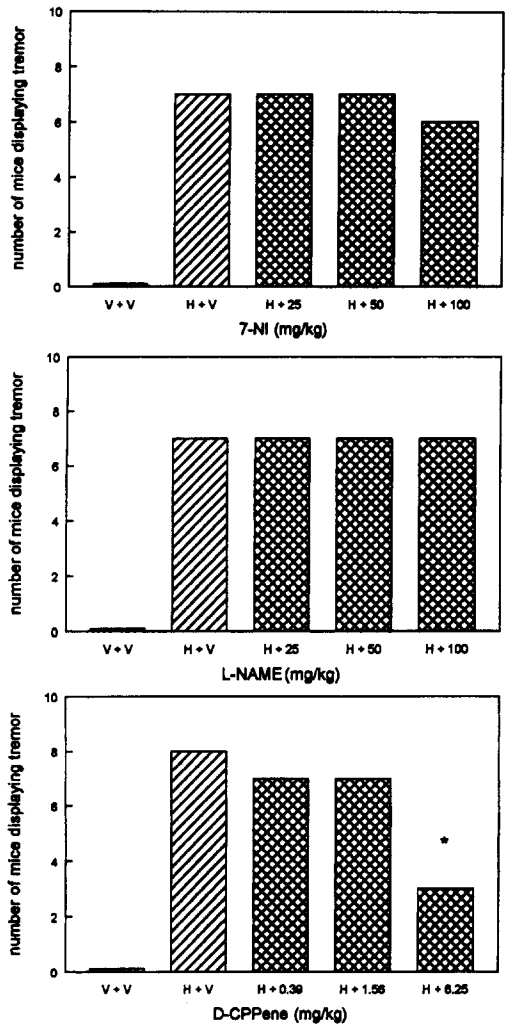

Fig. 3. Effect of 7-nitroindazole (7-NI, 12.5–100 mg/kg), L-NAME (25–100 mg/kg) and D-CPPene (0.39–6.25 mg/kg) on tremor in male mice treated with vehicle (V) or harmaline (H; 50 mg/kg). The bars indicate the number of animals displaying tremor. Experimental groups consisted of 8 animals. Significances: * $P < 0.05$ vs. harmaline, Kruskal-Wallis test.

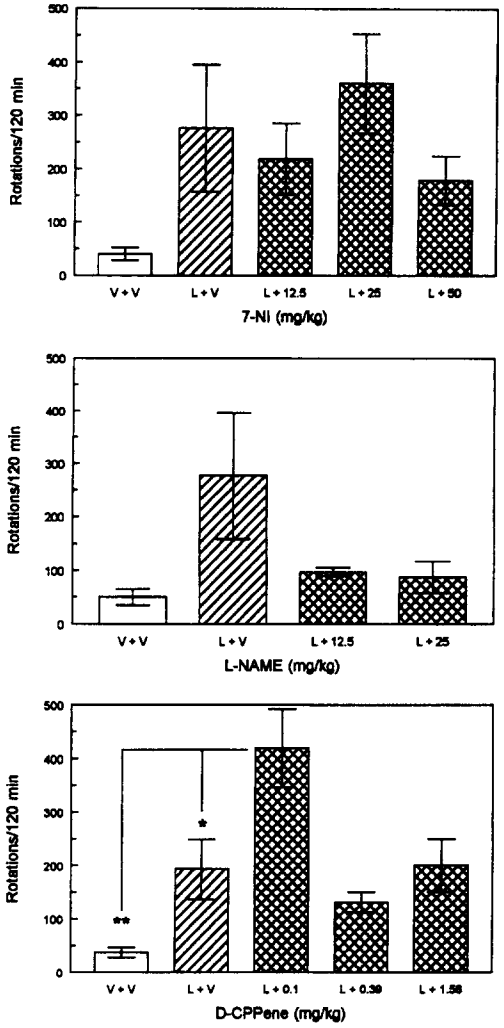

Fig. 4. Effect of vehicle (V), 7-nitroindazole (7-NI, 12.5–50 mg/kg), L-NAME (12.5–50 mg/kg) and D-CPPene (0.1–1.56 mg/kg) on the number of contralateral rotations in unilaterally 6-hydroxydopamine-lesioned rats treated with lisuride (L; 0.005 mg/kg). Experimental groups consisted of 8 animals. Data are presented as means ± S.E.M. Significances: * $P < 0.05$, ** $P < 0.01$ vs. vehicle or lisuride, Tukey test.

L-NAME (37.5 mg/kg) or D-CPPene (6.25 mg/kg) lowered cerebellar cGMP levels and reversed the cGMP increase induced by harmaline (40 mg/kg) (Fig. 2).

3.2. Harmaline-induced tremor

In mice and rats, harmaline induced a postural tremor. In mice, the incidence of harmaline-induced tremor depended on the dose (3.13–50 mg/kg) and did not change within the observation period of 60 min (data not shown). Because there were no major fluctuations during the observation period only the 15 min rating is reported. For drug interaction experiments a dose of harmaline (50 mg/kg) was selected which reliably led to tremor in almost all animals. Harmaline-induced tremor was prevented by D-CPPene (0.39–6.25 mg/kg). In contrast, 7-nitroindazole (25–100 mg/kg) and L-NAME (25–100 mg/kg) had no effect on harmaline-induced tremor (Fig. 3). Similarly, D-CPPene (6.25 mg/kg), but not 7-nitroindazole (25 mg/kg) or L-NAME (37.5 mg/kg), suppressed the tremor induced by harmaline (40 mg/kg) in rats ($P < 0.05$, Kruskal-Wallis test; data not shown).

3.3. Locomotor activity

7-Nitroindazole (3.13–50 mg/kg), L-NAME (12.5–50 mg/kg) and D-CPPene (0.39–6.25 mg/kg) had no significant action on the spontaneous locomotor activity of mice (data not shown).

3.4. Rotational behavior in 6-hydroxydopamine-lesioned rats

7-Nitroindazole (50 mg/kg), L-NAME (25 mg/kg) and D-CPPene (1.56 mg/kg) did not induce ipsilateral or contralateral rotations in rats bearing unilateral 6-hydroxydopamine lesions of the substantia nigra when administered alone (data not shown). D-CPPene (0.1 mg/kg) potentiated lisuride-induced contralateral rotations, whereas higher doses were less effective. 7-Nitroindazole (12.5–50 mg/kg) and L-NAME (12.5–25 mg/kg) did not affect the number of contralateral rotations induced by lisuride (0.005 mg/kg) (Fig. 4). L-NAME (50 mg/kg) combined with lisuride (0.005 mg/kg) was not tested because it was lethal in more than half of the animals. Because D-CPPene was effective at very low doses we performed seperate experiments using lower doses of 7-nitroindazole (0.39–6.25 mg/kg) and L-NAME (0.39–6.25 mg/kg). The lower doses of 7-nitroindazole and L-NAME were equally ineffective (data not shown).

3.5. NMDA-induced seizures

Intracerebroventricular injection of NMDA (0.7 nmol) in mice led to seizures in all animals tested. 7-Nitroindazole (12.5–25 mg/kg) and L-NAME (12.5–25 mg/kg) did not prevent NMDA-induced seizures. 7-Nitroindazole (50 mg/kg) and L-NAME (50 mg/kg) partially protected against seizures occurring in 6 and 5 out of 8 animals, respectively. These effects were not statistically significant. In contrast, CPPene (6.25–25 mg/kg) led to a complete prevention of seizures depending on the dose. 7-Nitroindazole (12.5–50 mg/kg) and L-NAME (12.5–50 mg/kg) had a tendency to delay the latency to onset of wild running and clonic seizures after intracerebroventricular injection of NMDA (0.7 nmol) in mice. In contrast, D-CPPene (6.25–25 mg/kg) significantly increased the latency to onset of wild running and clonic seizures (Fig. 5).

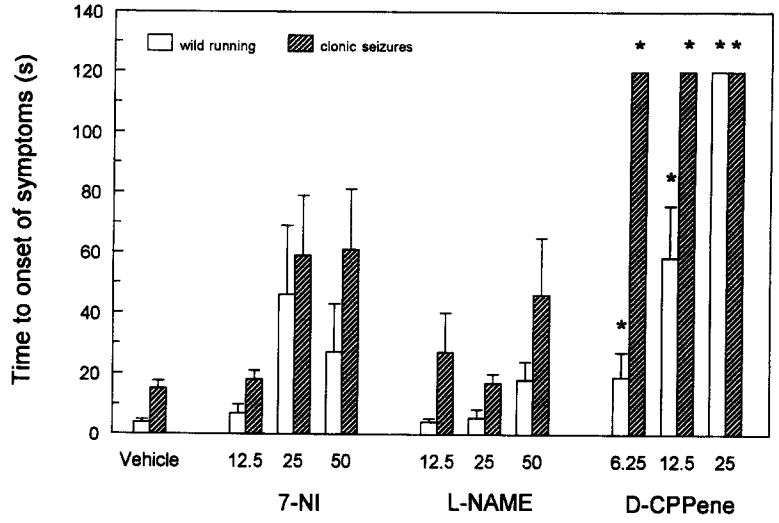

Fig. 5. Effect of 7-nitroindazole (7-NI, 12.5–100 mg/kg), L-NAME (12.5–50 mg/kg) and D-CPPene (6.25–25 mg/kg) on the latency to onset of wild running and clonic seizures induced by intracerebroventricular injection of NMDA (0.7 nmol/3.5 μl) in male mice. Experimental groups consisted of 6–8 animals. Data are presented as means ± S.E.M. Significances: * $P < 0.05$ vs. vehicle, Tukey test.

4. Discussion

The present behavioral experiments were undertaken to study whether neuronal NO synthase mediates the abnormal consequences of increased NMDA receptor-mediated synaptic transmission in models of postural tremor, Parkinson's disease and epilepsy. This study confirms the well-documented role of NMDA receptors in the pathophysiology of Parkinson's disease and epilepsy and reports the novel finding that NMDA receptors are also involved in the expression of postural tremor. The principal finding of our research, however, is that 7-nitroindazole, an inhibitor of neuronal NO synthase, and L-NAME, an unspecific inhibitor of NO synthase, did not share the antitremor, antiparkinsonian and anticonvulsant action of the competitive NMDA receptor antagonist D-CPPene, suggesting that neuronal NO synthase does not serve as a mediator of increased NMDA receptor-mediated synaptic transmission in animal models of Parkinson's disease, epilepsy and postural tremor.

7-Nitroindazole is a novel NO synthase inhibitor which differs from conventional NO synthase inhibitors such as L-NAME in that 7-nitroindazole is devoid of hypertensive actions (Moore et al., 1993a, b, Yoshida et al., 1994). This observation suggests that 7-nitroindazole selectively inhibits neuronal NO synthase without affecting endothelial NO synthase. The results of in vitro studies, however, call the specificity of 7-nitroindazole for neuronal NO synthase into question. As demonstrated by Wolff and Gribin (1994), 7-nitroindazole is also an inhibitor of constitutive NO synthase in bovine endothelial cells. The reasons for the discrepancy between the in vivo and in vitro studies with respect to the action of 7-nitroindazole on endothelial NO synthase are unclear. Possibly, 7-nitroindazole is metabolized in vivo to a compound with a more specific action on neuronal NO synthase. Alternatively, 7-nitroindazole may possess relaxing actions on vascular smooth muscles which reverse the hypertensive action of NO synthase blockade (Medhurst et al., 1994). Nevertheless, 7-nitroindazole is a valuable tool to study the behavioral consequences of blockade of neuronal NO synthase, because it is devoid of cardiovascular actions after systemic application which could interfere with the animal's behavioral responses.

We used 7-nitroindazole in a number of behavioral paradigms and compared it with the action of the conventional hypertensive NO synthase inhibitor L-NAME and the competitive NMDA receptor antagonist D-CPPene. In parallel ex vivo experiments we measured the effects of 7-nitroindazole, L-NAME and D-CPPene on the harmaline-induced increase of cerebellar cGMP levels. It is well established that the action of harmaline on cerebellar cGMP levels is mediated by neuronal NO synthase present in the cerebellar cortex (Wood et al., 1990). The results of the ex vivo experiments show that the doses of NO synthase inhibitors we used are sufficient to block neuronal NO synthase.

The competitive NMDA receptor antagonist D-CPPene effectively blocked harmaline-induced tremor in rats and mice. In contrast, the NO synthase inhibitors 7-nitroindazole and L-NAME were not effective, although all compounds blocked the harmaline-induced increase of cerebellar cGMP levels to almost the same degree. The antitremor action of D-CPPene was not due to unspecific suppression of motor activity since D-CPPene did not reduce locomotor activity at doses which were effective in suppressing tremor. Harmaline induces oscillations of inferior olive neurons in a guinea-pig in vitro slice preparation (Llinas and Yarom, 1986). In cerebellar Purkinje cells, which are the major targets of the olivary efferent climbing fiber projection, rhythmic excitatory postsynaptic potentials are generated by harmaline, whereas rhythmic inhibitory potentials can be recorded from neurons of the cerebellar nuclei, which are innervated by Purkinje cells and receive collaterals from the olivocerebellar climbing fibers (Brodal, 1981). These rhythmic postsynaptic potentials are synchronous with the harmaline-induced oscillations in the inferior olive (Llinas and Muhlethaler, 1988). Since climbing fibers send collaterals to other cerebellar cortical interneurons (Brodal, 1981), one may assume that harmaline leads to widespread neuronal oscillations in the cerebellar cortex and deep cerebellar nuclei. These electrophysiological effects of harmaline are paralleled by NMDA receptor- and NO-mediated increases of cGMP. The discrepancy between the ability of NO synthase inhibitors to prevent the harmaline-induced rise of cerebellar cGMP and their failure to suppress the behavioral consequences of harmaline show that the NMDA receptor- and NO-mediated increase of cerebellar cortical cGMP is not essential for the evolution of harmaline-induced tremor. The unexpected dissociation between the biochemical and behavioral actions of harmaline is less surprising if one takes into account that synaptic transmission within the olivocerebellar climbing fiber pathway to Purkinje cells does not involve NMDA receptors and that Purkinje cells are devoid of NMDA receptors and NO synthase (Llano et al., 1991; Vincent and Kimura, 1992; Petralia et al., 1994; Wood et al., 1994). These considerations raise the question by which mechanisms the NMDA receptor antagonist D-CPPene exerts its antitremor action. Stimulation of NMDA receptors promotes oscillatory behavior of neurons in a variety of regions within the central nervous system, an effect which can be dampened by application of NMDA receptor antagonists (Brodin et al., 1991; Buzsáki, 1991). It is conceivable that the anti-oscillatory effect of NMDA receptor blockade contributes to the antitremor action of D-CPPene described in this study.

The pathophysiology of human tremor is poorly understood. Whereas the pacemakers for the resting tremor of parkinsonian patients are located in the basal ganglia and thalamus (Bergman et al., 1994), oscillations occurring in the olivocerebellar loop and the cerebellar efferent pathways to the red nucleus and thalamus are assumed to

underlie the postural and kinetic tremors which occur in patients with cerebellar damage, multiple sclerosis and essential tremor. In addition, postural and kinetic tremors may be generated by oscillation in peripheral reflex loops. Harmaline-induced tremor is considered to represent a relevant animal model of postural tremor. The novel finding that D-CPPene suppressed harmaline-induced tremor suggests that NMDA receptor antagonists should be considered as a potential new treatment for human postural tremor. This suggestion is of direct clinical relevance because a number of weak non-competitive NMDA receptor antagonists are available for use in humans (Choi, 1987; Kornhuber et al., 1989, 1991; Klockgether et al., 1993).

The observation that 7-nitroindazole and L-NAME did not share the antiparkinsonian action of D-CPPene is in accordance with a recent study of Starr and Starr (1995), who reported that 7-nitroindazole and L-NAME failed to reverse reserpine-induced akinesia in mice and did not potentiate the action of dopamine agonists in this model. Glutamate antagonists, such as D-CPPene exert their remedial action in animal models of Parkinson's disease by blocking enhanced glutamatergic transmission within the striatum, the subthalamic nucleus, and the basal ganglia output nuclei. Glutamatergic neurotransmission in these nuclei is increased as a consequence of the loss of striatal dopamine innervation (Klockgether and Turski, 1993). The failure of NO synthase antagonists to share the antiparkinsonian actions of NMDA receptor antagonists suggests that NO does not serve as a mediator of pathologically enhanced glutamatergic transmission in parkinsonism. There are a number of reasons for this finding. First, the distribution of NO synthase within the brain does not match the distribution of NMDA receptors. Histochemical studies using the NADPH-diaphorase technique show that NO synthase is present in striatal interneurons (Hope et al., 1991; Vincent and Kimura, 1992). In addition, the striatum contains high levels of cGMP (Greenberg et al., 1978). In contrast, cellular and biochemical markers of NO are weak or absent in other basal ganglia nuclei, such as the pallidum, subthalamic nucleus and substantia nigra which are targets for the antiparkinsonian action of glutamate antagonists. Second, in vitro studies show that exogenously applied NO or NO donors increase the release of dopamine from cultured striatal neurons and striatal slices (Hanbauer et al., 1992; Zhu and Luo, 1992). NO synthase inhibition may thus lead to reduced concentrations of extracellular dopamine. Finally, depending on the redox state NO may downregulate NMDA receptor function by interacting with thiol groups of the receptor's redox modulatory site (Lipton et al., 1993).

7-Nitroindazole and L-NAME had only weak actions against seizures induced by intracerebroventricular injection of NMDA in mice. At the highest dose tested, both compounds conferred partial protection against NMDA-induced seizures. In addition, both compounds had a tendency to delay the onset of seizures. These effects, however, were not statistically significant. In contrast to our findings, the results of earlier studies suggest an involvement of NO in NMDA-induced seizures. De Sarro et al. (1991) have reported that local pretreatment with the NO synthase inhibitor N^G-monomethyl-L-arginine prevents seizures induced by local injection of NMDA into the deep prepiriform cortex of rats. Mollace et al. (1991) have observed a proconculsant effect of intracerebroventricularly administered L-arginine, the precursor of NO, on seizures induced by intracerebroventricular injection of NMDA. This effect was reversed by coadministration of L-NAME. In seizures induced by other chemoconvulsants NO synthase inhibitors have both anticonvulsant and proconvulsant actions (Bagetta et al., 1992; Osonoe et al., 1994; Penix et al., 1994). The results of the studies investigating the possible anticonvulsant actions of NO synthase inhibitors are difficult to compare because different experimental approaches have been used. In addition, systemic administration of unspecific NO synthase inhibitors may lead to impairment of cerebral autoregulation by inhibition of endothelial NO synthase and may have a major impact on seizure threshold and seizure spread.

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