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Short communication

7-Nitro indazole, an inhibitor of neuronal nitric oxide synthase, attenuates pilocarpine-induced seizures

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Abstract

7-Nitro indazole (25–100 mg/kg i.p.), an inhibitor of neuronal nitric oxide (NO) synthase, attenuated the severity of pilocarpine (300 mg/kg i.p.)-induced seizures in mice. This indicates that the decreased neuroexcitability of the central nervous system (CNS) following administration of 7-nitro indazole may be due to inhibition of neuronal NO synthase, implying that NO acts as an excitatory and proconvulsant factor in the CNS.

Keywords: Epilepsy; Nitric oxide (NO); Nitric oxide (NO) synthase; 7-Nitro indazole; Pilocarpine; Seizure

1. Introduction

As a retrograde messenger, nitric oxide (NO) induces presynaptically the release of several neurotransmitters, including the excitatory amino acid, L-glutamate (Montague et al., 1994). This indicates that NO deranges the neurotransmitter balance in the central nervous system (CNS) and affects neuronal excitability. Therefore, the role of NO in epilepsy has been investigated by several authors, who used various NO synthase inhibitors. However, these experiments have yielded conflicting results.

The NO synthase inhibitors, L-NAME (L-N^G nitroarginine methyl ester) and L-NMMA (L-N^G-monomethylarginine) attenuated pentylenetetrazol-induced seizures in rats (Osonoe et al., 1994) and kainate-induced seizures in mice (Przegalinski et al., 1994). These results suggested that NO is an endogenous *proconvulsant* agent. In contrast, administration of L-NAME increased convulsions induced by NMDA (*N*-methyl-D-aspartate) in mice (Buisson et al., 1993). Another NO synthase inhibitor, L-NOARG (L-N^G nitroarginine), potentiated seizures induced in rats by various convulsant compounds, such as quinolinate (Haberny et al., 1992), kainic acid (Rondouin et al., 1993) and bicuculline (Wang et al., 1994). These data indicated that NO is an endogenous *anticonvulsant* substance.

All NO synthase inhibitors used in these studies are alkyl esters of arginine and affect both neuronal NO synthase and endothelial NO synthase. These alkyl esters induce hypertension, caused by inhibition of endothelial NO synthase. It is known that an acute increase in blood pressure may cause arousal from sleep, indicating that hypertensive agents may affect excitability of central neurons (Fevell and Johnson, 1994). Another relevant fact is that L-NAME and other alkyl esters of arginine are muscarinic receptor antagonists (Buxton et al., 1993). This means that, in addition to inhibiting NO synthesis, they may affect neuronal excitability by derangement of other neurotransmitters as well.

In order to avoid the effect of arginine-derived NO synthase inhibitors on blood pressure and muscarinic receptors, we used 7-nitro indazole, an inhibitor of neuronal NO synthase, which does not affect endothelial NO synthase (in vivo) and blood pressure (Moore et al., 1993). We examined the effect of 7-nitro indazole on pilocarpine-induced seizures in mice since, in

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cholinergic models of epilepsy, L-NAME exerted both stimulation (Starr and Starr, 1993) and inhibition of seizure activity (Bagetta et al., 1992).

2. Materials and methods

The experiments were performed on 100 male Swiss mice (29–47 g). One group of animals ($n = 20$) received vehicle (arachis oil) intraperitoneally (i.p.), while the other three groups (each 20 animals) were treated with one of three doses of 7-nitro indazole (25, 50 and 100 mg/kg i.p.). An additional group of animals ($n = 20$) was given L-arginine (300 mg/kg i.p.), injected 60 min prior to pilocarpine. Seizures were induced with pilocarpine (300 mg/kg i.p.), administered 30 min after injection of vehicle or 7-nitro indazole. A maximum inhibition of neuronal NO synthase in mice occurred 18–30 min following administration of 7-nitro indazole (50 mg/kg i.p.; Moore et al., 1993), while pilocarpine-induced seizure started 10–15 min after injection of pilocarpine (300 mg/kg i.p.; Turski et al., 1984). The schedules of injections of drugs guaranteed an occurrence of pilocarpine seizure during the most pronounced decrease of brain NO synthase activity. In order to minimize peripheral cholinergic effects, scopolamine (1 mg/kg) was injected subcutaneously (s.c.), 30 min prior to administration of pilocarpine (Turski et al., 1984). The seizures were evaluated by observation of the latency, behavioral pattern of seizures (clonic/tonic) and mortality. The animals were observed for a period of 90 min following pilocarpine injection.

7-Nitro indazole (Lancaster, UK) was suspended in arachis oil by sonication and was used in a dose range shown to inhibit neuronal NO synthase (Moore et al., 1993). Pilocarpine hydrochloride, scopolamine methylnitrate (Sigma) and L-arginine hydrochloride (Sigma) were freshly dissolved in distilled water.

The comparisons were done with binomial test. Statistical significance was accepted at a probability (P) value of 0.05 or less.

3. Results

Administration of pilocarpine (300 mg/kg i.p.) to mice ($n = 20$), 30 min after the vehicle (arachis oil i.p.) induced a sequence of behavioral alterations consisting of initial akinesia, grooming, body tremor and salivation. These symptoms appeared within 5–10 min following pilocarpine injection. This preconvulsant behaviour built up to tonic/clonic seizures. In some animals paroxysmal activity resulted in status epilepticus, which was generally lethal to mice. The mean latency of the first tonic-clonic seizure in the control group was 13 min. The percentages of pilocarpine-induced convulsions and lethality in the control group were 90% and 70%, respectively. Administration of vehicle had no overt effect on the behaviour of the animals.

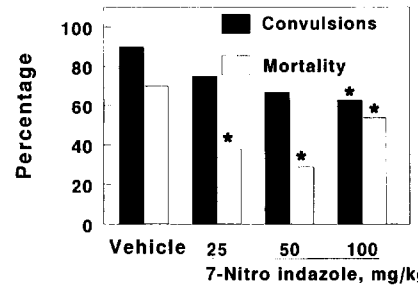

Fig. 1. Frequency of pilocarpine (300 mg/kg i.p.)-induced tonic/clonic seizures and lethality in mice ($n = 80$, divided in 4 equal groups), pretreated with vehicle (arachis oil i.p.) or 7-nitro indazole (i.p.). Pilocarpine was administered 30 min following vehicle or 7-nitro indazole. The data are expressed as percentages of the effect of pilocarpine in vehicle-treated animals ($n = 20$). Significance at $P < 0.05$ level (binomial test) with respect to vehicle-treated group. Note the dose-dependent and bell-shaped decrease in seizure frequency and lethality following administration of 7-nitro indazole.

Administration of 7-nitro indazole (25, 50 and 100 mg/kg i.p., to 60 animals divided into three equal groups for each dose, 30 min prior to pilocarpine), decreased significantly the frequency of seizures and lethality in pilocarpine (300 mg/kg i.p.)-treated mice (Fig. 1). The plot of the decrease in seizure frequency and lethality was bell-shaped. The latency of seizures was not significantly affected by 7-nitro indazole. The mean value of seizure latency in animals treated with 7-nitro indazole (25, 50 and 100 mg/kg) was in the range of 12–16 min, while in the control group it was 13 min. L-Arginine (300 mg/kg i.p., 30 min prior to 7-nitro indazole and 60 min prior to pilocarpine, $n = 20$) did not affect the anticonvulsant effect of 7-nitro indazole (50 mg/kg i.p.).

4. Discussion

This study showed that pilocarpine-induced seizures and lethality in mice can be prevented by 7-nitro indazole. However, the anticonvulsant effect of 7-nitro indazole and the decrease in lethality were less pronounced after a high dose of this drug. This might indicate that higher doses of 7-nitro indazole (above 50 mg/kg i.p.) possess some additional non-specific effect, unrelated to brain NO synthase inhibition. The anticonvulsant effect of 7-nitro indazole contrasts with the effect of the NO synthase inhibitor, L-NAME, which was found to be proconvulsant when injected in conjunction with a subconvulsant dose of pilocarpine (100 mg/kg i.p.) and which failed to protect mice against

seizures induced by high dose of pilocarpine (400 mg/kg i.p., Starr and Starr, 1993). A high dose of pilocarpine (400 mg/kg i.p.) is generally considered as lethal to mice (Turski et al., 1984) and this may prevent the detection of the corresponding effect of L-NAME. The mechanism of the proconvulsant effect of L-NAME in the presence of a subconvulsant dose of pilocarpine (100 mg/kg i.p.) is not clear. Although we have no explanation of this effect of L-NAME, it might be possible that the discrepancies between results of two studies, with the same animal model of epilepsy are due to differences between actions of L-NAME and 7-nitro indazole, unrelated to brain NO synthase. Our results are, however, consistent with the anticonvulsant effect of L-NAME in another cholinergic model of seizure, using as convulsant agent an acetylcholinesterase inhibitor, tacrine (1,2,3,4-tetrahydro-9-amonoacrodine; Bagetta et al., 1992). The present results are also consistent with those of a recent study showing that 7-nitro indazole (40 mg/kg i.p.) attenuates kainate-elicited convulsions in rats (Mülsch et al., 1994). All these data support the idea of a proconvulsant role of central NO.

However, in our study this idea was not confirmed when the NO precursor, L-arginine, was co-administered with 7-nitro indazole. Although, L-arginine injected in the prepiriform cortex potentiates excitatory amino acid-induced seizures in rats (De Sarro et al., 1993) and mice (Przegalinski et al., 1994), no overt effect was observed on the behaviour of mice challenged with a threshold convulsant dose of pilocarpine (100 mg/kg i.p.; Starr and Starr, 1993). Furthermore, concomitant administration of L-arginine (500 mg/kg i.p.) prevented the convulsant effect of 5 mg/kg L-NAME, but was ineffective against 25 mg/kg L-NAME (Starr and Starr, 1993). Evidently, the species (pharmacokinetic factors), doses and brain concentrations of L-arginine, and some other factors related to the NO synthase inhibitor (possible complex interactions of 7-nitro indazole with other parts of the enzyme, for example with tetrahydrobiopterin) may significantly determine the effect of L-arginine on seizure phenomena affected by NO synthase inhibitors. In this context, a failure of L-arginine to antagonize the anticonvulsant effect of 7-nitro indazole does not confirm, but does not exclude, a possibility that observed effects of 7-nitro indazole are mediated by inhibition of brain NO synthase. Although it is most likely that effects of moderate doses of 7-nitro indazole (25–50 mg/kg) are mediated by inhibition of brain NO synthase, it seems that the non-specific effects (unrelated to brain NO synthase inhibition) of high doses of 7-nitro indazole (above 50 mg/kg), should not be ruled out.

In conclusion, this study showed that 7-nitro indazole, a selective inhibitor of neuronal NO synthase, decreases the frequency and lethality of pilocarpine-induced seizures in mice. This implicates that NO acts as a central neurotransmitter with proconvulsant properties. Although the pharmacology of 7-nitro indazole is not yet completely clarified, particularly the effects of high doses of 7-nitro indazole, we are suggesting that 7-nitro indazole, devoid of a vasopressor effect is presently an appropriate tool to study the role of NO in seizure and other central phenomena.

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