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The Role of Nitric Oxide in Soman-Induced Seizures, Neuropathology, and Lethality

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LALLEMENT, G., T.-M. SHIH, I. PERNOT-MARINO, D. BAUBICHON, A. FOQUIN AND J. H. MCDONOUGH. *The role of nitric oxide in soman-induced seizures, neuropathology, and lethality.* PHARMACOL BIOCHEM BEHAV **54**(4) 731–737, 1996.—The effects of the inhibitors of endothelial and neuronal nitric oxide (NO) synthases, *N*-nitro-L-arginine methyl ester (L-NAME) and 7-nitroindazole (7-NI), respectively, and the precursor of NO, glyceryl trinitrate, on soman-induced seizures, lethality, and neuropathology were studied in rats. It was found that pretreatment of rats with L-NAME and 7-NI potentiated the severity of motor convulsions and enhanced lethality produced by soman. On the other hand, glyceryl trinitrate, administered transdermally at doses ranging from 2.5–5 mg/day 1 day before soman, decreased seizure susceptibility and lethality in soman-intoxicated animals. This was accompanied by a subsequent reduction of central neuronal damage 24 h after soman treatment. Pretreatment with glyceryl trinitrate also reversed seizure latency produced by 7-NI treatment during soman intoxication. These results indicate that neuronal NO may play a prominent role in seizures by acting as an anticonvulsant and neuroprotectant in soman intoxication.

Soman Seizures Nitric oxide Glyceryl trinitrate Nitric oxide synthase inhibitors

NITRIC oxide (NO) has been identified as a neuronal messenger in the central nervous system and a modulator of several brain functions (3,7,23,32). Its role in convulsive phenomena has been studied in different experimental models, though the reported results are far from unequivocal. For example, the proconvulsant activity of NO in the seizures induced by the excitatory amino acids *N*-methyl-D-aspartate (NMDA) or kainate, as well as by the acetylcholinesterase (AChE) inhibitor tacrine, has been demonstrated (1,5,6,22). Conversely, the results of other studies indicate that NO may play a role of an endogenous anticonvulsant substance (4,8,28,29,33). NO was also shown to be either neuroprotective or neurodestructive (16) depending on its redox state nitric oxide (NO) or nitrosonium ion (NO^+). NO, generated endogenously after NMDA receptor activation, can lead to neurotoxicity (16) by reaction with superoxide anion (O_2^-), leading to the formation of peroxynitrite ($ONOO^-$) (16). In contrast, the neuroprotective effects of NO result from downregulation of NMDA-receptor activity by reaction with thiol groups of the receptor's redox modulatory site (15). This reaction is not mediated by NO itself, but occurs under conditions supporting S-nitrosylation of NMDA receptor thiol after transfer of NO^+. Therefore, depending on its chemical state, the NO group can lead to the destruction or the protection of neurones. These findings have led to therapeutic approaches using drugs resembling nitrosonium such as glyceryl trinitrate (18) to decrease NMDA receptor overactivity during ischemia.

In the present study, we examined the role of NO in soman (O-1,2,2-trimethylpropylmethylphosphonofluoridate) intoxication in rats. Soman is an organophosphorus compound that inhibits AChE both peripherally and centrally resulting in accumulation of acetylcholine (11,30). Acute administration of soman produces a rapid onset of limbic seizures, generalized convulsions and subsequent neuropathology (13,21). Effective

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management of soman-induced seizures is critical for minimization of brain damage and full recovery from the central effects of exposure to this agent. Previous research has established that glutamate is released centrally after soman exposure and plays a prominent role during intoxication particularly in the maintenance of seizures and the development of subsequent neuropathology via the activation of hippocampal NMDA receptors (2,12). NO could be synthetized centrally by a calcium-calmodulin-dependent NO synthase both in hippocampal neurons in response to the activation of NMDA receptors and in endothelial cells. We, therefore, investigated the possible antiepileptic and/or neuroprotective effects against soman of a systemic administration of *N*-nitro-L-arginine methyl ester (L-NAME), an inhibitor of endothelial NO synthase (26), or 7-nitroindazole (7-NI), a brain-selective NO synthase inhibitor (17) or of glyceryl trinitrate, an NO donor (20). These studies were performed in two separate laboratories using only slightly different soman exposure protocols as noted below in the Method section.

METHOD

Effects of Pretreatment With L-NAME or 7-NI and Effectiveness of Glyceryl Trinitrate on the Effects of 7-NI

Subjects. Adult male Sprague–Dawley rats (Crl: CDBR VAF/plus; Charles River Labs., Wilmington, MA), weighing 250–350 g, served as subjects. They were individually housed in an American Association for Accreditation of Laboratory Animal Care (AAALAC) accredited facility with free access to food and water in a room maintained at 20 ± 2°C with 50 ± 10% humidity and a 12 L:12 D cycle in effect (with lights on at 0600 h).

Surgery. Animals were anesthetized (pentobarbital, 60 mg/kg, IP) and stereotaxically implanted with cortical recording electrodes. Stainless steel screws served as both cortical electrodes and anchors for the dental cement. Animals were allowed 1 week to recover following surgery before testing.

Drug Treatment. Two sets of experiments were performed following the protocol reported by Penix et al. (25). In the first experiment, animals were pretreated with L-NAME (50 mg/kg, IP), 7-NI (50 mg/kg, IP), or saline (0.5 ml/kg, IP) at 24, 18, and 2 h before injection of soman (180 μg/kg, SC; equivalent to 1.6 LD_{50}). In the second experiment, animals were pretreated with saline or different doses of L-NAME (20, 50, or 100 mg/kg, IP) or 7-NI (30, 40, or 50 mg/kg, IP) 30 min before soman challenge.

In a separate experiment, rats were anaesthetized with pentobarbital and a continuous transdermal delivery system of glyceryl trinitrate (Nitriderm, 3M Santé France) was then applied to the depilated skin of the back of each animal. Glyceryl trinitrate was delivered at 2.5 (n = 10) mg/day. The following day, the animals were divided into two groups. One group (n = 6) received 7-NI (40 mg/kg, IP) and the other group received saline to serve as control 30 min prior to soman administration.

In all experiments, animals were also treated with HI-6 (125 mg/kg, IP) 30 min prior to soman administration and with atropine methyl nitrate (2 mg/kg, IM) therapy immediately after soman. These treatments prolong the survival time of soman-intoxicated animals without affecting the central seizure activity (21,31).

Procedure. Electroencephalographic (EEG) activity was recorded from each animal in a shielded chamber using a Grass Model 12 Neurodata Acquisition System with Model 12A5 Grass amplifiers (1/2 low filter = 0.3; 1/2 high filter = 100; 60 Hz filter = on) and displayed as chart recordings on a Grass Model 78D polygraph. The EEG and behavioral response of the animals were monitored for onset of electrographic seizure activity (repetitive high amplitude spike/sharp wave activity, > 10 s duration) and behavioral signs of convulsions. The EEG and behavioral response of the animals were monitored for 4 h after soman injection. Seizure termination was defined as the last rhythmic spike/sharp wave activity in the EEG record, and the absence of continuous rhythmic spike/sharp wave activity for the rest of the recording period. Mortality was recorded for up to 24 h after soman administration.

Data Analysis. A one-way analysis of variance (ANOVA) compared the treatment groups with respect to latency of seizure onset and time to death. If an overall significant difference was observed, a Newman–Keuls multiple range test was used to assess significant paired treatment differences. The difference in percent mortality between groups was assessed with a chi-square test. Statistical significance is defined as $p < 0.05$.

Effects of Glyceryl Trinitrate Pretreatment Against Soman

Experimental Procedures. Male Wistar rats weighing 300–320 g were deeply anesthetized with pentobarbital (70 mg/kg, IP) and prepared for EEG recordings as follows: three monopolar screw electrodes were inserted through the parietal bone 4 mm lateral to the longitudinal fissure and 4.5 (left side), 2.3 and 4.8 mm (right side), respectively, posterior to bregma. After 1 week of recovery, rats were transiently anesthetized with ether and a continuous transdermal delivery system of glyceryl trinitrate (Nitriderm, 3M Santé France) was then applied to the depilated skin of the back of each animal. Glyceryl trinitrate was delivered at 1.25, 2.5, 5 mg/day (n = 9 in each group) or at 10 mg/day (n = 7). Control animals (n = 10) were similarly transiently anesthetized without treatment. The following day, the animals were connected to an EEG recorder (Alvar, Minidix France) and received, 30 min before soman, an IP injection of the oxime HI-6 (125 mg/kg). All rats were then treated with 126 μg/kg, SC (equivalent to 1.4 LD_{50}) of soman followed 1 min later with 4 mg/kg, IM of atropine sulfate (Merck, Germany). We previously demonstrated that following soman exposure the administration of this low dose of atropine is devoid of anticonvulsant activity by itself and is necessary to ensure the efficacy of any subsequent antiepileptic treatment (14). Cortical EEG activity was recorded from 10 min before soman until 1 h after intoxication to evaluate the possible effects of the different doses of glyceryl trinitrate on the prevention or the evolution of epileptic activity after soman. Mortality was recorded for up to 24 h after soman administration.

Data Analysis. Three EEG patterns were observed (see the Results section): status epilepticus, episodic epileptic activity, and normal. In each treated group, the comparison of the distribution of rats vs. controls was made using the nonparametric Kolmogorov-Smirnov's test. Moreover, the 24-h mortality rate observed in each treated group was compared vs. controls using YATES chi-square test.

Neuropathology. Twenty-four hours after soman administration, rats were anesthetized (pentobarbital, 70 mg/kg, IP) and transcardially perfused with heparinized saline followed by a fixative solution made of formaldehyde (4%) and acetic acid (3%). Eight adjacent coronal sections (10 μm thick) were cut on a cryostat at bregma −3.8 [Paxinos and Watson's Atlas

TABLE 1

THE EFFECTS OF NO SYNTHASE INHIBITION WITH L-NAME OR 7-NI ON SOMAN-INDUCED TOXICITY

Treatment*	Seizure Latency (min)	Time to Death (min)†	Mortality
Saline	4.58 ± 0.27 (12)	36.50 ± 10.81 (6)	4/12
L-NAME	8.30 ± 2.26 (6)	52.67 ± 15.98 (6)	6/6‡
7-NI	11.11 ± 1.98‡ (6)	271.67 ± 123.23 (6)	6/6‡

*Rats were pretreated with saline (0.5 ml/kg, IP., L-NAME (50 mg/kg, IP.; 3×), or 7-NI (50 mg/kg, IP; 3×) at 24, 18, and 2 h before administration of soman (180 μg/kg, SC.; equivalent to 1.6 LD_{50} dose). All animals also received HI-6 (125 mg/kg, IP) 30 min prior to soman plus atropine methyl nitrate (2 mg/kg, IM) therapy immediately after soman.

†Results expressed as mean ± SEM (number of animals). The treatment groups were compared with respect to latency of seizures and time of death with Newman–Keuls test. The difference in percentage mortality was assessed with chi-square test: ‡$p < 0.05$ vs. saline control group.

(24)] and thaw mounted on gelatine-coated slices. Sections were stained with hematoxylin and eosin (H&E) for the investigation of the presence and severity of neuropathology.

RESULTS

Effects of NO Synthase Inhibition With L-NAME or 7-NI

Pretreatment with L-NAME or 7-NI, either once or three times during a 24-h period, did not affect the brain EEG activity, but animals were notably less active following treatment with either drug. After soman (180 μg/kg, SC) challenge, animals pretreated with L-NAME showed prominent changes in peripheral color, the tip of the tail and paws became flushed with blood before and during the initial seizure episodes. Very similar effects were observed in 7-NI–pretreated animals, although these changes were less notable than in the L-NAME group. Until brain seizures were fully developed, L-NAME–pretreated rats were behaviorally less mobile than controls and showed severe difficulty in breathing, whereas animals in the 7-NI pretreatment group were able to stand up on all four limbs.

The results obtained from the study of pretreatment with either L-NAME (50 mg/kg × 3, IP) or 7-NI (50 mg/kg × 3, IP) are shown in Table 1. In saline controls, soman produced a seizure latency of 4.6 min and 33% mortality. Using this high dose of soman (180 μg/kg, SC), all control animals (100%) exhibited sustained status epilepticus (21). Pretreatment with 7-NI significantly prolonged the mean time to seizure with a latency to onset of 11.1 min, $F(9, 56) = 14.44$, $p < 0.05$. The seizures waxed and waned before becoming continuous [type IV as previously described (14)]. Both L-NAME and 7-NI pretreatment then markedly enhanced soman toxicity to cause 100% lethality. Thus, the control group had significantly fewer deaths than either pretreatment group.

The dose–response effects of a single 30-min pretreatment with L-NAME (20, 50, and 100 mg/kg, IP) or 7-NI (30, 40, or 50 mg/kg, IP) are shown in Table 2. L-NAME, by a single pretreatment, did not reliably modify the time to onset of type IV soman seizure (14). On the other hand, 7-NI produced a dose-related increase in soman-induced seizure latency, $F(9, 56) = 14.44$, $p < 0.05$. Similar to results obtained with multiple L-NAME or 7-NI pretreatment, a single pretreatment of either inhibitor of NO synthase also enhanced soman-produced lethality.

Because NO synthase inhibition by 7-NI produced a significant delay in soman-induced seizure activity (Tables 1 and 2), the effectiveness of glyceryl trinitrate pretreatment on the effects of 7-NI during soman intoxication was studied. The results are shown in Table 3. It shows that glyceryl trinitrate applied transdermally 24 h earlier at 2.5 mg/day significantly reversed, $F(9, 56) = 14.44$, $p < 0.05$, the effects of 7-NI at 40 mg/kg on seizure latency induced by soman (180 μg/kg, SC). Pretreatment with glyceryl trinitrate for 24 h did not affect the time to seizure onset and all animals survived for 24 h postsoman administration.

Effects of Glyceryl Trinitrate Pretreatment Against Soman

Table 4 shows the effects of graded doses of glyceryl trinitrate applied transdermally on the development of seizure activity and mortality following soman exposure. Using a lower dose of soman (126 μg/kg, SC), 80% of the control group animals developed sustained seizures in 12.0 ± 2.6 min (mean ± SEM) while the remaining 20% were free of epileptic activity. After administration of glyceryl trinitrate at either 2.5, 5, or 10 mg/day only 22 to 45% of animals exhibited sustained type IV seizures (14) after soman with a latency to onset of status epilepticus of 15.3 ± 3.3 min (mean ± SEM; $n = 9$). Twenty-two to 33% of remaining rats experienced

TABLE 2

THE DOSE EFFECTS OF NO SYNTHASE INHIBITION WITH L-NAME OR 7-NI ON SOMAN-INDUCED TOXICITY

Treatment*	Seizure Latency (min)†	Time to Death (min)†	Mortality
Saline	4.58 ± 0.27 (12)	36.50 ± 10.81 (4)	4/12
L-NAME			
20 mg/kg	5.23 ± 1.19 (6)	70.00 ± 18.79 (5)	5/6
50 mg/kg	7.09 ± 1.28 (6)	89.83 ± 50.34 (6)	6/6‡
100 mg/kg	7.47 ± 1.01 (6)	86.83 ± 23.03 (6)	6/6‡
7-NI			
30 mg/kg	6.74 ± 1.20 (6)	80.67 ± 41.56 (6)	6/6‡
40 mg/kg	13.61 ± 1.73‡ (6)	111.67 ± 16.58 (3)	3/6
50 mg/kg	18.69 ± 1.69‡ (6)	191.40 ± 105.50 (5)	5/6

*Rats were pretreated with saline (0.5 ml/kg, IP, L-NAME (20, 50, or 100 mg/kg, IP.; 1×), or 7-NI (30, 40, 50 mg/kg, IP; 1×) at 30 min before administration of soman (180 μg/kg, SC.; equivalent to 1.6 LD_{50} dose). All animals also received HI-6 (125 mg/kg, IP) 30 min prior to soman plus atropine methyl nitrate (2 mg/kg, IM) therapy immediately after soman.

†Results expressed as mean ± SEM (number of animals). Statistical analysis was performed as indicated in Table 1: ‡$p <$ 0.05 vs. saline control group.

TABLE 3

THE EFFECTIVENESS OF GLYCERYL TRINITRATE PRETREATMENT ON THE EFFECTS OF NO SYNTHASE INHIBITION WITH 7-NI ON SOMAN-INDUCED TOXICITY

Treatment*	Seizure Latency (min)†	Time to Death (min)†	Mortality
Glyceryl Trinitrate 2.5 mg/day	5.75 ± 0.48 (4)	None (0)	0/4
Glyceryl Trinitrate 2.5 mg/day + 7-NI 40 mg/kg	7.73 ± 1.47‡ (6)	406 ± 194 (2)	2/6

*Rats were pretreated with glyceryl trinitrate dermal patches 24 h before administration of soman (180 μg/kg, SC; equivalent to 1.6 LD_{50} dose). 7-NI (40 mg/kg, IP) was given 30 min before soman. All animals also received HI-6 (125 mg/kg, IP) 30 min prior to soman plus atropine methyl nitrate (2 mg/kg, IM) therapy immediately after soman.

†Results expressed as mean ± SEM (number of animals). Statistical analysis was performed as indicated in Table 1: ‡$p < 0.05$ vs. respective 7-NI pretreatment alone group (Table 2).

only type II episodic epileptic activity (14) consisting of either spikes or repetitive bursts sequences (duration 10–15 s) or unstable seizures lasting 1–2 min (Fig. 1). Finally, 28 to 45% of intoxicated animals pretreated with glyceryl trinitrate at dose ranging from 2.5 to 10 mg/day were free of any epileptic activity after soman. Although glyceryl trinitrate pretreatment appeared to decrease the incidence of seizure activity after 2.5 to 10 mg/day, this decrease was significant only after the 2.5 mg/day dose (Table 4). On the other hand, glyceryl trinitrate treatment at 2.5 or 5 mg/day significantly improved the 24-h survival rate (Table 4). Thus, there was no strong dose–effect relationship of glyceryl trinitrate pretreatment on the development of nerve agent-induced seizures.

The examination of H&E stained brain sections 24 h after

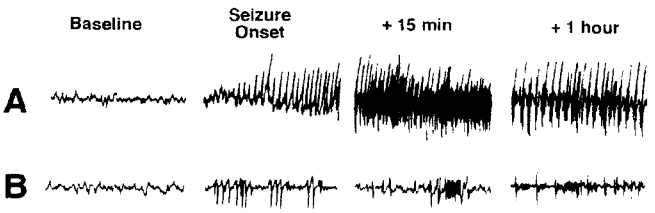

FIG. 1. Examples of the different effects of a pretreatment with glyceryl trinitrate (2.5 mg/day) on soman-induced EEG epileptic activity. In two animals, glyceryl trinitrate has no effect on seizure development after soman (126 μg/kg, SC) (A) while in three other animals EEG pertubations after soman were limited to either spikes or transient bursts sequences (B). See details of protocol and results in the text.

soman (Figs. 2 and 3) demonstrated that all animals that experienced seizures, regardless of treatment group (controls or after glyceryl trinitrate treatment), displayed numerous red suffering neurones (34) in cingulate and frontoparietal cortices, hippocampal CA1 and CA3 pyramidal layers, central and dorsolateral thalamic nuclei, the dorsal hypothalamic area, amygdaloid nuclei, and in piriform/entorhinal cortices. Moreover, neuronal suffering in the hippocampal CA1 area, in dorsolateral thalamic nuclei and in the piriform/entorhinal cortices was accompanied by spongiform change of the corresponding neuropil, leading to a disruption of neuronal tissue integrity. In comparison, in animals exhibiting only episodic epileptic activity after soman, there was a marked reduction of neuropathology with red neurones visible only in central thalamic area, the dorsal hypothalamic region, amygdaloid nuclei, and piriform/entorhinal cortices. However, in these last two areas, the density of suffering neurones was clearly decreased compared to that observed in seizing animals. Moreover, in all animals that showed only episodic epileptic activity after soman, there was no alteration of the neuronal tissue integrity (i.e., spongiform change).

TABLE 4

PREVENTIVE ADMINISTRATION OF GLYCERYL TRINITRATE: EFFECTS ON SOMAN-INDUCED EPILEPTIC ACTIVITY AND 24 H MORTALITY RATE (RATIO AND PERCENTAGE)

Treatment*	Status† Epilepticus	Episodic Epileptic Activity†	Normal EEG†	Mortality‡	Time to Death Mean ± SEM)
Controls (n = 10)	8/10 (80%)	0.10 (0%)	2/10 (20%)	5/10 (50%)	117.0 ± 31.4
Glyceryl Trinitrate 1.25 mg/day (n = 9)	7/9 (78%)	0/9 (11%)	0/9 (11%)	0/9 (22%)	98.4 ± 8.2
2.5 mg/day (n = 9)	2/9§ (22%)	3/9§ (33%)	4/9§ (45%)	1/9§ (11%)	104.5
2.5 mg/day (n = 9)	4/9 (45%)	2/9 (22%)	3/9 (33%)	1/9§ (11%)	84.2
2.5 mg/day (n = 7)	3/7 (43%)	2/7 (28.5%)	2/7 (28.5%)	1/7 (14%)	79.8

*Rats were pretreated with glyceryl trinitrate dermal patches 24 h before administration of soman (126 μg/kg, SC.; equivalent to 1.4 LD_{50} dose). All animals also received HI-6 (125 mg/kg, IP) 30 min prior to soman and atropine sulfate (4 mg/kg, IM) 1 min after soman.

†The comparison of the distribution of rats in each treated group vs. controls was made using the non-parametric Kolmogorov–Smirnov test: §$p < 0.05$.

‡The comparison of the mortality rate in each treated group vs. controls was made using Yates chi-square test: §$p < 0.05$.

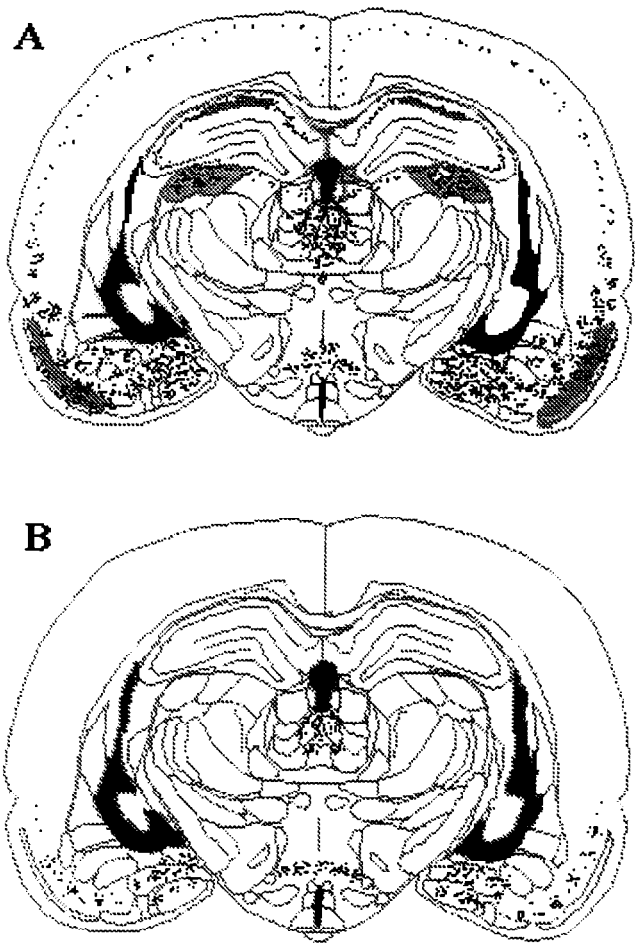

FIG. 2. Schematic reconstruction of the distribution of red suffering neurones (H&E stain) in the rat brain at 24 h after soman (126 μg/kg, SC) exposure. Panel A displays the distribution of suffering in animals exhibiting sustained seizures, while panel B shows neuropathology of animals that had only episodic epileptic activity. Gray areas correspond to regions where a spongiform neuropil was observed. The line drawing was taken from plate 22 of Paxinos and Watson's Atlas (24) (Bregma −.8 mm).

DISCUSSION

The results of the present study indicate that NO may play a role as an anticonvulsant substance in soman-induced seizures. Despite clear dose–effect relationships, we found globally that inhibition of NO synthase by either L-NAME, an endothelial NO synthase inhibitor, or 7-NI, a brain-selective NO synthase inhibitor, produced an increase in the severity of motor convulsive activity that resulted in significant enhancement of lethality induced by soman. On the other hand, a decrease in seizure susceptibility and lethality was observed in animals pretreated with the NO donor, glyceryl trinitrate. This anticonvulsant action of glyceryl trinitrate was associated with a subsequent partial neuroprotection in soman-intoxicated animals. Moreover, pretreatment with glyceryl trinitate reversed the effects of 7-NI on seizure latency during soman intoxication, demonstrating that continuous availability of NO could affect the inhibitory action of 7-NI on brain NO synthase activity during soman intoxication.

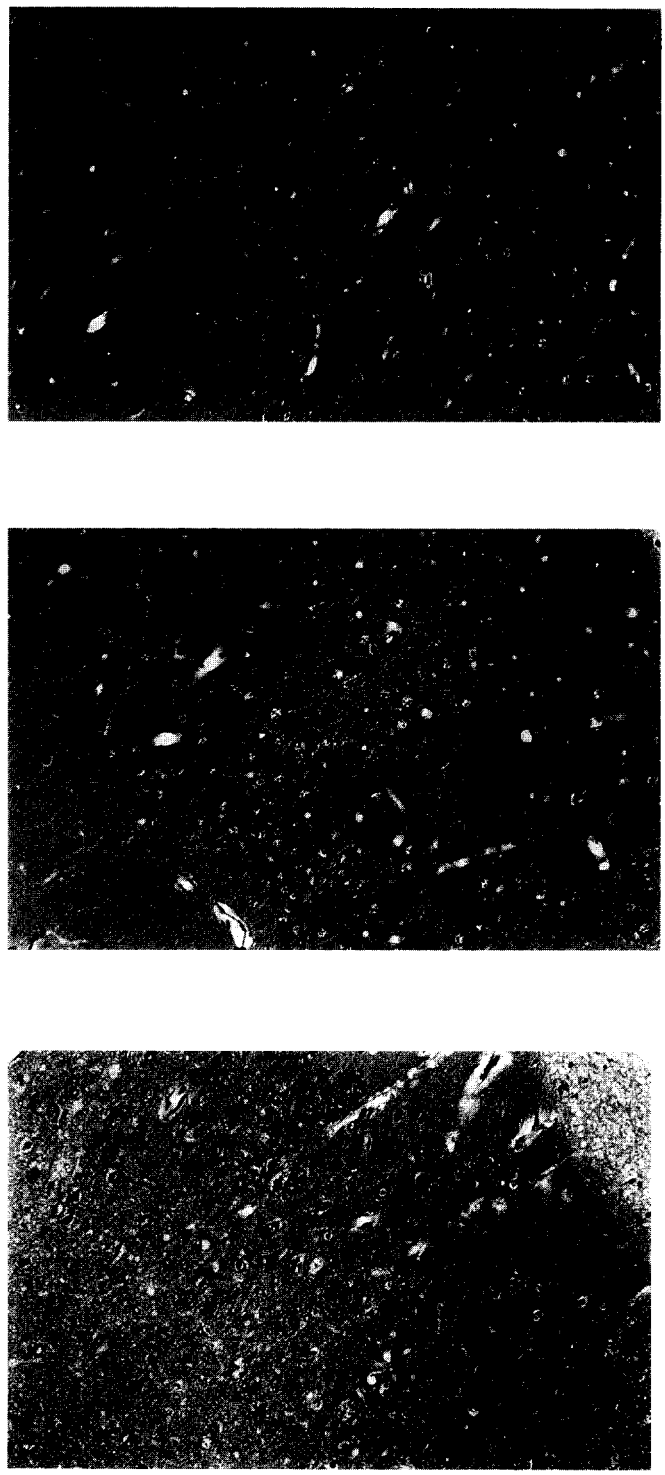

FIG. 3. H&E colorations of piriform cortex observed 24 h after soman (126 μg/kg, SC) intoxication in rats pretreated with glyceryl trinitrate 2.5 mg/day. (A) Animal free of epileptic activity. (B) Animal with episodic epileptic signs. Note the presence of only few suffering neurones (black arrow). (C) Animal showing sustained seizures after soman. Note the presence of numerous suffering neurones (black arrow) and the damaged aspect of the tissue (open arrow). See details in the text. Bar: 125 μm in all cases.

Our results are in agreement with recent reports that showed the anticonvulsant action of NO. Indeed, concerning the effects of L-NAME and 7-NI, inhibition of NO synthase was previously found to similarly increase the severity of seizures induced by pilocarpine (33) or by intraamygdala injection of kainate (28), and to increase the amygdala kindling rate in rats (29). In the same way, a decrease in the convulsive dose of kainate was observed in animals treated with L-NAME (26). NO synthase inhibition was also shown to potentiate the seizures induced by administration of quinolinate to rats (8) and to prolong the duration of seizure activity evoked by NMDA in mice (4).

Although both L-NAME and 7-NI potentiated soman-induced motor convulsive activity and lethality, only 7-NI pretreatments resulted in a significant delay in seizure onset. This effect of 7-NI occurred whether brain NO synthase was inhibited three times within 24 h or acutely, and showed a strong dose dependency. These results with the centrally active 7-NI indicate a stronger influence of neuronal NO on soman-induced seizure activity than peripheral.

The anticonvulsant role of NO in seizures induced by excitatory amino acids has been suggested to be related to its formation in response to the activation of NMDA receptors and to a negative feedback exerted by NO on the activity of these receptors through either a competitive blockade of the NMDA recognition site (19) or an interaction with the redox modulatory site of the NMDA receptor (15). Because there are numerous reports that NMDA receptors are activated during soman-induced seizures (2,12), it is possible that, in our study, inhibition of NO synthase by 7-NI disrupts this negative feedback mechanism and thereby enhances the excitability of postsynaptic neurons. This hypothesis receives strong support from our experiments with glyceryl trinitrate. This drug is a generator of NO^+ (16), for instance, the nitrosonium ion involved in the negative feedback of NMDA receptor activity via S-nitrosylation of NMDA receptor thiol (15,16). Moreover, it has been shown that systemic administration of glyceryl trinitrate produces NO concentrations in the brain sufficient to stimulate guanylate cyclase, for instance, the enzyme activated by NO (36). Therefore, it is tempting to speculate that, in our study, the ability of glyceryl trinitrate to decrease the seizure susceptibility of soman-treated rats and to reverse the effects of 7-NI on soman-induced seizure latency and lethality is likely related to the capacity of this compound to centrally generate sufficient quantities of NO^+ ions, thus, modulating the activity of NMDA receptors in epileptogenic areas like hippocampus.

NMDA receptor overactivation has been shown to be involved not only in the maintenance of seizures after soman but also in the subsequent neuropathological changes, probably via a large influx of Ca^{2+} through NMDA associated channels (2,13). In the present study, severe neuronal damage was observed in all animals exhibiting sustained seizures after soman while a drastic reduction of neuropathology was found in rats that had only episodic epileptic activity. Thus, it appears that the neuroprotection afforded by the NO donor glyceryl trinitrate is closely related to its blockade of NMDA receptors as mentioned earlier. This notion is supported by Rondouin et al. (28) who conversely demonstrated that inhibition of NO synthesis by L-nitroarginine resulted in a facilitation of both development of status epilepticus and secondary neuronal death provoked by intraamygdala injection of kainate.

Although experiments with 7-NI argue in favor of the participation of neuronal NO in soman-induced seizures and subsequent neuropathology, the involvement of NO synthase in the vascular epithelium could not be completely ruled out. NO is a powerful vasodilator and NO released from the cerebrovascular endothelium is a principal factor in the autoregulation of cerebral blood flow (9,35,37). We have reported earlier that during seizures induced by soman there is increased glucose utilization and a corresponding increase in cerebral blood flow (31). Therefore, inhibition of NO production by the vascular endothelium would lead to an uncoupling of the relationships between metabolic demand and blood flow, leading to a mismatch of blood flow for metabolic demand (10,27). Such a mismatch would lead to conditions that further facilitate spread and increase severity of convulsions and seizures, as well as enhanced toxicity and lethality. Accordingly, we have observed that inhibition of endothelial NO synthase by L-NAME enhanced soman-induced motor convulsive activity and lethality. Conversely, continuous supply of NO via transdermal application of glyceryl trinitrate tends to reduce severity of seizure and lethality after soman intoxication.

In conclusion, our results suggest that the toxicity and severity of soman-induced seizures may be exacerbated after inhibition of NO synthase. Comparatively, the epileptic activity observed in soman-treated rats and the subsequent neuropathology are sensitive to modulation by glyceryl trinitrate, a NO forming compound. These results indicate that NO synthetized in response to the activation of NMDA receptors may play an anticonvulsant and neuroprotective role during soman intoxication.

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