Cyclic Alteration in the Anticonvulsant Effect of Nitrous Oxide in Rats

Koh SHINGU, Masami OSAWA and Kenjiro MORI

The anticonvulsant action of nitrous oxide and its time course were studied in rats. Bicuculline, a GABA-receptor antagonist, was administered intravenously at a rate of 0.2 $mg \cdot kg^{-1} \cdot min^{-1}$ during exposure to air (n = 60) or 75% nitrous oxide in oxygen (n = 80). The convulsant dose of bicuculline was determined. The rats were divided into subgroups according to the duration of exposure to air or nitrous oxide, from 0 to 120 min at 15 min intervals. Although the convulsant dose of bicuculline was consistent in the air group (1.03 ± 0.06 $mg \cdot kg^{-1}$, mean ± SEM), it showed two peaks at 30- and 90 min exposures to nitrous oxide. The threshold dose in the nitrous oxide group was significantly higher than in the air group at only 15- and 30 min exposures (1.50 ± 0.16, 2.15 ± 0.25 $mg \cdot kg^{-1}$, respectively, $P < 0.05$). We conclude that nitrous oxide has an anticonvulsant action against bicuculline-induced seizure, and that a cyclic nature exists in its action.
(Key words: Nitrous Oxide, Anticonvulsant Action, Convulsion, Bicuculline, Drug Tolerance)

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Nitrous oxide has various effects on the central nervous system (CNS) such as hallucinogenic, EEG slowing, blocking the wakefulness-sleep cycle as well as hypnotic and analgesic actions. However, continued administration of nitrous oxide results in developing acute drug tolerance to some of these actions in both humans and laboratory animals: the development of tolerance to the actions of EEG slowing and the blockade of wakefulness-sleep cycle[1] was the first of the reports. It was followed by the reports of tolerance to the analgesic action[2–4], the suppression of righting reflex[5], and the anticonvulsant action[6,7]. The tolerance to analgesic action, however, was not confirmed in our laboratory[8]. Since different functions of CNS should involve different neuronal networks and/or different transmitters, it is rational that different CNS functions demonstrate different susceptibility and different time courses in developing drug tolerance to a continuous administration of a given drug. The present study examined the effects of continuous administration of nitrous oxide on the bicuculline-induced convulsion in rats.

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Methods and Materials

One hundred and forty male Wistar rats, weighing 289 ± 3.0g (mean ± SEM), were used. The rats were given water and rat chow at libitum, and were kept in a 12 hr light-dark cycled environment. Experiments were carried out between 10:00–17:00. The rat were anesthetized with halothane in oxygen via a plastic mask. Three stainless steel screws, 1.0 mm in diameter, were fixed to the skull: one in the frontal bone as a ground, the other two over the frontal and occipital cortex for recording EEG. The electrodes were connected to a socket, which was

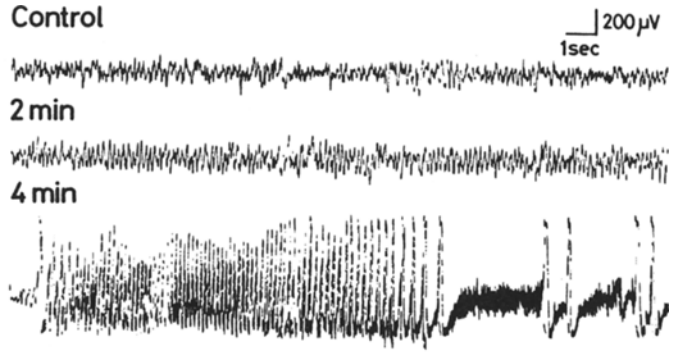

Fig. 1. Cortical EEG changes during bicuculline infusion in a rat exposed to air.

The upper trace shows the EEG before bicuculline infusion. The middle trace shows that 2 min after bicuculline administration. This EEG shows rhythmic theta waves. The lowest trace shows that 4 min after bicuculline infusion. This EEG shows repetitive high amplitude spikes.

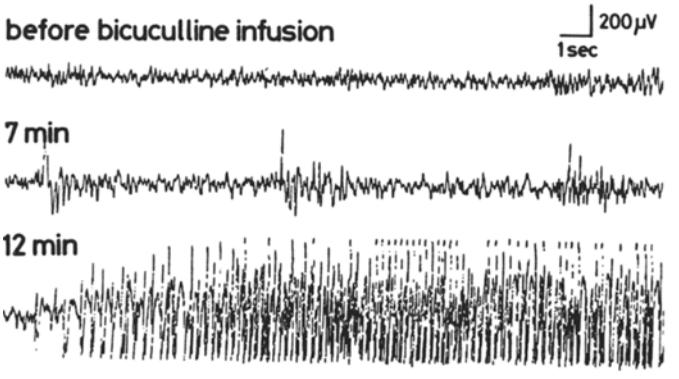

Fig. 2. Cortical EEG changes during bicuculline infusion in a rat exposed to nitrous oxide.

The upper trace shows the EEG 30 min after nitrous oxide exposure. The middle trace shows that at 7 min after bicuculline infusion. Occasional spikes can be seen. The lowest trace shows that at 12 min after bicuculline infusion. Repetitive high amplitude spikes can be seen.

fixed to the skull with dental cement. After 10 days, the rats were again anesthetized with halothane in oxygen, and a polyethylene tube, 0.5 mm in diameter, filled with 0.9% saline, was inserted into the femoral vein. After the placement of a rectal thermistor probe, the hind limbs and tail were taped to the cannula and the thermistor probe. Care was taken not to restrict respiration. After connecting the socket to a pickup plug and cable, each rat was placed in a Plexiglas tube with rubber stoppers with one hole on the gas inlet side and two holes on the gas outlet side. The tail, cannula, and thermistor were passed out through one of the outlet holes, and the EEG cable was passed through the other outlet hole. The tail was taped to the outside of the tube. After exposure to 100% oxygen for 4 hr in the tube, the rats were exposed to either air or 75% nitrous oxide in oxygen. The flow rate of these gases was approximately 1 $l \cdot min^{-1}$ in each tube. Rectal temperature was maintained at 36.5–38.5°C using a heating lamp.

Bicuculline was infused through the femoral cannula at a rate of 0.2 $mg \cdot kg^{-1} \cdot min^{-1}$. The dose of bicuculline to induce seizures in EEG and behavior was determined. Sixty rats were exposed to air and were allocated to 9 subgroups according to the duration of exposure to air prior to bicuculline infusion: 0, 15, 30, 45, 60, 75, 90, 105, and 120 min. Each subgroup consisted of 5 rats, except the 0 min group, which consisted of 20. Eighty rats were exposed to nitrous oxide and were allocated to 8 subgroups according to the duration of exposure to nitrous oxide prior to bicuculline infusion. Each subgroup exposed to nitrous oxide consisted of 10 rats. Bicuculline was infused during continuous administration of the gas studied. The effect of the different exposure times was analyzed using single analysis of variance. The threshold doses of bicuculline were compared using unpaired t-test between the same-time exposure subgroups to air

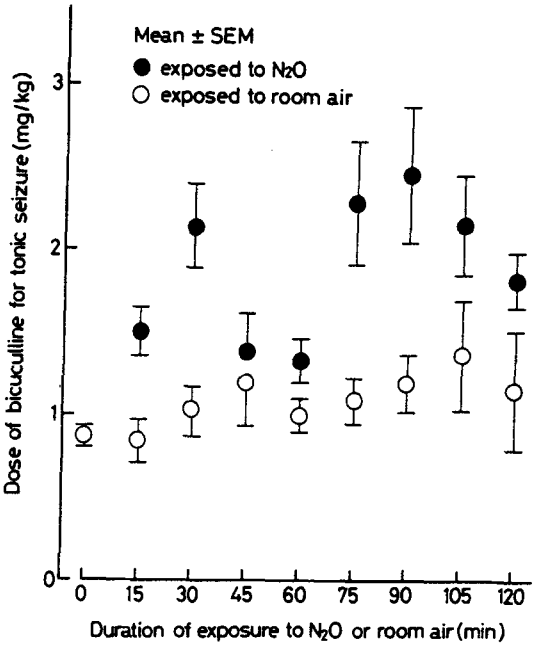

Fig. 3. The threshold dose of bicuculline to induce convulsion during exposure to nitrous oxide (closed circle) or air (open circle) in rats. The values are means ± SEM.

and nitrous oxide. Differences were considered statistically significant when $P < 0.05$ in each comparison.

Results

There was no marked difference in the EEG patterns of the air and nitrous oxide groups before and during the induction of seizures. The preconvulsive EEG during infusion of bicuculline consisted of low voltage fast waves and 6–7 Hz, 120–200 μV theta waves. These were followed by occasional spikes and then by the sudden appearance of high amplitude repetitive spikes (figs. 1 and 2). The repetitive EEG spikes were associated with tonic convulsion. The threshold dose for bicuculline-induced seizure was consistently 1.03 ± 0.06 mg·kg^{-1} in the air group (fig. 3). In contrast, the threshold dose in the nitrous oxide group showed two peaks at 30 and 90 min (2.15 ± 0.25, 2.47 ± 0.41 mg·kg^{-1}, respectively, fig. 3), but there were no significant differences among the subgroups. The threshold dose in the nitrous oxide group was significantly higher than in the air group at only 15 (1.50 ± 0.16 mg·kg^{-1}) and 30 min ($P < 0.05$).

Discussion

Nitrous oxide elevated the threshold dose of bicuculline required to induce seizure in the early phase of exposure. De Jong et al.[9], Oshima et al.[6] and Stevens et al.[7] respectively reported inhibitory action on lidocaine-, amygdaloid kindling-, and enflurane-induced convulsions in cats. These findings suggest that nitrous oxide has anticonvulsant actions against various types of convulsion.

The significant differences in bicuculline dose inducing seizure between the nitrous oxide group and the air group were noted only at 15 and 30 min. This may be attributed to the development of tolerance to the anticonvulsant action. Oshima et al.[6] and Stevens et al.[7] reported the development of tolerance to the anticonvulsant action of nitrous oxide in cats. If such tolerance had developed, the threshold dose of bicuculline would have decreased after the initial peak and then become stable. Our study, however, showed a second peak at 90 min. This peak can not be explained by the development of tolerance alone. Mori and Winters[1] reported that the EEG effect of nitrous oxide disappeared dur-

ing 2–3 hr exposure and that wakefullness-sleep cycle reappeared. This suggests that the CNS has a cyclic rhythm even under nitrous oxide anesthesia. Animals have cyclic biorhythms such as the circadian rhythm. Further, an ultradian rhythm, which has a shorter cycle, also exists in animals: 2–3 hr in cats and 60–90 min in rats[10]. Our results suggest the presence of an ultradian rhythm even under nitrous oxide anesthesia. Munson et al.[11] noted changes in the MAC values of halothane and cyclopropane with the circadian rhythm in rats. Although the mechanism(s) of the effect of the ultradian rhythm on the anticonvulsant action of nitrous oxide is uncertain, the CNS activities may change with the biorhythms, resulting in modifications of the action of nitrous oxide. The anticonvulsant action of nitrous oxide has a cyclic nature during continuous exposure in rats.

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