

Acute Tolerance to the Analgesic Action of Nitrous Oxide Does Not Develop in Rats

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The time course of nitrous oxide analgesia was studied in rats with a behavioral criterion, the tail-flick test to radiant heat. All rats were placed individually in a Plexiglas® tube and exposed to either nitrous oxide, 75% in oxygen, or room air (control) for 2 hr. Analgesic potency was evaluated by prolongation of the time required to induce tail-flick. Although individual animals showed variability in the tail-flick time during exposure to nitrous oxide, no animal showed a tendency toward the development of tolerance, and a statistically significant sustained prolongation of tail-flick time was produced. (Key words: Analgesia; tolerance. Anesthetics, gases: nitrous oxide. Tolerance: nitrous oxide.)

NITROUS OXIDE has various central nervous system (CNS) actions manifested by such effects as hypnosis, analgesia, hallucinations, *etc.* The development of acute tolerance to some of these actions has been reported from several laboratories.¹⁻⁸ With regard to its analgesic action, divergent time courses of development of tolerance have been reported, ranging from less than 1 hr^{7,8} to longer than 10 h.⁴ The absence of tolerance to the analgesic action of anesthetics is a prerequisite for the determination of MAC value, and Eger⁹ reported that, at least after the first half hour, the time of exposure to halothane and cyclopropane did not modify the value of MAC in humans and laboratory animals. Tolerance to the analgesic action of anesthetics, if it occurs, is not only of scientific interest but is of importance in the daily practice of anesthesia. The present study attempted to verify, first, whether it occurs and, second, its time course if it occurs.

Materials and Methods

A total of 56 adult male Wister rats, weight 200-250 g, were used, and data were collected from 36. Rats were placed individually in Plexiglas® tubes, having a volume of 592 ml and rubber stoppers in both ends.

The tail of the rat protruded through a hole in one stopper, and the portion 3 cm from the tip was exposed to a heat lamp (Pain meter NYT-5®, Kudo-Denki).¹⁰ At the opposite end of each tube, there was a polyethylene tube leading to a gas manifold. Either nitrous oxide and oxygen or air was delivered to the gas manifold from calibrated flow meters. The flow through each tube was approximately 1 l·min⁻¹. The potency of the analgesic action of nitrous oxide was measured by the prolongation of time required to induce tail-flick after applying radiant heat to the skin of tail. Prior to administration of nitrous oxide, five control tests were performed in each rat at an interval of 15 min. During the initial two tests, the heat lamp energy was adjusted so that rats withdrew the tail within 3-5 s. Control tail-flick time was obtained by averaging the last three tests. When adjustment of heat energy was not possible because of too much variability, the animals were discarded from the study. In order to avoid damage to the skin of the tail, the maximum length of heat application was set at 10 s. Twenty-one rats served as control animals and were exposed to air for 3 h after the control procedures. Fifteen rats were exposed to 75% nitrous oxide in oxygen for 2 h after the control study and then to air for 1 h. In one rat, nitrous oxide concentration in the tube was measured using a mass spectrometer (Parkin-Elmer,® 1100 Medical Gas Analyzer) when the inspired gas was changed from 100% oxygen to 75% nitrous oxide in oxygen and then to 100% oxygen again in the same condition as used in the study.

All test procedures were performed at an interval of 15 min. Per cent analgesia was calculated as follows:

$$\frac{(\text{Experimental tail-flick time} - \text{control tail-flick time}) \times 100}{(10 - \text{control tail-flick time})}$$

The analgesic effect of nitrous oxide was evaluated using the Mann-Whitney test to compare the rats exposed to nitrous oxide and those given air at the same periods of exposure. The possible development of tolerance to the analgesic action of nitrous oxide was evaluated using Friedman's test. Differences were considered significant when $P < 0.05$. Values are presented as means \pm SEM.

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Received from the Department of Anesthesiology, Kyoto University Hospital, Sakyo-ku, Kyoto 606, Japan. Accepted for publication November 7, 1984. Supported in part by a Grant-in-Aid for Scientific Research No. 59440064 from the Ministry of Education, Science and Culture, Japan, and a grant from Niwa Medical Research Foundation.

Results

The control tail-flick time of control rats and those to be exposed to nitrous oxide were 4.1 ± 0.1 s and 4.3 ± 0.1 s, respectively (not significant). The control rats showed a consistent tail-flick time during the 3-h study (fig. 1). Administration of nitrous oxide and its withdrawal induced some agitation: the rats struggled in the tube for approximately 5 min at both phases. During exposure to nitrous oxide, the tail-flick time of individual rats varied from time to time, but no animal showed a tendency for decline in the analgesic potency of nitrous oxide. In comparison with the control rats, there was a sustained, significant prolongation of tail-flick time during exposure to nitrous oxide and also a significant residual analgesia during the succeeding 60-min emergence period ($P < 0.001$ from 15 to 135 min, $P < 0.005$ at 150 min, and $P < 0.05$ at 165 and 180 min) (fig. 1).

The mass spectrometer showed a rapid increase in nitrous oxide, which plateaued within 2 min when 100% oxygen was changed to 75% nitrous oxide, and a rapid decrease when nitrous oxide inflow was changed to 100% oxygen again.

Discussion

The consistency of the tail-flick time in our control animals confirmed the consistency of sensitivity to heat. The statistically significant and sustained prolongation of tail-flick time induced by nitrous oxide confirmed the analgesic action of nitrous oxide in the concentration studied and ruled out the possibility of developing tolerance to its analgesic action. An enhancement in the algesic sensitivity of skin is known to occur after heat injury.¹¹ This was ruled out in our study by the absence of sensitization to heat in the control animals. Such sensitization has to be taken into consideration when the data indicate a tendency toward the development of tolerance, since the development of a hyperalgesic state induces apparent attenuation of drug-induced analgesia.

Using tibial pressure and hot-wire tests in human subjects, Whitwam *et al.*² reported the development of tolerance to the analgesic action of nitrous oxide in three of seven subjects. However, the degree of attenuation was definite in only one subject, and four of seven did not show even the slightest sign of tendency to develop tolerance. Further, the threshold stimulation required to induce pain sensation in their study differed from person to person. Ruppreht *et al.*^{7,8} used pressure-induced noxious stimulation in rats and reported the development of tolerance. Two types of nociceptors are known, *i.e.*, the high threshold mechanoreceptor and the polymodal nociceptor, and their central projections differ from each other.¹¹ Whitman *et al.*² reported a higher susceptibility of tibial pressure-induced pain sensation to

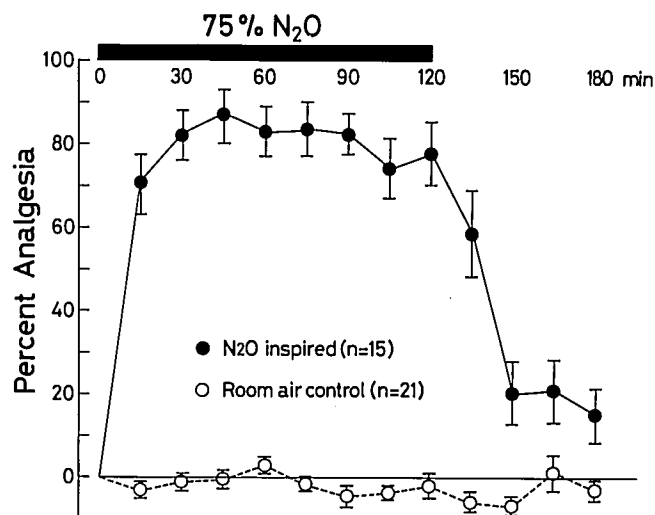


FIG. 1. Time course of analgesic action of nitrous oxide in rats. The abscissa represents the time and the ordinate mean \pm SEM for 15 rats exposed to 75% nitrous oxide in oxygen (filled circle) and for 21 control rats placed in air (open circle). The period of nitrous oxide administration is indicated by the black horizontal bar at the top of the figure. There was no tendency toward attenuation of analgesic action during the period of nitrous oxide administration.

attenuation by nitrous oxide than the hot wire-produced one. Differences between our findings and those of Ruppreht *et al.* might be due to the difference in the nature of test stimulations: we used heat and stimulated polymodal nociceptors only, while Ruppreht *et al.* stimulated the high threshold mechanoreceptor possibly in addition to the polymodal nociceptors.

Berkowitz *et al.*⁴ noted attenuation of the analgesic potency of nitrous oxide within the first 15 min in rats when it was readministered 30 min after a prior continuous administration of 16–18 h. The present study did not examine such a long-term administration. Smith *et al.*,³ on the other hand, observed a rapid development of tolerance in mice occurring within the first 10 min of administration; they showed that, depending on uptake and distribution, such early onset of tolerance to an anesthetic could be masked and might not be detected with ordinary experimental procedures. Since we did not examine for tolerance until 15 min following onset of nitrous oxide, we could not observe such early onset of tolerance. Ruppreht *et al.*⁸ recently reported the development of tolerance and its prevention by pretreatment with enkephalinase inhibitor. They thus attributed tolerance to an acute depletion of endorphins, indicating that nitrous oxide analgesia was exerted through activation of the endorphin-opioids receptor system. However, the contribution of the endorphin-opioids receptor system to nitrous oxide analgesia has been reported to be little, if any, in studies by us¹² and by Way *et al.*¹³

One of us (K.M.) first documented the development of acute tolerance to nitrous oxide actions on the EEG and the wakefulness-sleep cycle in cats.¹ The development of tolerance to an anticonvulsant action of nitrous oxide in cats also was reported from our laboratory.^{5,6} However, with regard to the analgesic action of nitrous oxide, the present study revealed the absence of evidence for the acute development of tolerance after the first 15 min. The CNS actions of anesthetics have various manifestations, including EEG alteration, analgesia, hypnosis, anticonvulsant action, sedation, *etc.* Such different actions may involve different neuronal pathways and/or different neurohumoral transmitters. Tolerance to the CNS actions of anesthetics, if it occurs, may take different time courses according to the neurochemical processes involved. Accordingly, further studies need to be done before definite conclusions can be reached with regard to tolerance to nitrous oxide analgesia.

The authors thank Dr. H. Nakahama, Professor of Physiology, Tohoku University Institute of Brain Diseases, for technical advice.

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