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Antagonism of Nitrous Oxide Analgesia by Naloxone in Man

J. C. Yang, M.D., W. Crawford Clark, Ph.D.,† S. H. Ngai, M.D.‡*

The possible reversal of nitrous oxide analgesia by naloxone was investigated. Two studies were conducted in 21 healthy male subjects, who responded to ischemic pain produced by tourniquet applied to the upper arm for 15 min, while breathing air or nitrous oxide, 33 per cent. Using a double-blind procedure, the subjects received intravenous injections of naloxone and saline solution on different days. In eight subjects, naloxone, 8 mg, administered without nitrous oxide, had no effect on pain report. However, unlike saline solution, naloxone, 8 mg, decreased significantly the analgesia induced by nitrous oxide. In 13 subjects, naloxone, 4 mg, also decreased significantly the effect of nitrous oxide analgesia in comparison with saline solution. Naloxone showed its reversal effect mainly on sensory response ratings obtained during the painful stages of ischemia, between 11 and 15 min. The results suggest that analgesia induced by nitrous oxide may be partly related to the opiate receptor–endorphin system in man. (Key words: Analgesia: measurement. Anesthetics, gases: nitrous oxide. Antagonists, narcotic: naloxone. Pain: experimental ischemic.)

BERKOWITZ *et al.*[1] reported that naloxone, 5 mg/kg, partially antagonized nitrous oxide-induced analgesia in mice, measured by the writhing response to intraperitoneal injection of phenylquinone. They proposed that the analgesic effect of nitrous oxide may be related to the release of endogenous opiates. However, Smith *et al.*[2] observed that naloxone, 2 or 16 mg/kg, intraperitoneally, failed to antagonize the effect of nitrous oxide on the righting reflex in mice. These studies are not contradictory, since the former was concerned with analgesia, the latter with response to nonpainful stimuli. However, they do suggest that the effects of naloxone on anesthesia as assessed by responses to nonpainful and painful stimuli should be studied under similar conditions in the same subjects.

Studies in animals have produced conflicting results. Finck *et al.*[3] reported partial reversal of halothane, enflurane, or cyclopropane anesthesia by naloxone in rats, while Harper *et al.*,[4] who also used the noxious tail-clamp technique in rats anesthetized with halothane, reported that naloxone did not alter the anesthetic requirement for halothane. Other studies using well-accepted techniques for measurement of analgesia have yielded ambiguous results. For instance, Bodnar *et al.*[5] found in rats that naloxone attenuated analgesia induced by cold-water immersion in a dose-dependent manner, but had no effect upon the flinch–jump threshold. Part of this confusion may be due to the use of animals as subjects. In these instances, the response to pain involves skeletal muscle movement, and any substance or procedure that alters motor function could be misinterpreted as a change in pain threshold. Other problems include species difference in responses to analgesics, anesthetics, and antagonists.

Recently, Chapman and Benedetti[6] reported that in man the peak-to-peak amplitude in cerebral evoked potentials to painful tooth pulp electrical shocks was decreased by nitrous oxide. This action of nitrous oxide could be partially reversed by naloxone, 0.4 mg, intravenously. The present study is concerned with the effect of much higher doses of naloxone on nitrous oxide-induced analgesia in human subjects experiencing ischemic pain produced by a tourniquet applied to the upper arm. Placebo effects were controlled by the double-blind administration of saline solution. The effect of naloxone on pain report in the absence of nitrous oxide was also studied.

Methods

The protocol was approved by the institutional Human Investigation Committee. Healthy young male volunteers were informed that they would receive nitrous oxide with injections of naloxone and saline solution on different days. Possible physical and psychological effects of the experiment were described, and the subjects signed an informed consent form. The tests were done at about the same time each day to control for possible diurnal variation,[7] and approximately a week apart. The subject and the experimenter who recorded the sensory response were "blind" with respect to which of the two substances was injected. Each session began with a preinjection control period during which the ischemic pain test was administered using the subject's nondominant arm. In a sitting position, the subject extended his arm upwards while an Esmarch bandage was wrapped around the hand and arm to force out venous blood. An automatic tourniquet§ was placed around the arm and inflated to 250 torr. The bandage was removed, and the subject

* Assistant Professor of Anesthesiology.

† Research Scientist V, New York State Psychiatric Institute, and Associate Professor of Clinical Psychology.

‡ Professor of Anesthesiology and Pharmacology.

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Address reprint requests to Dr. Yang.

§ Walter Kidde & Co., Bloomfield, N. J. 07003.

TABLE 1. Sensory Report Scale*

1	2 3 4	5 6 7	8 9 10	11 12 13	14 15 16	
Nothing	Slight sensation (nonpainful)	Strong sensation (nonpainful)	Slightly painful	Definitely painful	Severely painful	Stop

* The numbers 1 to 16 across the top of the table were the scores used for analysis; they were not on the scale presented to the subject.

squeezed a hand dynometer to half of his maximum strength for two 10-sec periods. Ten seconds later, the subject started to report his sensory experience every 10 sec for a period of 15 min; then, the cuff was deflated. Following an interval of about 15 min, when the sensation of the arm was completely recovered, the subject breathed nitrous oxide, 33 per cent–oxygen, 67 per cent, from a semiclosed circle system at a total gas flow of 3 l/min for 10 min. The subject then received either the saline or the naloxone injection intravenously; 1 min later the tourniquet procedure was repeated on the nondominant arm. Administration of nitrous oxide continued throughout the 15 min of ischemia.

In the first study, eight volunteers received either naloxone, 8 mg, or saline solution. In addition, to test the possibility that naloxone might affect pain report in the absence of an analgesic, naloxone, 8 mg, was administered alone. Each subject in this group was studied on three occasions: naloxone alone, nitrous oxide plus naloxone, and nitrous oxide plus saline. Each of these sessions included a period of preinjection control. To control for anxiety, the treatments were randomized over days. In the second study, 13 volunteers received naloxone, 4 mg, or saline solution, as described above. The session with naloxone alone was omitted, since it had failed to influence the sensory reports in the first study.

The subjects reported their subjective sensation by pointing at the scale shown above in table 1. Under each verbal category a column of numbers from 1 to 26 appeared, the higher numbers meaning stronger sensation; this numerical score was used by the subject to report small changes in sensory experience occurring within a verbal score category.

For analysis of the data, the verbal report category "nothing" was assigned a rating of 1, while each of the remaining five report categories, "slight sensation" to "severe pain," was divided into three values: low, average, and high. These three ratings were obtained by dividing the range of the numerical score of each subject within a verbal category. The subject's sensory measure was based on the median value obtained from the six responses (10 sec each) made at each min. The median is the point at which 50 per cent of the rating value falls, meaning 50 per cent of the observations are below and 50 per cent above the median. Using the median, the data are much less influenced by extreme values. Thus, the sensory rating ran from 1, "nothing," through three levels of "slight sensation," 2, 3, 4, to three levels of "severely painful," 14, 15, 16.

Our tourniquet procedure differs in a number of ways from that of Smith *et al.*[8]: The subject responds every 10 sec, not every min; the category scale covers all sensory experience, not only pain. Furthermore, in this study the median of the reports of the sensory experience, not the mean time to a particular sensation, is the dependent variable.

The subjects' response medians were first treated by analysis of variance (ANOVA) to test whether there was any difference among treatments at each minute. When the F values obtained indicated that the mean of one treatment group was different from that of another, critical differences (CD), based on two-tailed t tests,[9] with each subject serving as his own control, were then computed. CD is the difference at each minute between values of means that must be met or exceeded in order to achieve significance.

Results

Results from the group treated with naloxone, 8 mg, appear in figure 1. The ANOVA at each minute interval revealed significant simple main effects among treatments from 9 through 15 min, F (3,315) $\geq$ 3.91, $P < .01$. According to the CD test, nitrous oxide plus saline solution significantly decreased the pain report from 9 through 15 min, compared with the preinjection control. Nitrous oxide plus naloxone yielded significantly more pain reports than nitrous oxide plus saline solution during each of the six periods from 11 through 15 min. The preinjection control and treatment with naloxone alone did not differ from each other at any time. The subjects' medians averaged over the 15-min preinjection control period did not differ significantly among sessions.

Results from the group treated with 4 mg naloxone appear in figure 2. The ANOVA at each minute interval revealed significant simple main effects among treatments from 4 through 15 min, F (2,360) $\geq$ 6.40, $P < .01$. According to the CD test, nitrous oxide plus saline solution significantly reduced the strengths of

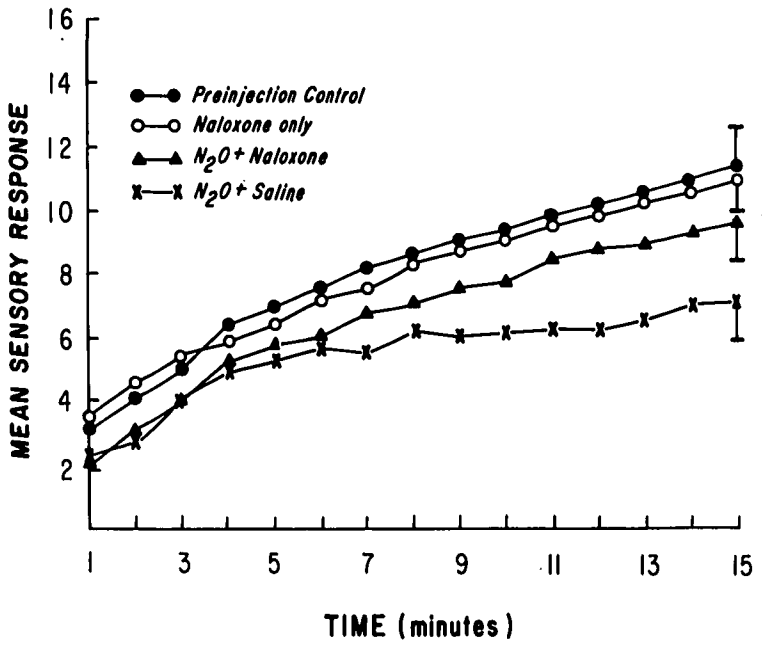

FIG. 1. Effect of naloxone, 8 mg, iv, on ischemic pain responses in eight subjects (mean ± SE), with and without nitrous oxide, 33 per cent. The minimum median responses were: 2, slight sensation; 8, slightly painful; 14, severely painful. $t(315) \geq 1.96, CD > 1.89$ on the report scale, $P < 0.05$. During nitrous oxide analgesia, naloxone, compared with saline solution, significantly increased pain sensation during all periods from min 11 to min 15.

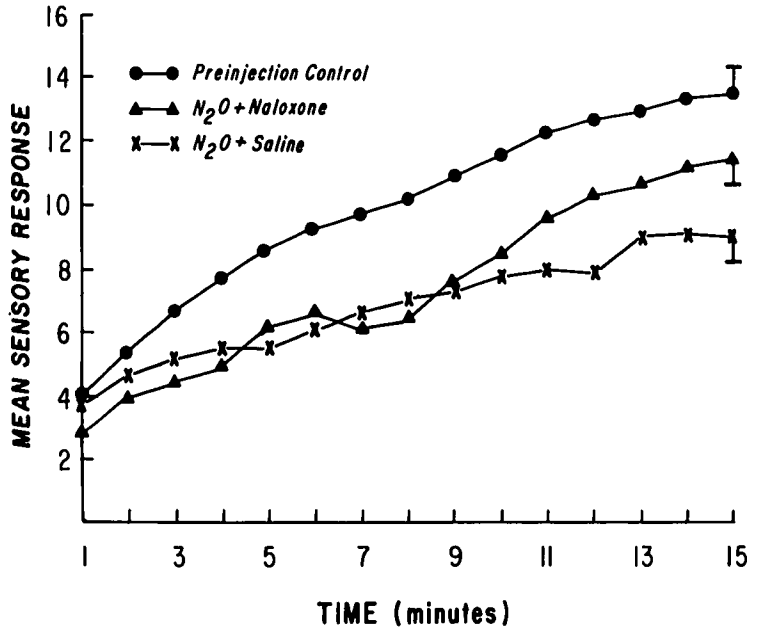

FIG. 2. Effect of naloxone, 4 mg, iv, on ischemic pain responses in 13 subjects (mean ± SE), with and without nitrous oxide, 33 per cent. The minimum median responses were: 2, slight sensation; 8, slightly painful; 14, severely painful. $t(360) \geq 2.58, CD > 2.12$ on the report scale, $P < 0.01$. During nitrous oxide analgesia, naloxone, compared with saline solution, significantly increased pain sensation during all periods from min 11 to min 15.

both nonpainful and painful sensations from 4 through 15 min, compared with the preinjection control. Nitrous oxide plus naloxone yielded significantly more pain reports than nitrous oxide plus saline solution during each of the six periods from 11 through 15 min. Again, the subjects' medians averaged over the 15 min preinjection control period did not differ significantly among sessions.

Discussion

Our results with tourniquet ischemia demonstrated that the inhalation of nitrous oxide decreased both the reports of pain and the reports of somesthetic sensation. Naloxone partially reversed nitrous oxide-induced analgesia, but had little effect on nonpainful sensation. This supports the hypothesis of Berkowitz *et al.*[1] that the analgesia produced by nitrous oxide may be mediated by endogenous opiates.

Berkowitz *et al.*[10] reported that in rats, using the tail-flick test, naloxone, 1 mg/kg, was not effective in antagonizing nitrous oxide-induced analgesia, and that higher doses of naloxone were necessary to antagonize nitrous oxide than to antagonize morphine. Our study was designed to find out whether nitrous oxide analgesia is involved in endogenous opiates in

man, hence much higher dosages of naloxone (4 mg and 8 mg iv) were used.

Chapman and Benedetti[6] studied the effect of naloxone, 0.4 mg, iv, on nitrous oxide-induced analgesia to painful tooth-pulp stimulation in man. They reported that nitrous oxide decreased the pain reports and lowered the peak-to-peak amplitudes of late components of evoked potentials, and naloxone partially reversed these effects. Our results complement their findings, but we also observed that naloxone has very little effect on nonpainful sensation. Furthermore, the naloxone reversal effect we observed was much greater, perhaps because of the higher dose used. However, the available data do not permit speculation on whether the effect of naloxone is dose-related or whether both doses we used were supramaximal.

Smith *et al.*,[2] using loss of righting reflex, measured anesthetic potency of nitrous oxide in mice and observed that naloxone did not alter the ED_{50} of nitrous oxide. They proposed that the antagonism by naloxone is to the analgesic component of anesthesia only. Our results were compatible with their postulation, although they did not measure the effect of naloxone on nitrous oxide-induced analgesia. Both analgesia and depression of reflexes are components of anesthesia. It is possible that naloxone might reverse one component but not the other.

Harper *et al.*,[4] after observing that the administration of naloxone intravenously did not alter halothane requirement, concluded that if any effect of naloxone on anesthetic action existed, it was the result of a nonspecific analeptic action of naloxone. If this were the case, naloxone alone should increase pain response by itself. However, in agreement with Grevert and Goldstein,[11] who administered naloxone, 10 mg, iv, to man, we found that naloxone, 8 mg, alone, has no effect on the perception of ischemic pain. Hence the change in nitrous oxide-induced analgesia by naloxone must be related to the interaction between the two drugs. Arndt and Freye[12] have demonstrated that perfusion of the fourth cerebroventricle with *levo*-naloxone reverses the circulatory and hypnotic effects of halothane in dogs. They pointed out the pharmacokinetic problem inherent in using a single injection of lipophilic naloxone and suggested the possibility that insufficient brain naloxone levels in rats and mice might be responsible for the negative results reported.[2,4] The results of Arndt and Freye[12] support the hypothesis that opiate receptors and endorphins might be involved in analgesia induced by inhalational anesthetics.

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