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Nitrous Oxide Effects on Cerebral Evoked Potential to Pain:

Partial Reversal with a Narcotic Antagonist

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The effect of naloxone, 0.4 mg, on the analgesia induced by nitrous oxide, 33 per cent, in oxygen, was studied in 12 volunteers. Results of previous investigations in animals suggested that endogenous opiate-like substances may play a major role in the analgesic mechanism of nitrous oxide, but the issue had not been studied in man. Cerebral evoked potentials (CEP) to painful tooth-pulp electrical shocks were obtained before and after inhalation of nitrous oxide, and after nitrous oxide plus naloxone, 0.4 mg, in one session; and before and after inhalation of room air, and after room air plus naloxone, 0.4 mg, in another session. CEP waveforms observed between 80 and 350 msec were quantified in terms of three peak-to-peak amplitudes and peak latencies. Nitrous oxide decreased each of the waveform peak-to-peak amplitudes 48 per cent. Naloxone restored the peak-to-peak amplitude of the negative-going wave occurring between 100 and 175 msec. Nitrous oxide also increased the negative peak latency at 175 msec, and naloxone restored this peak latency to normal levels. Neither room air nor room air plus naloxone altered CEP amplitudes or latencies. Over time a significant trend in subjective reports of decreased pain intensity with nitrous oxide and partially increased pain with naloxone was evident. These findings demonstrate that some of the effects of nitrous oxide on the central nervous system can be reversed by naloxone. (Key words: Analgesia: measurement. Anesthesia, dental. Anesthetics, gases: nitrous oxide. Antagonists, narcotic: naloxone. Brain: cerebral evoked potential. Pain: measurement.)

RECENT EVIDENCE suggests that endogenic opiate-like substances, called endorphins, may play a role in the analgesic effects of anesthetic agents, including nitrous oxide. Studying the writhing response to intraperitoneal injection of phenylquinone in mice, Berkowitz *et al.*[1] observed that the dose-related analgesia produced by nitrous oxide was reversed by pretreatment with the narcotic antagonist, naloxone. Finck *et al.*[2] demonstrated that the proportion of rats responding to noxious tail clamp during light halothane anesthesia increased significantly after the administration of naloxone. They proposed that one of the mechanisms of action of inhalational anesthetics is the release of endorphins. In contrast, Harper and associates[3] determined the antianesthetic effect of naloxone in rats breathing halothane by evaluating shifts in the anesthetic dose–response curve induced by variable doses of narcotic antagonist. Using a tail-clamp-response test, they observed that naloxone in doses as high as 250 mg/kg did not alter anesthetic requirement.

Smith and colleagues,[4] reasoning that endorphins must be released at opiate receptor sites along pain transmission pathways, hypothesized that the effects of naloxone are solely on the analgesic component of nitrous oxide anesthesia, and that narcotic antagonists do not affect responses to anesthetics not involving pain. They demonstrated that naloxone did not alter the loss of the righting reflex in mice exposed to nitrous oxide. Bennett[5] also supported this hypothesis by showing that naloxone did not alter the effects of halothane on the righting reflex in rats.

To date, all studies evaluating the role of endorphins in inhalational anesthesia have used laboratory animals. Applying such findings to man is difficult because of the relative insensitivity of analgesic tests used in animals, the high dosages of narcotic antagonist typically needed, and interspecies differences in responsivities to anesthetics, analgesics and antagonists. The purpose of this paper is to address this issue in man by measuring components of the cerebral evoked potential (CEP) to painful tooth-pulp stimulation. This index, obtained from scalp recordings, has been shown by several groups of investigators[6] to be correlated with verbal expressions of perception of pain. The amplitudes of the late components of the CEP (80–350 msec) increase with increments in stimulus intensity and correlate well with subjective judgments of pain.[7–9] Dental CEP amplitudes are decreased by narcotic analgesia (fentanyl, 0.1 mg, iv) and are largely or completely restored by naloxone, 0.4 mg.[10] Furthermore, it has been demonstrated that the dental CEP is absent in a patient with congenital insensitivity to pain, and that it cannot be demonstrated following dental nerve block.[11] We hypothesized that nitrous oxide, 33 per cent in oxygen, would decrease the wave amplitude of the CEP to painful stimulation, and that naloxone would partially restore the normal CEP waveform.

Materials and Methods

Twelve paid male volunteers ranging in age from 19 to 31 years served as subjects in this experiment. All signed fully descriptive informed-consent agreements approved by the Human Subjects Research Committee at the University of Washington. Each

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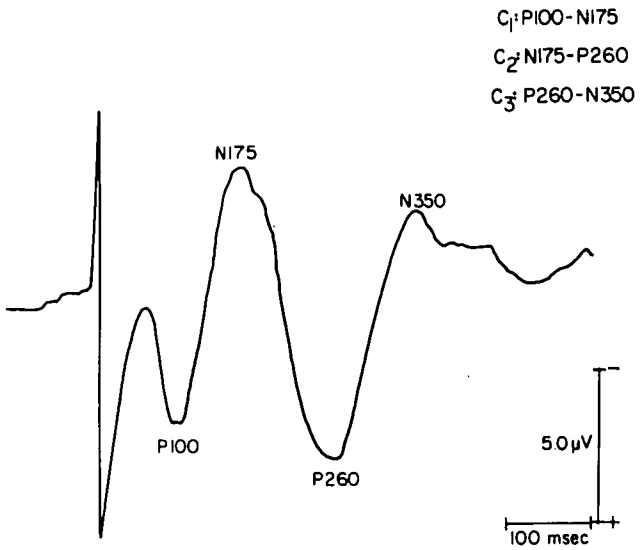

FIG. 1. The cerebral evoked potential (CEP) to painful dental stimulation. The waveform represents summation of 192 responses to "strong pain" in one subject. The dental stimulus artifact is evident at 100 msec. Analyses of data were limited to the components of the wave that occurred more than 80 msec beyond stimulus offset, identified here as C1, C2, and C3.

subject inhaled a mixture of nitrous oxide, 3 l/min, and oxygen, 6 l/min, in one testing session, and room air in another separate control testing session. Half the subjects inhaled nitrous oxide first and the other half, room air first. Subjects were fully informed of what they were breathing. They breathed both the nitrous oxide–oxygen mixture and room air through a nasal mask from a standard anesthesia machine and a nonrebreathing system. All were trained to avoid oral breathing during the experiment. During each session CEP tracings were obtained before (baseline) and during inhalation of nitrous oxide or air. Naloxone, 0.4 mg, was then administered intravenously and a third CEP was obtained 5 min later. Subjects were told that we wanted to know whether the injection would alter the brain-wave response, but they were not told that the injection might reverse the effects of nitrous oxide.

In order to elicit precisely controlled, reliable and relatively pure pain sensations, we electrically stimulated unfilled healthy upper central or lateral incisors. This technique has previously been employed in our laboratory and elsewhere.[12–14] Square-wave pulses of 5-msec duration were provided by a Grass S-44® stimulator with constant current and stimulus isolation units and delivered via a 4-mm conductive rubber electrode (cathode) mounted in a plastic hand-held probe. Stimuli were delivered at a level considered to be "strong pain" by the subject during baseline testing. The subject was trained to hold the probe at a constant locus on the incisal edge of the carefully dried tooth. Stimulus waveforms were constantly monitored on an oscilloscope to ensure that reliable electrical contact was maintained during testing. The dental stimuli were hand-triggered, in blocks of 64 trials, and a 600-msec sample of the EEG was taken beginning 100 msec before the stimulus for each trial. Three sets of 64 trials each were recorded and summed to yield a single CEP waveform reflecting 192 trials. Eye movements, blinks, electrode contact problems, and other potential contaminants were carefully monitored. Care was taken to ensure that the subject held the stimulating electrode at a constant locus on the tooth at all times, including during inhalation of nitrous oxide. Any block of 64 trials involving such difficulties was aborted and replaced by another set of 64 trials. An electroencephalogram (EEG) was recorded from the vertex referred to the inion with a ground electrocardiogram electrode taped to the zygomatic arch. Signals from an Analog Devices® isolation amplifier 277J and optional amplifier were fed into a Nicolet 1072® signal averager (system gain 10^3 and frequency range 0.6 to 100 Hz). Input signal range on the signal digitizer (Nicolet SD-72/4A®) was ±1 volt, with a filter time constant of 20 msec, resulting in an effective bandwidth of 0.5 to 15 Hz.

Measures of CEP peak amplitude and latency were obtained for the 192 trial waveforms by the technologist through the use of the Nicolet 1072 numeric-display option. No effort was made to blind the technologist to the treatments administered in each session. Hard copies of the evoked responses were made using an X-Y recorder. A 100-msec, 10-μV square wave

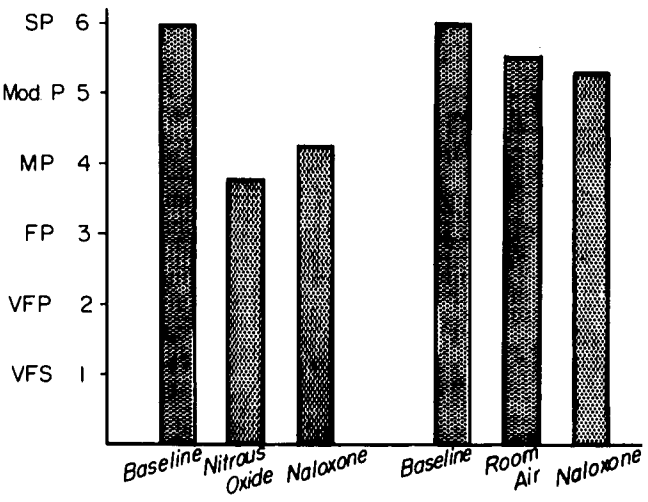

FIG. 2. Mean subjective response data over test conditions in the two sessions. Subjects described the stimuli experienced over each 192-trial test as: strong pain (SP), moderate pain (ModP), mild pain (MP), faint pain (FP), very faint pain (VFP), or very faint sensation (VFS).

was used to calibrate the amplifiers and signal-summating system (Grass SWC 1B®).

The data were quantified in terms of the peak-to-peak amplitudes and peak latencies of the three stable waveform components defined as C1, C2, and C3 (fig. 1). C1 was a negative-going wave occurring between 100 and 175 msec (P_{100}–N_{175}). C2 was the large positive-going component between 175 and 260 msec (N_{175}–P_{260}), immediately following C1. The final component, C3, was negative-going between 260 and 350 msec (P_{260}–N_{350}).

Treatment effects were evaluated by performing t tests for paired data on treatment-induced changes in latency and amplitude characteristics of the CEP that were observed between 80 and 350 msec after the painful dental shock, and by observing changes in verbal reports of pain. At the end of each set of trials the subject reported the level of dental stimulation he had experienced according to one of six categories (fig. 2) visually rank-ordered on a large chart. Each category was assigned a number from 1 to 6, which served as a score when a subject selected it for a pain-evaluation report. Group data underwent statistical evaluation by analysis of variance, including trend analysis.[15]

Results

Inhalation of nitrous oxide significantly decreased the mean amplitudes of C1, C2, and C3 by 48 per cent, while inhalation of room air had no significant effect (table 1). Injection of naloxone inhalation of nitrous oxide resulted in a significant restoration of mean amplitude for C1 to 81 per cent of its initial value, but the other components were not affected. Naloxone had no significant effect on any of the waveform components during breathing of room air. Inhalation of nitrous oxide significantly increased peak latency at N_{175} (table 2), and naloxone returned latency at N_{175} to nearly normal levels. Other peak latencies were not significantly affected by experimental manipulations. In the session when subjects received nitrous oxide, the mean judgment scores were significantly decreased during inhalation, $F(2,22) = 37.1$, $P < 0.001$. After naloxone was given the mean judgment scores increased. The decrease followed by an increase in the mean scores was statistically significant as a quadratic trend, $F = 16.3$, $P < 0.001$, indicating a significant decrease in subjective report of pain intensity with inhalation and a partial reversal with naloxone. A similar analysis for the room air-inhalation session showed a significant decrease in subjective report over experimental conditions, $F(2,22) = 5.95$, $P < 0.01$, but with no quadratic trend. The decrease over conditions was slight, although significant, and may have reflected subjects' increasing familiarity with the test stimulus over time. It must be noted that statistical significance in the subjective report data reflects, in part, the low between-subjects variance among scores, particularly during inhalation of room air. While the subjective-report data served to confirm our assumption that the CEP amplitude changes reflect variation in the subjec-

TABLE 1. Peak-to-peak Amplitudes (Mean ± SE) of Waveform Components before (Baseline) and during Inhalation of Nitrous Oxide or Room Air and after Treatment with Naloxone

	C1 (μV)	C2 (μV)	C3 (μV)
Nitrous oxide (33 per cent)			
Baseline x̄	4.8 ± 0.9	5.7 ± 0.8	4.6 ± 0.5
Treatment x̄	2.5* ± 0.6	3.0* ± 0.5	2.4* ± 0.4
Naloxone x̄	3.9† ± 0.5	3.4 ± 0.4	2.4 ± 0.3
Room air			
Baseline x̄	4.3 ± 0.8	5.1 ± 0.7	4.1 ± 0.6
Treatment x̄	3.5 ± 0.5	4.7 ± 0.5	3.2 ± 0.6
Naloxone x̄	3.4 ± 0.6	4.2 ± 0.6	3.8 ± 0.6

* Significantly altered from baseline by 33 per cent N_2O, $P < .01$.
† Significantly altered from treatment level by naloxone, $P < .01$.

TABLE 2. Peak Latencies (Mean ± SE) of the CEP Waveform

	P_{100} (msec)	N_{175} (msec)	P_{260} (msec)	N_{350} (msec)
Nitrous oxide (33 per cent)				
Baseline x̄	104.4 ± 6.4	167.9 ± 7.3	263.9 ± 4.8	366.9 ± 8.1
Treatment x̄	119.2 ± 12.2	186.1* ± 10.0	279.8 ± 5.7	355.0 ± 12.8
Naloxone x̄	108.9 ± 7.4	170.9† ± 7.7	258.6 ± 6.9	358.0 ± 11.6
Room air				
Baseline x̄	101.5 ± 4.9	166.1 ± 5.1	260.9 ± 4.7	352.7 ± 3.5
Treatment x̄	101.5 ± 4.9	165.9 ± 6.3	259.7 ± 4.7	356.5 ± 4.3
Naloxone x̄	104.6 ± 5.1	167.2 ± 6.6	262.0 ± 8.1	354.4 ± 4.5

* Significantly altered from baseline by 33 per cent N_2O, $P < .01$.
† Significantly altered from treatment level by naloxone, $P < .05$.

tive appreciation of pain, such measures are too crude to be considered alone as evidence for or against the hypothesis in question.

Discussion

Nitrous oxide decreased the peak-to-peak amplitudes of all three components of the CEP, in addition to increasing the latency of the peak at N_{175}. Naloxone partly restored the amplitude of the early component and it returned the N_{175} peak latency to normal values. This demonstrates that naloxone can alter, in part, the effects of nitrous oxide on the response of the central nervous system (CNS) to painful stimulation.

The contention emerging from animal studies[3,4] that naloxone alters only the analgesic component of the anesthetic effect of nitrous oxide is supported by results of studies in man, including our data. Using auditory click stimulation, several investigators have observed decreases in CEP-component amplitudes with inhalation of nitrous oxide,[16–18] and a similar diminution of the visual evoked response has been reported.[19] These studies demonstrate that nitrous oxide has a general cortical depressant effect. Our data suggest that C1, particularly the negative peak at 175 msec, reflects primarily the analgesic effects of nitrous oxide. The later components could, perhaps, reflect associative processes of interpretation, or possibly the CNS arousal associated with painful stimulation.

While our findings are consistent with the hypothesis that the mechanism of nitrous oxide analgesia involves the action of endorphins at selected sites along pain pathways,[20] they must be interpreted cautiously, since analgesia during inhalation of nitrous oxide occurs against a complex background of other CNS effects. Furthermore, such background effects may not necessarily increase linearly with increased inhaled dosage.

Our data imply that human research may be a fruitful area for further study of these issues, particularly because the phenomena can be observed with relatively low doses of nitrous oxide and standard doses of narcotic antagonist. They also indicate that investigation of CNS responses may be a useful approach for animal research. Animal paradigms tend to confound measurement of nitrous oxide analgesia with general cortical depression because they depend on behavioral responses alone. Concomitant use of CNS recordings may increase the precision of measurement procedures in animal investigations.

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