Failure of Naloxone to Reverse the Nitrous Oxide–Induced Depression of a Brain Stem Reflex: An Electrophysiologic and Double-blind Study in Humans

Jean-Claude Willer, M.D.,* Sabine Bergeret, M.D.,† Jean-Henri Gaudy, M.D.,‡ Claude Dauthier, M.D.§

The effects of 33% nitrous oxide on the two components of the blink reflex were studied on seven healthy volunteers. The blink responses were elicited by supraorbital nerve stimulation and recorded from the ipsilateral orbicularis oculi muscle. The intensity of stimulation was chosen at two to three times the reflex threshold in order to obtain stable suprathreshold reflex responses as well as a tolerable pain sensation reported by the volunteers. Nitrous oxide administration resulted in a potent depression of the two components of the blink reflex. This depressive effect was more marked upon the late (R2) nociceptive component (83%) than upon the early (R1) component (41%). Simultaneously, subjects reported either a decrease in pain sensation or an indifference toward the painful stimulus. None of these effects were reversed by a double-blind intravenous naloxone (1.4 mg) injection. The analgesic effect of nitrous oxide is a nonspecific depressant action on the transmission of the nociceptive messages in central nervous structures, independent of pain-suppressive endogenous morphine-like systems. (Key words: Anesthetics, gases; nitrous oxide. Antagonists, narcotic: naloxone. Pain: experimental.)

Although the analgesic properties of nitrous oxide have been largely demonstrated in humans,1-5 its mechanisms of producing analgesia remain a matter of debate. Several hypotheses have been proposed. One of the most likely is that nitrous oxide could activate some endogenous morphine-like systems involved in pain control. This is supported by the observations that the opioid antagonist naloxone can reverse nitrous oxide analgesia in mice6 and that a cross-tolerance between morphine and nitrous oxide was obtained in both mice and rats.7 Supporting the same idea, it was also observed that nitrous oxide produced reduction in ischemic pain8 and depression of cerebral evoked potentials to painful tooth pulp stimulation in humans9 that were naloxone reversible. From these data, it appears clearly that if some endogenous morphine-like substances are involved in the mechanisms of nitrous oxide analgesia, one can expect a depressive effect of this gas mixture on the transmission of nociceptive messages. Furthermore, in order to confirm the hypothesis, this depression must be reversed by naloxone.

An interesting model for studying drug action on the central nervous system (CNS) can be the electrophysiologic features of the electrically evoked blink reflex in humans. Since the pioneer study of Kugelberg10 this brain stem reflex has been extensively investigated.11-13 It is well known that electrical stimulation of supraorbital nerve elicits a double-component reflex response in the ipsilateral orbicularis oculi muscle; the first one (R1) is of short latency (9-11 ms) and is transmitted through an oligosynaptic reflex arc involving the nucleus caudalis of the trigeminal nerve and projecting to the motoneuronal pool of the facial nerve14-16; the second response (R2) is of longer latency (25-35 ms) and its multisynaptic central pathway includes numerous brain stem structures involving some regions of the mesencephalic reticular formation.14-16 Although the functional aspect of the R1 response remains unclear, all authors agree on the nociceptive function of the R2 component of the blink response, since its appearance produces the eyelid closure and thus ensures protection of the eyeball against a nociceptive stimulus.10-12 Furthermore, this brain stem reflex has been proposed as a good model for studying nociception in humans, since it is strongly modulated by peripheral and

* Associate Professor of Physiology.
† Resident in Anesthesiology.
‡ Fellow in Anesthesiology.
§ Professor in Anesthesiology.
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Address reprint requests to Dr. Willer.
central influences and can be depressed by analgesic maneuvers such as electroacupuncture or transcutaneous electrical nerve stimulation.\textsuperscript{17,18}

Thus, the aim of the present study was to investigate the following: 1) the depressive effect of nitrous oxide on the two components of the blink reflex; 2) the possible naloxone reversibility of this effect, using a double-blind paradigm in normal volunteers.

Materials and Methods

The experiments were carried out with seven unpaid volunteers (six men, one woman, 27–41 yr old). They were carefully briefed on the aim and procedure of this study in order to avoid any element of surprise or anxiety, which are known to modify spinal reactivity.\textsuperscript{19,20} They gave their informed consent according to the principles of the Helsinki Convention. During the sessions, they sat comfortably in armchairs, eyes gently closed, so as to obtain a state of good muscular relaxation.

Electrophysiologic Procedure

The methodologic details for eliciting and recording the blink reflex responses have been described extensively in previous papers.\textsuperscript{17,18} Briefly, electrical stimulation of the supraorbital nerve was achieved using a pair of surface electrodes placed on the skin above the nerve. The stimulus consisted of a double shock of rectangular pulses of 0.2 ms duration, each spaced by 1 ms, delivered continuously throughout the 40–60-min period of each session by a constant-current stimulator at a rate of 20 Hz.

Reflex activities were recorded from the ipsilateral orbicularis oculi muscle by means of surface electrodes placed on the degreased skin above the muscle in question. After amplification, the reflex responses were displayed in parallel on a storage oscilloscope (photographs and monitoring) and on an analog converter, where the reflex activity was full wave rectified and the surface area was digitized in arbitrary units (AU) by means of a digitizer integrator. Figure 1 summarizes the experimental set up. The stimulus intensity was chosen in order to elicit an electromyographic reflex response of two to three times the reflex threshold (usually between 12 and 15 mA) according to a procedure described previously.\textsuperscript{17,18} These stimulation parameters have been selected for the present study because they are able to elicit stable control responses and to produce a slight sensation of pain that parallels the variations of the nociceptive component (R2) of the blink reflex.\textsuperscript{17,18}

Pharmacologic Procedure

After a 15-min control period of air breathing, subjects were connected to an open-circuit mask in which a constant flow (10 l/min) of nitrous oxide (33%) and oxygen (66%) was given for a period of 25–30 min. Ten to fifteen minutes after the onset of nitrous oxide administration, naloxone hydrochloride (1.2 mg; 3 ml total doses) or placebo (saline, 3 ml) were injected (iv) into the canula of an isotonic glucose infusion placed into a vein of the forearm at the beginning of the session. Drugs were slowly injected (2 to 4 min) using a random cross-over technique and a double-blind paradigm, while nitrous oxide was still given. Thus, for a given session, lasting between 40–60 min, the general experimental procedure consisted in a continuous study of two components of the blink reflex before and during breathing a mixture of 33% nitrous oxide, during which naloxone or placebo were given intravenously. At the end of each session, subjects were asked to describe their subjective sensations related to the electrical stimulus and to nitrous oxide inhalation.

For each subject, numeric values for R1 and R2 responses were expressed as percentage of control value so as to allow an interindividual comparison. Global data then were studied in terms of a variance analysis. The
FIG. 2. Individual example showing the depressive effect of 33% nitrous oxide inhalation (lower black bar) and the lack of naloxone (arrow) reversal upon the two components of the blink reflex. Upper: Blink responses before (a), during nitrous oxide, (b), and after subsequent naloxone administration (c). Each trace is a superimposition of two responses. Calibration: horizontal: 10 ms; vertical: 200 μV. Lower: Graph showing the evolution of the digitized R1 (broken line) and R2 (solid line) responses before and during nitrous oxide administration and after naloxone injection. Data are expressed in terms of percentage of control values (100%).

significance in variations was studied with a factorial analysis and with the paired t test.

Results

Characteristics of Control Responses

In all subjects, during air breathing, suprathereshold stimulation of supraorbital nerve elicited a classical double components blink reflex response recorded from the ipsilateral orbicularis oculi muscle (fig. 1). The first response R1 was of short latency (10 ± 1 ms) and usually of diphasic or triphasic shape, while the second response R2 was largely multiphasic, of longer duration, and of longer latency (28 ± 6 ms). These reflex responses had the same threshold (Thr. = 6 ± 1 mA) and did not show any sign of facilitation or of habituation as a function of repetition.

FIG. 3. Individual example showing the depressive effect of nitrous oxide (lower black bar) on the blink responses before and after intravenous injection of saline (arrow). Upper: Blink responses before (a), during nitrous oxide, (b), and after subsequent saline administration (c). Each trace is a superimposition of two responses. Calibration: horizontal: 10 ms; vertical: 200 μV. Lower: Graph showing the evolution of the digitized R1 (broken line) and R2 (solid line) responses before and during nitrous oxide administration and after saline injection. Data are expressed in terms of percentage of control values (100%).
of stimulation for a suprathreshold intensity (2–3 Thr.) delivered at the low rate (0.20 Hz) used in this study. Such a stimulus also produced a painful sensation compared with a needle prick, locally around the electrodes and projecting into the cutaneous receptive field of supraorbital nerve. This painful sensation was always well tolerated by the subjects.

EFFECTS OF NITROUS OXIDE

In all subjects, inhalation of 33% nitrous oxide resulted in a rapid and significant depression of the two components of the blink reflex response. As shown in figures 2 and 3 for an individual example, the depressive effect of nitrous oxide occurred after 2–3 min, reached a maximum by the 6–8 min, and remained stable at this level as long as the gas mixture was administered. It also clearly appears from figures 2 and 3 that, at the maximal effect, nitrous oxide induced a more potent and significant ($t = 8.7; N = 7; P < 0.001$) depression upon the noxious component (R2) of the blink reflex (83% depression; $t = 16.45; N = 7; P < 0.001$) than upon the early component R1, which was, however, significantly lowered by 41% of its control values ($t = 9.06; N = 7; P < 0.001$). Global data are shown in figure 4. Parallel to these electrophysiologic modifications, nitrous oxide also produced homogenous and stereotypical subjective sensations, which were reported by all subjects. After five or six ventilatory cycles of nitrous oxide inhalation, the subjects described a sensation of sleepiness with heaviness of the body as well as paresthesias of the lips and of the distal parts of the upper and lower limbs. These sensations were reported as pleasant by most of the subjects. Simultaneously, they described that the electrically induced pain was either absent or decreased, and in all cases subjects reported a kind of indifference towards the painful stimulation.

EFFECTS OF NALOXONE

As can be seen from figures 2 and 3 for an individual example and from figure 4 showing global data, neither naloxone nor saline were able to produce any significant modification in the nitrous oxide-induced depression of the two components of the blink reflex. Furthermore, naloxone or saline administration did not produce any subjective side effect that was reportable by the subjects. No significant subjective after effect resulting from nitrous oxide or to naloxone were reported at the end of the experiments, i.e., after a 15–20-min period of air breathing following nitrous oxide administration.

Discussion

This study shows that inhalation of a gas mixture of 33% nitrous oxide 66% oxygen is able to produce a potent depression of the electrophysiologic features, particularly the noxious component (R2) of the human blink reflex. This depressive effect was paralleled by hypoalgesia with a kind of indifference to painful stimulation. All these effects were observed as long as nitrous oxide was administered and were not reversed by intravenous naloxone injection, using a cross-over double-blind method with saline as placebo. To a certain extent, our present data are similar to those described by others that have shown that nitrous oxide inhalation resulted in a decrease in amplitude of the tooth pulp stimulation evoked cerebral potentials as well as of the visual, auditory, and somatosensory evoked potentials in humans. In acute spinal cats, nitrous oxide has been shown to depress the amplitude of spinal reflexes in a dose-dependent manner. However, in this latter study, the authors observed that the mono-
synaptic reflexes were much more affected than the polysynaptic ones by nitrous oxide.\textsuperscript{22} In contrast, in our study, we found that the oligosynaptic component (R1) was less affected than the nociceptive largely multisynaptic component (R2) of the blink reflex. This discrepancy can be explained from several points of view.

A methodologic aspect is related to the site of stimulation (dorsal root) used by Sugai et al.\textsuperscript{22} for eliciting both monosynaptic and polysynaptic ventral root reflex responses. Large-diameter proprioceptive fibers are activated by the polysynaptic-induced stimulation and can modify this polysynaptic reflex activity, since the inhibitory effect of such large-diameter fibers has been largely demonstrated in the peripheral control of nociceptive input (23–25). One might expect different data if the authors had stimulated a muscular nerve and a cutaneous nerve separately to obtain a monosynaptic and a polysynaptic ventral root reflex, respectively. Another point that can explain the discrepancy between our data and those of Sugai et al.\textsuperscript{22} consists in the model used for each study: while we were investigating a brain stem reflex in normal awake humans, their work concerned lumbar reflex activity in acute spinal and anesthetized cats. Thus, our data are more related to the effects of nitrous oxide upon the brain stem and other related central nervous structures involved in both pathway and control of the blink reflex activity than in the spinal action of this gas mixture. In this respect, it is easy to explain that in our study, the nociceptive (R2) component of the blink reflex of which the central path involves reticular formation was more affected by nitrous oxide than the early (R1) component, since it has been shown that synaptic transmission in this area is very sensitive to the depressive action of various anesthetic drugs.\textsuperscript{26,27} This idea is reinforced by other observations that have shown that the R2 component of the blink reflex is more depressed than the early one (R1) in comatose states of various origin but of which the common feature is a nonspecific depression of brain stem structure activity.\textsuperscript{28} Concerning the depressive effects of nitrous oxide on the first component (R1) of the blink reflex, one can assume that, because of its disynaptic pathway,\textsuperscript{29} the main effect of the gas mixture could be located directly on the motoneuronal pool of the facial nerve, as described previously for monosynaptic reflexes in both humans and animals (see references in Sugai et al.\textsuperscript{22}). At this level, nitrous oxide can induce inhibitory processes involving either presynaptic or postsynaptic mechanisms responsible for this inhibition. However, it would be interesting to pursue animal studies, using intracellular microelectrodes recordings and other related techniques, in order to specify the cellular mechanism of the depressive action of nitrous oxide.

While, in previous studies, we observed that morphine chlorhydrate produced a naloxone-reversible dose-dependent depression of nociceptive flexion reflexes,\textsuperscript{30} and of the blink reflex responses (ongoing unpublished study), our present data shows the failure of naloxone to reverse the depressive effects of nitrous oxide on both blink reflex and pain sensation. These data are consistent with those of Smith et al.,\textsuperscript{31} who reported that naloxone had no effect on nitrous oxide anesthesia. In contrast, they are at odds with those of others,\textsuperscript{8,9} which have shown that the depression of the cerebral evoked potential as well as the analgesia produced by 33% nitrous oxide inhalation were partially reversed by naloxone, when using a nociceptive tooth pulp electrical stimulation as a model for experimental pain. These different data clearly show that the mechanism of nitrous oxide analgesia in humans still remains a matter of debate according to the model that has been used for studying the nociceptive message.

However, from the present work, it appears that the analgesic effect of nitrous oxide could be related to a general nonopiate related depressive mechanism in the synaptic transmission of nociceptive messages within central nervous integrative structures. Further experiments involving both electrophysiologic and pharmacologic techniques would be necessary for assessing this hypothesis.

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