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Naloxone Has No Effect on Nitrous Oxide Anesthesia

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It has been reported that naloxone antagonizes general anesthesia in rats when the tail clamp is used as a painful stimulus to assess anesthesia.[1] The authors' hypothesis is that this antagonism is to the analgesic component of anesthesia only, and that anesthesia assessed by a non-painful stimulus would not be antagonized by naloxone. Therefore, the anesthetic potency of nitrous oxide in mice was measured using loss of the righting reflex as a non-painful stimulus. Naloxone, 2 and 16 mg/kg, intraperitoneally, failed to antagonize nitrous oxide anesthesia measured 14–39 min after injection. Thus, 19 min after injection of naloxone, 2 mg/kg, the nitrous oxide ED_{50} was 1.25 ± 0.060 atm (n = 35), compared with 1.19 ± 0.053 atm (n = 35) after injection of saline solution (control). Following naloxone, 16 mg/kg, the nitrous oxide ED_{50} was 1.18 ± 0.059 atm (n = 35), compared with 1.22 ± 0.059 atm (n = 35) for saline solution. At neither dose of naloxone was the ED_{50} different from the control ED_{50}, a finding that supports the authors' hypothesis. (Key words: Analgesia: measurement. Anesthetics, gases: nitrous oxide. Antagonists, narcotic: naloxone.)

BERKOWITZ AND CO-WORKERS[2] have shown that the dose-related analgesia (measured by the writhing response to intraperitoneal injection of phenylquinone in mice) produced by nitrous oxide is reversed by naloxone. They suggested that the analgesia associated with general anesthesia may be related to the release of endogenous opiates. Finck *et al.* recently studied the effect of naloxone on general anesthesia, based on the possibility that anesthetics may act partly by releasing a morphine-like substance.[1] Using constant doses of halothane, enflurane, or cyclopropane such that about 40 per cent of the animals responded to a tail clamp applied for 30 sec, they found that the average number of animals responding increased from 40 to 70 per cent when naloxone was given. Since analgesia is part of the state of anesthesia, they concluded that naloxone partially antagonizes general anesthesia. We believe this conclusion may be related to the painful stimulus they used to assess anesthesia.

Any measure of anesthesia must involve both a stimulus and a purposeful response. The concentration of anesthetic necessary to block a response increases with the intensity of the stimulus until a plateau of supramaximal stimulation is reached, after which no further increase in dose is needed.[3] In the experiments of Finck *et al.*[1] the physical stimulus (tail clamp) was the same for both naloxone-treated and control animals. However, at a given anesthetic concentration, the perception of this painful stimulus in the central nervous system may have been greater in the naloxone-treated than in the control animals, so that they responded more. We have performed experiments using the rotation of an animal's cage as a nonpainful stimulus. This stimulation of the righting reflexes provides an observable anesthetic end-point that yields ED_{50} values close to those obtained using painful stimuli.[4]

Methods

We used a 34-l steel pressure chamber equipped with a circulating fan, carbon dioxide scrubber, temperature control device, and motors to rotate the cages.[5] Eight mice received intraperitoneal injections of naloxone hydrochloride, 2 or 16 mg/kg, and eight control mice received injections of physiologic saline solution. The latter dose of naloxone is at least 32 times the dose that was found to antagonize completely the analgesic effect of either fentanyl alone or fentanyl combined with droperidol for at least 30 min when injected intraperitoneally in mice.[6] Seven each of the naloxone-treated and the control mice were placed in individually marked wire mesh cages. Two sets of seven cages were mounted in front of different windows in the pressure chamber. Chamber temperature was monitored and adjusted to maintain the rectal temperatures of the two remaining restrained mice between 36.5 and 38 C. The chamber was sealed and the oxygen partial pressure increased to 0.5 atm. Nitrous oxide was then immediately added to the desired partial pressure, a total of 9 min after injection. The rolling response was measured 5, 10, 20, and 30 min after exposure to nitrous oxide. Two observers, who were unaware of the treatment each mouse had received, each recorded the response of seven animals. The cages were rotated at 4 rpm. All mice that rolled over completely during more than one revolution of a five-revolution sequence were scored 0. Mice remaining upright or falling over during one revolution only were scored 1. We used new groups of mice for each of five doses of nitrous oxide (0.98, 1.13,

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TABLE 1. ED_{50} Values for Loss of Righting Responses during Exposures to Five Doses of Nitrous Oxide in Control and Naloxone-treated Mice

	Time after Injection of Naloxone (Min)	Time after Nitrous Oxide Exposure (Min)	Saline Solution $ED_{50} \pm SE$ (Atm) n = 35 (Seven Animals at Each N_2O Dose)	Naloxone $ED_{50} \pm SE$ (Atm) n = 35 (Seven Animals at Each N_2O Dose)	Significance of Difference Between ED_{50} Values
Naloxone, 2 mg/kg, ip	14	5	1.08 ± 0.036	1.11 ± 0.034	$P = 0.92$
	19	10	1.19 ± 0.053	1.25 ± 0.060	$P = 0.90$
	29	20	1.20 ± 0.057	1.31 ± 0.077	$P = 0.84$
	39	30	1.16 ± 0.035	1.23 ± 0.039	$P = 0.82$
Naloxone, 16 mg/kg, ip	14	5	1.17 ± 0.095	1.05 ± 0.107	$P = 0.38$
	19	10	1.22 ± 0.059	1.18 ± 0.059	$P = 0.60$
	29	20	1.25 ± 0.059	1.29 ± 0.061	$P = 0.60$
	39	30	1.18 ± 0.060	1.27 ± 0.062	$P = 0.14$

1.17, 1.22, and 1.35 atm for the 2 mg/kg naloxone series of experiments and 1.01, 1.14, 1.26, 1.40, and 1.52 atm for the 16 mg/kg naloxone series).

The resulting five-point dose–response curves were analyzed by logit methods.[7] First, the curves obtained with naloxone and with saline solution were analyzed allowing their slopes to vary independently. Since there was no significant difference between the two slopes, a second analysis assuming a common slope for the two curves was performed to obtain ED_{50} values with their standard errors.

Results

The ED_{50} values for the control and naloxone-treated mice were not significantly different (table 1). A t test for paired data of the individual responses in each group at each dose also failed to show significant differences between the groups. The ED_{50} values 5 min after exposure showed evidence of a rapidly developing acute tolerance, previously reported by Smith *et al.*§ The later ED_{50} values were higher than the 5-min ED_{50} values in both control and naloxone-treated mice. Therefore, we conclude that naloxone had no effect on the development of acute tolerance.

Discussion

We must consider the possibility that the ineffectiveness of naloxone in our experiments resulted from its rapid removal from the brain. Ngai *et al.*[8] have shown that in rats given naloxone, 5 mg/kg, intravenously, drug levels decayed with a half-life of 30 min in serum, and that brain levels paralleled serum levels. Furthermore, in mice, Smith[6] has shown that as little as 0.5 mg/kg naloxone administered intraperitoneally completely reversed both the analgesic and the respiratory depressant effects of fentanyl (0.4 and 0.8 mg/kg), but that there was an observable return of analgesia at 45–60 min. It thus seems improbable that ineffective levels of naloxone were present in our experiments, particularly at the highest dose used.

Thus, our results show that naloxone has no effect on the ability of nitrous oxide to prevent mice from perceiving spatial disorientation, whereas Berkowitz *et al.*[2] have shown that naloxone reverses nitrous oxide-induced analgesia assessed by a noxious writhing test. There is no contradiction in these observations, since the latter deals purely with the analgesic effect of nitrous oxide whereas the former does not involve any anti-nociceptive response. Classically, general anesthesia consists of several components, which include loss of consciousness, amnesia, analgesia, and depression of reflexes. The experiments of Finck *et al.*[1] measured the ability of rats that had central nervous system depression to respond to a painful stimulus. This provides a composite end-point that includes analgesia and, probably, loss of consciousness and the depression of reflexes. The rolling response, which we used, is also a composite end-point, which probably includes both loss of consciousness and depression of reflexes. Although we do not know what proportions of these components of anesthesia are represented by the tail clamp and the rolling response end-points, the three sets of observations are consistently explained if naloxone can antagonize the analgesic but not the other components of anesthesia caused by nitrous oxide.

Several studies have suggested that naloxone may also antagonize certain effects of other non-opiate central nervous system depressants. Blum *et al.* have reported that ethanol-induced sleeping time in mice is decreased significantly by naloxone, 5 mg/kg, intraperitoneally.[9] Fürst *et al.* showed that naloxone, 1 mg/kg, delayed the onset and decreased the duration of pentobarbital- or methohexital-induced loss of

§ Smith RA, Winter PM, Smith M, et al: Rapidly developing tolerance to an acute exposure of nitrous oxide (abstr. American Society of Anesthesiologists Annual Meeting, 1976, pp 313–314.

righting reflex (sleeping time) in rats.[10] However, without knowledge of the brain levels of the depressant drugs used in these experiments, and because of the problems of associating changes in sleeping time with shifts in dose–response curves, it is difficult to assess the true difference between results in naloxone-treated and control animals in these studies. In our studies the inspired doses of nitrous oxide and the rectal temperatures of the mice were kept constant, and thus brain levels of nitrous oxide would be constant and equal in both naloxone-treated and control mice. Thus, even though anesthetics and other non-opiate central nervous system depressant drugs might release an opiate-like factor in the brain,[1] there is at present no unequivocal evidence that this contributes to any other than the analgesic component of the state of general anesthesia.

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