Antagonism of General Anesthesia by Naloxone in the Rat

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The effect of naloxone, a narcotic antagonist, on the response of animals to painful stimuli during anesthesia was studied. Rats were anesthetized with cyclopropane, halothane, or enflurane in groups of 12. Following induction, inspired anesthetic concentration was gradually reduced to a point at which 35-60 per cent of animals responded to tail clamping. Thereafter the anesthetic concentration was held constant for 30 minutes. Rats in each group then received saline solution or naloxone, 10 mg/kg, given intravenously. The response to tail clamping was retested 5 minutes later. In additional experiments EEG's were recorded from rats anesthetized with one of these anesthetics. After a stable light plane of anesthesia had been attained, each animal was given naloxone, 10 mg/kg, iv, and the EEG recorded for an additional 5 minutes. In the tail-clamping experiments, naloxone approximately doubled the number of rats responding during cyclopropane, halothane, or enflurane anesthesia. The EEG patterns of several animals anesthetized with either cyclopropane or halothane changed to patterns consistent with lighter planes of anesthesia after naloxone administration. That naloxone alters the depth of inhalational anesthesia suggests that anesthetics may release an endogenous morphine-like factor (MLF) in the central nervous system. (Key words: Analgesics, narcotic, opiate receptor; Anesthetics, gases, cyclopropane; Anesthetics, volatile, enflurane; Anesthetics, volatile, halothane; Antagonists, narcotic, naloxone; Theories of anesthesia.)

IN THE LAST SEVERAL YEARS specific "opiate receptors" have been found in the mammalian central nervous system.[1-4] Several groups of investigators have also isolated substances from mammalian brain[5-7] and pituitary,[8] known variously as "enkephalin," "endogenous morphine-like substance" or "endorphine." They bind specifically to purified opiate receptor preparations *in vitro*. The binding of these endogenous morphine-like factors (MLF) is antagonized by naloxone, a narcotic antagonist with little or no known agonistic activity. Some of these substances have been chemically identified as pentapeptides.[9] More recently, Akil, Mayer and Liebeskind[10] found in rats that naloxone partially antagonized the analgesia produced by focal electrical stimulation of the periaqueductal grey area of the brain, using the tail-flick test to measure analgesia. Their results suggested that electrical stimulation-induced analgesia may be (partly) caused by the release of MLF.

We have tested the effects of nitrous oxide upon the writhing response to the intraperitoneal injection of phenylquinone in mice. The dose-related analgesia produced by nitrous oxide was reversed by pretreatment with naloxone, 5 mg/kg, sc.[11] On the basis of this observation, we decided to investigate the effect of naloxone on the state of general anesthesia, based upon the possibility that anesthetics may act, in part, by causing the release of MLF.

Methods

Male Sprague-Dawley rats weighing between 140 and 200 g were placed in individual metal chambers (volume 0.40 l) having fitted clear plastic covers. Twelve animals were used for each experiment. The tail of each animal protruded through a rubber grommet-covered hole at one end of the chamber. The opposite end of each chamber was attached by means of small-bore polyethylene tubing to a gas manifold. Halothane or enflurane was delivered to the manifold from temperature-compensated calibrated vaporizers (Fluotec Mark II for halothane, Ethranetec for enflurane, both from Cyprane, Ltd.) at a flow rate of 6 l/min. Both vaporizers were checked for linearity of vapor concentrations delivered at dial settings, at the same gas flow, by gas chromatography (Perkin-Elmer Model 154D). Cyclopropane in oxygen was delivered to the manifold via calibrated flowmeters (Ohio Medical); flow rates ranged from 2.5 to 4.2 l/min. Anesthesia was induced using 3 per cent halothane or 4 per cent enflurane for 5 min, or 33 per cent cyclopropane for 10 min. The concentration of halothane or enflurane was then reduced in decrements of no more than 0.5 per cent initially, and after 25 min, no more than 0.25 per cent at 15-min intervals. The concentration of cyclopropane was reduced to 22 per cent after induction and then further reduced in decrements of no more than 2 per cent at 8-min intervals. Rectal temperature was measured using thermocouple probes attached to an electronic thermometer (Yellow Springs Instruments) and maintained between 36-38 C using an infrared heating lamp.

A hemostat applied to the tail for 30 sec was used as the stimulus, and movement of a leg or lifting of the head was taken as a positive response. Prior to tail clamping, the anesthetic concentration was kept at a given level for at least 10 min when using halothane or enflurane, at least 5 min using cyclopropane, and then increased or decreased if necessary.

TABLE 1. Results of Experiments Using Halothane

Injection	Responding before Injection		Responding after Injection		Net Change	Δ Per Cent
	Number	Per Cent	Number	Per Cent		
Saline solution	8/12		7/12		−1	
Saline solution	3/12		2/12		−1	
TOTAL	11/24	46	9/24	38	−2/24	−8
Naloxone	7/12		12/12		+5	
Naloxone	2/12		7/12		+5	
Naloxone	7/12		9/12		+2	
Naloxone	1/12		5/12		+4	
TOTAL	17/48	35	33/48	69	+16*/48	+33

* Different from results following iv injection of saline solution, $P < 0.005$.

TABLE 2. Results of Experiments Using Enflurane

Injection	Responding before Injection		Responding after Injection		Net Change	Δ Per Cent
	Number	Per Cent	Number	Per Cent		
Saline solution	7/12		9/12		+2	
Saline solution	5/12		5/12		0	
Saline solution	6/12		7/12		+1	
TOTAL	18/36	50	21/36	58	+3/36	+8
Naloxone	6/12		9/12		+3	
Naloxone	5/12		10/12		+5	
Naloxone	6/12		9/12		+3	
Naloxone	4/12		9/12		+5	
TOTAL	21/48	44	37/48	77	+16*/48	+33

* Different from results following iv injection of saline solution, $P < 0.005$.

TABLE 3. Results of Experiments Using Cyclopropane

Injection	Responding before Injection		Responding after Injection		Net Change	Δ Per Cent
	Number	Per Cent	Number	Per Cent		
Saline solution	3/10		4/10		+1	
Saline solution	4/9		2/9		−2	
Saline solution	4/11		3/11		−1	
TOTAL	11/30	37	9/30	30	−2/30	−7
Naloxone	3/12		6/12		+3	
Naloxone	5/11		9/11		+4	
Naloxone	5/12		8/12		+3	
Naloxone	4/11		10/11		+6	
TOTAL	17/46	37	33/46	72	+16*/46	+35

* Different from results following iv injection of saline solution, $P < 0.005$.

For each group of animals, the aim was to find, in this manner, an effective dose (concentration) of the anesthetic at which 35–60 per cent of the animals did not respond to the stimulus, i.e., ED_{35-60}. Forty-five minutes or more after induction of anesthesia, the inspired anesthetic concentration was kept constant for at least three consecutive test periods (10 min per period for halothane and enflurane, 5 min per period for cyclopropane), and the response of the group was reproducible. Thereafter, beginning 5 min after the last test period, each animal was given an intravenous injection (0.7 ml) of either physiologic saline solution or naloxone HCl, 10 mg/kg. Five minutes after the injection each animal was retested for response to the stimulus.

Statistics were performed using a two-tailed chi-squared test with continuity correction for 2 × 2 contingency tables.[12]

In additional experiments, individual rats were anesthetized following the sequence already described, i.e., using overpressure for induction and decrementally reducing the anesthetic concentration. Electroencephalograms from these rats were recorded on an Offner Dynograph using 30-gauge steel needles placed bitemporally and subcutaneously as electrodes. An attempt was made to reduce the anesthetic concentration gradually to one at which the animal did not move in response to a hemostat applied to the tail, which could not be further reduced without the animal's responding. This inspired concentration, always achieved by going from higher to lower concentrations, was maintained for at least 10 min, after which the animal was given naloxone, 10 mg/kg, iv, and the EEG recorded for a further 5-min period.

Results

Six experiments with 12 rats each were performed using halothane as the anesthetic. The concentration needed to abolish the response to tail clamping in 35–60 per cent of the animals was 1.0–1.1 per cent. Two groups received physiologic saline solution and four groups were treated with naloxone. Of 24 animals that received saline solution, 11 responded to the stimulus prior to injection and nine responded 5 min after injection, a net decrease of two animals responding. Of 48 animals treated with naloxone, 17 responded prior to and 33 responded after injection, an increase of 16 animals responding. Results are summarized in table 1.

Another seven experiments were performed using enflurane. The concentrations needed to abolish the response to tail clamping in 40–60 per cent of the animals ranged from 1.9 to 2.5 per cent (average 2.1 per cent). Three groups of animals received saline solution and four, naloxone. Of the 36 animals receiving saline solution, 18 responded prior to injection and 21 after injection, an increase of three

animals responding. Among the 48 animals treated with naloxone, 21 responded prior to and 37 after injection, an increase of 16 animals responding. Results are summarized in table 2.

Cyclopropane was used in another seven experiments with 12 rats each. With the protocol described, an occasional animal died during or shortly after induction from what appeared to be airway obstruction. The concentration of cyclopropane needed to abolish response to tail clamping in 35–60 per cent of the animals was 19–20 vol per cent. Three groups of animals received saline solution and four, naloxone. Of the 30 rats that received saline solution, 11 responded prior to and nine responded after injection, a net decrease of two animals responding. Among the 46 animals receiving naloxone, 17 responded before and 33 responded after injection, an increase of 16 animals responding. These results are summarized in table 3.

For each anesthetic, among saline-treated control groups there was no significant change in the number of animals responding to tail clamping after the injection. However, following treatment with naloxone, the increase in the number of animals responding to the stimulus was significant, $P < .005$.

In two experiments each with halothane and enflurane, those animals that did not respond to the stimulus prior to naloxone injection but did respond 5 min after the injection of naloxone failed to respond one hour later, *i.e.*, they became reanesthetized. The responses of the rest of the animals (whether present or not) remained constant throughout this period.

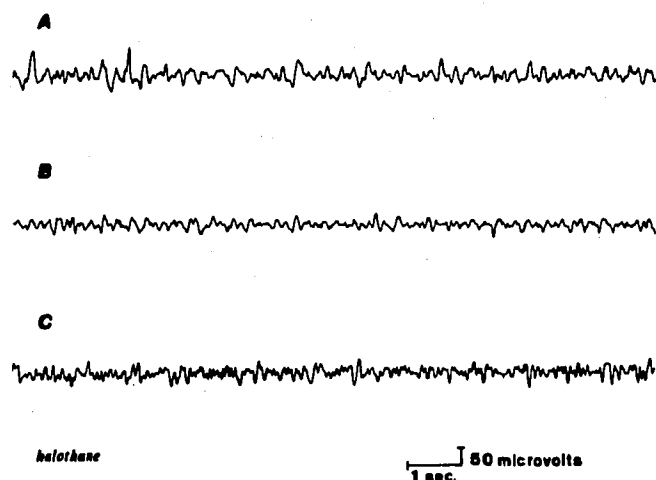In the EEG experiments, halothane was used in

FIG. 1. EEG from a rat during halothane anesthesia. A, baseline record obtained with 1.1 per cent halothane maintained constant throughout. B, EEG from the same animal 1 min after the iv injection of naloxone, 10 mg/kg; note decrease in slow-wave activity. C, EEG from the same animal 5 min after naloxone injection; increased fast-wave activity is present.
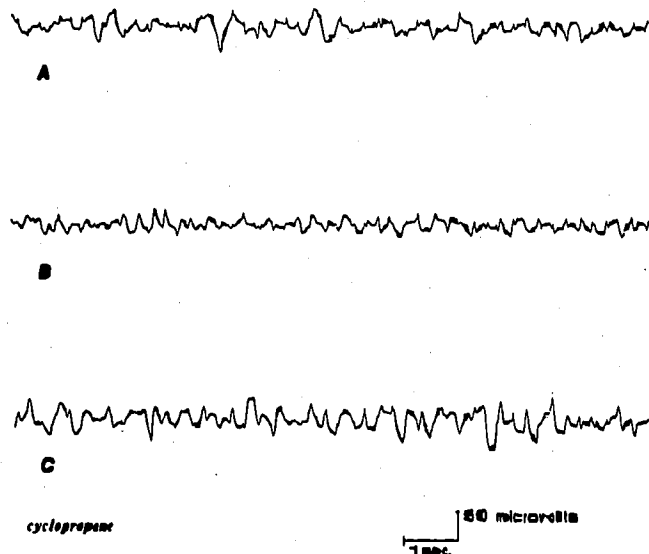

FIG. 2. EEG from a rat during cyclopropane anesthesia. A, baseline record obtained using 20 per cent cyclopropane, maintained constant throughout. B, EEG from the same animal 2 min after the iv injection of naloxone, 10 mg/kg. C, EEG from the same rat 5 min after naloxone administration. The record is more rhythmic than that in A.

six animals at an inspired concentration of 1.0–1.25 vol per cent during the time naloxone was injected. Three of these animals showed no apparent change in EEG pattern after naloxone administration, and none of the six animals responded to tail clamping 5 min after naloxone administration. However, in three animals the EEG pattern appeared to change after naloxone administration. Parts of the records obtained from one of these are shown in figure 1. The initial EEG pattern (fig. 1A) showed slow-wave activity at 2–6 Hz and an amplitude of 50 microvolts, with only a minimal fast-wave component. A minute after the administration of naloxone, 10 mg/kg, iv, there was a decrease in this slow-wave activity (fig. 1B). At 5 min (fig. 1C) a noticeable increase in fast-wave activity at 10–15 Hz and an amplitude of 10–25 microvolts with a continued reduction in slow-wave activity was apparent.

The EEG's of four rats anesthetized with enflurane (2.0–2.3 per cent) failed to show any apparent change in pattern after the administration of naloxone. The presence of hypersynchronous burst activity complicated the interpretation of these records.

Only one of four rats anesthetized with cyclopropane (20 per cent) had a change in EEG pattern after injection of naloxone. Portions of this record are shown in figure 2. In figure 2A the animal is anesthetized with 20 per cent cyclopropane. The EEG consisted of slow waves at 2–4 Hz and amplitude of 50–75 microvolts (the record also contains EKG artifact). Two minutes after administration of

naloxone (fig. 2B) the EEG pattern became more rhythmic at approximately the same frequency and an amplitude of 20–40 microvolts. Five minutes after naloxone injection the EEG pattern was still rhythmic at 2–6 Hz with an amplitude of 40–60 microvolts.

Discussion

These results demonstrate that naloxone is capable of partially antagonizing the anesthetic actions of cyclopropane, halothane and enflurane. That these rats were on the linear portion of an anesthetic log dose–response curve (as evidenced by the ED_{35-60} range used) is important. At a higher anesthetic concentration some of the animals might have been anesthetized so deeply that the effect of naloxone would not have been detected using the endpoint we had chosen. Obviously, had a lower concentration of anesthetic been used, most of the animals would have already been responding to the stimulus prior to treatment with naloxone. We did not succeed in adjusting the anesthetic concentration to the range of ED_{35-60} in all experiments. However, all results are included in the tables.

There are several possible explanations of these results. There was some variation in the depths of anesthesia, as demonstrated by the changing responses of rats receiving injections of saline solution. However, this variation was small, as can be seen in tables 1–3. A change in body temperature is known to alter the anesthetic requirement.[13] However, rectal temperature was monitored and maintained between 36 and 38 C. Although there was some variation within this range, the rate of change of temperature at the time of naloxone injection was slow. Were temperature changes the reason for the results, one would expect that the saline-treated control groups should have been affected to the same extent.

Of interest is the finding that animals treated with naloxone appeared to become reanesthetized an hour later. This is additional evidence for a drug effect. We reported previously that the naloxone concentration in rat brain an hour after intravenous injection of 5 mg/kg had decreased to less than 15 per cent of the concentration attained at 5 min.[14] The short duration of action of naloxone against anesthesia might therefore be expected, as is seen in its clinical use as a narcotic antagonist.[15]

Changes in EEG pattern were not demonstrated in every animal, but this is not surprising in view of individual variations in anesthetic requirements. Presumably any effect of naloxone in partially antagonizing the effects of anesthesia upon the EEG would be masked at deeper levels. Naloxone administration to awake rats with chronically implanted cortical electrodes is without effect upon the EEG.[16] The EEG records that did show a change in pattern after naloxone invariably changed to a pattern consistent with a lighter plane of anesthesia. For halothane this was evidenced by a decrease in slow-wave activity and an increase in fast-wave activity. For cyclopropane the change was from a complex rhythm to a more rhythmic pattern.[17] The EEG results serve as supportive evidence that naloxone is capable of antagonizing the effects of general anesthetics.

Naloxone may have some hitherto unrecognized action in antagonizing general anesthesia, though a nonspecific analeptic action of the drug has not been reported.

The results suggest to us that anesthetics may cause the release of an endogenous morphine-like factor (MLF), which binds to the receptor complex. Naloxone would then antagonize this effect by altering the receptor activation state or by displacing MLF from the receptor. Anesthetics could also act directly upon the membrane–receptor complex, thereby increasing receptor affinity for the agonist, MLF. These possibilities are not mutually exclusive.

Direct evidence that naloxone inhibits the effects of MLF upon opiate receptors has been demonstrated *in vitro*. This is based upon tissue-bath studies using the guinea pig myenteric plexus–longitudinal muscle preparation and the mouse vas deferens. Both tissues contain specific opiate receptors, and the twitch-like response to electrical stimulation is inhibited by narcotics. This inhibition is reversed by naloxone. Two specific MLF's (leucine–enkephalin and methionine–enkephalin) act like opiates in these preparations, and these effects are completely reversed by naloxone.[9] The same has been shown for MLF isolated from the pituitary gland.[8] Additionally, using purified opiate receptors prepared from brain tissues, MLF competitively inhibits the binding of ^3H naloxone.[6,8,9,18]

Evidence that naloxone acts *in vivo* to inhibit MLF binding to opiate receptors is at present only inferential, as suggested by the work of Akil, Mayer and Liebeskind,[10] wherein naloxone antagonized electrically-produced analgesia. We have observed in mice that analgesia induced by a gaseous anesthetic, nitrous oxide, is reversed by naloxone, again suggesting the possible role of MLF in mediating analgesia.[11]

Our results with cyclopropane, halothane, and enflurane may be taken as indirect evidence that anesthetics act, in part, by releasing a humoral substance having opiate-like activity. Direct confirmation is necessary.

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