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 concentra-
tion 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 recorded 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 addi-
tional 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 recep-
tor; 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.14 Several groups of investiga-
tors have also isolated substances from mammalian brain25 and pituitary,3 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 antag-
ionist with little or no known agonistic activity.
Some of these substances have been chemically
identified as pentapeptides.8 More recently, Akil,
Mayer and Liebeskind16 found in rats that naloxone
partially antagonized the analgesia produced by
local electrical stimulation of the periaquaductal
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 injec-
tion 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 anes-
thesia 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 cali-
brated 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 mani-
fold 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 en-
flurane 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 concen-
tration of cyclopropane was reduced to 22 per cent
after induction and then further reduced in decre-
ments of no more than 2 per cent at 8-min intervals.
Rectal temperature was measured using thermo-
couple probes attached to an electronic thermometer
(Yellow Springs Instruments) and maintained be-
tween 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 hal-
thane or enflurane, at least 5 min using cyclopro-
pane, and then increased or decreased if necessary.
TABLE 1. Results of Experiments Using Halothane

<table>
<thead>
<tr>
<th>Injection</th>
<th>Responding before Injection</th>
<th>Responding after Injection</th>
<th>Net Change</th>
<th>( \Delta ) Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline solution</td>
<td>8/12 Number, 7/12 Per Cent</td>
<td>7/12 Number, 6/12 Per Cent</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>Saline solution</td>
<td>3/12 Number, 2/12 Per Cent</td>
<td>2/12 Number, 1/12 Per Cent</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>11/24 Number, 46 Per Cent</td>
<td>9/24 Number, 38 Per Cent</td>
<td>-2/24</td>
<td>-8</td>
</tr>
<tr>
<td>Naloxone</td>
<td>7/12 Number, 12/12 Per Cent</td>
<td>12/12 Number, 16/12 Per Cent</td>
<td>+5</td>
<td>+5</td>
</tr>
<tr>
<td>Naloxone</td>
<td>2/12 Number, 7/12 Per Cent</td>
<td>7/12 Number, 9/12 Per Cent</td>
<td>+5</td>
<td>+5</td>
</tr>
<tr>
<td>Naloxone</td>
<td>7/12 Number, 12/12 Per Cent</td>
<td>12/12 Number, 16/12 Per Cent</td>
<td>+4</td>
<td>+4</td>
</tr>
<tr>
<td>TOTAL</td>
<td>17/48 Number, 35 Per Cent</td>
<td>33/48 Number, 69 Per Cent</td>
<td>+16/48</td>
<td>+33</td>
</tr>
</tbody>
</table>

* Different from results following iv injection of saline solution, \( P < 0.005 \).

TABLE 2. Results of Experiments Using Enflurane

<table>
<thead>
<tr>
<th>Injection</th>
<th>Responding before Injection</th>
<th>Responding after Injection</th>
<th>Net Change</th>
<th>( \Delta ) Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline solution</td>
<td>7/12 Number, 9/12 Per Cent</td>
<td>9/12 Number, 12/12 Per Cent</td>
<td>+2</td>
<td>+2</td>
</tr>
<tr>
<td>Saline solution</td>
<td>5/12 Number, 5/12 Per Cent</td>
<td>5/12 Number, 5/12 Per Cent</td>
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<td>0</td>
</tr>
<tr>
<td>Saline solution</td>
<td>6/12 Number, 8/12 Per Cent</td>
<td>7/12 Number, 8/12 Per Cent</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>18/36 Number, 50 Per Cent</td>
<td>21/36 Number, 58 Per Cent</td>
<td>+3/36</td>
<td>+8</td>
</tr>
<tr>
<td>Naloxone</td>
<td>6/12 Number, 9/12 Per Cent</td>
<td>9/12 Number, 12/12 Per Cent</td>
<td>+3</td>
<td>+3</td>
</tr>
<tr>
<td>Naloxone</td>
<td>5/12 Number, 10/12 Per Cent</td>
<td>10/12 Number, 13/12 Per Cent</td>
<td>+5</td>
<td>+5</td>
</tr>
<tr>
<td>Naloxone</td>
<td>6/12 Number, 9/12 Per Cent</td>
<td>9/12 Number, 12/12 Per Cent</td>
<td>+3</td>
<td>+3</td>
</tr>
<tr>
<td>Naloxone</td>
<td>4/12 Number, 6/12 Per Cent</td>
<td>6/12 Number, 12/12 Per Cent</td>
<td>+5</td>
<td>+5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>21/48 Number, 44 Per Cent</td>
<td>37/48 Number, 77 Per Cent</td>
<td>+16/48</td>
<td>+33</td>
</tr>
</tbody>
</table>

* 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., E<sub>50-90</sub>. 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 x 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 Olfi<sub>n</sub> 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 enfurane, 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 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 enfurane (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

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**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.
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 ED₅₀–₆₀ 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 ED₅₀–₆₀ 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. 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. 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. The short duration of action of naloxone against anesthesia might therefore be expected, as is seen in its clinical use as a narcotic antagonist.

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. 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. 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. The same has been shown for MLF isolated from the pituitary gland. Additionally, using purified opiate receptors prepared from brain tissues, MLF competitively inhibits the binding of ³H naloxone.

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, 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.

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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References

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