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Naloxone Does Not Antagonize General Anesthesia in the Rat

Marilyn H. Harper, M.D., Peter M. Winter, M.D.,† Brynte H. Johnson, A.B.,‡ Edmond I. Eger, II, M.D.§*

The administration of naloxone 2, 10, 50, or 250 mg/kg intravenously did not alter halothane requirement (MAC) in Sprague-Dawley rats (12 rats per group). Two rats convulsed when given 50 mg/kg while anesthetized with halothane. In a separate group of awake rats, seven of nine animals convulsed when given naloxone, 100 mg/kg. It is concluded that any effect of naloxone on anesthetic requirement must be small (not significant in our study), and that if an effect exists it is the result of a nonspecific analeptic action of naloxone rather than a specific action at opiate receptors. (Key words: Anesthetics, volatile: halothane. Antagonists, narcotic: naloxone. Polypeptides: enkephalins. Receptors, opiate; Theories of anesthesia.)

FINCK AND COLLEAGUES recently proposed that inhaled anesthetics may act, at least in part, by releasing endogenous opiate-like substances (*e.g.*, enkephalins).[1] They found that 69 per cent of rats lightly anesthetized with halothane responded to a noxious stimulus (tail clamp) after administration of naloxone, 10 mg/kg, whereas only 35 per cent responded before injection. Naloxone competitively blocks the actions of both narcotics and enkephalins.[2,3] Similar increases in the incidences of responses were seen following naloxone injection in rats lightly anesthetized with enflurane or cyclopropane. The data of Finck *et al.* do not permit a quantitative estimate of the effect that a release of enkephalins, assuming it occurs, might have on anesthetic requirement. Although the increase from 35 to 69 per cent in the incidences of responses after naloxone administration appears to indicate a substantial effect, in fact such a change could result from only a small shift in the anesthetic dose–response curve. If only a slight increase in anesthetic requirement occurred then release of enkephalins by anesthetics would be unlikely to be a significant mechanism of anesthetic action. Accordingly, we have repeated the experiment of Finck *et al.*, modifying it to permit assessment of the shifts in the anesthetic dose–response curve induced by various doses of naloxone.

* Assistant Clinical Professor of Anesthesiology, University of California.

† Professor of Anesthesiology, University of Washington.

‡ Staff Research Associate, Department of Anesthesia, University of California.

§ Professor of Anesthesia, University of California.

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Address reprint requests to Dr. Eger.

Methods

First Experiment

Groups of five or six Sprague-Dawley rats, weighing between 290 and 340 g, were anesthetized with halothane in oxygen in individual plastic chambers. Tracheostomies were performed to facilitate sampling of end-tidal gases. Average rectal temperature was maintained between 37.2 and 37.5 C. End-tidal samples were obtained as described previously.[4] Inspired and end-tidal halothane and end-tidal CO_2 concentrations were measured with Beckman LB-2 infrared analyzers. End-tidal samples containing less than 5 per cent carbon dioxide usually were discarded and a second sample obtained. MAC was determined for each animal by decreasing the end-tidal halothane concentration in 20 per cent decrements or less, applying a tail clamp for 60 sec at each level and averaging the concentrations that just prevented and permitted movement in response to stimulation. Each end-tidal level was held constant for 15 min prior to stimulation. The end-tidal concentration then was restored to the concentration that just prevented movement in all rats or all but one rat. Lack of movement was confirmed by again stimulating each animal. Immediately following this confirmation and without altering the halothane concentration, either saline solution (control) or naloxone 2, 10, or 50 mg/kg was given intravenously, and MAC redetermined. The investigators were blind to which test solution was administered. The first tail-clamp test was done 5 min after injection in every animal. The halothane concentration then was altered as indicated by the response of the majority of the animals. The new concentration was maintained for 15 min, and the animal retested. We continued thereafter to manipulate the concentration so as to arrive at a MAC value for all animals.

Solutions were made by dissolving precisely weighed amounts of crystalline naloxone hydrochloride in half normal saline solution plus 5 per cent dextrose in water ("saline solution"). The amount of diluent was adjusted so that the volume injected always equalled 2 ml/kg.

Second Experiment

Because the administration of naloxone in the first experiment did not produce a significant change in MAC, the effect of a fourth naloxone dose, 250 mg/kg,

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TABLE 1. Results of the First and Second Experiments*

	Naloxone (mg/kg)				
	0	2	10	50	250
Number of rats	15	12	12	12	12
Weight (g)	290 ± 2	290 ± 3	284 ± 2	286 ± 3	339 ± 14
Values before injection					
$P_{ET_{CO_2}}$ (torr)	40.6 ± 1.0	44.0 ± 1.7	43.3 ± 1.4	43.6 ± 1.1	46.1 ± 1.5
Rectal temperature (C)	37.2 ± .15	37.3 ± .09	37.4 ± .12	37.5 ± .08	37.2 ± .14
Halothane MAC	.970 ± .031	.918 ± .026	.922 ± .027	.915 ± .027	.823 ± .031
Values after injection					
$P_{ET_{CO_2}}$ (torr)	42.7 ± 1.2	42.7 ± .8	45.9 ± 1.4	43.1 ± 1.0	44.2 ± 1.5
Rectal temperature (C)	37.4 ± .09	37.5 ± .11	37.2 ± .15	37.4 ± .10	37.5 ± .30
Halothane MAC	.891 ± .026	.899 ± .032	.926 ± .034	.958 ± .042	.786 ± .028
Per cent change in MAC	−7.1 ± 3.5	−1.4 ± 4.3	+1.0 ± 3.7	+5.8 ± 6.0	−4.1 ± 2.3

* The table combines the results of the first experiment where naloxone 0 (saline solution–dextrose alone), 2, 10, or 50 mg/kg were given intravenously on a "blind" basis to groups of five or six rats with those of the second experiment where 250 mg/kg were given intravenously but not on a blind basis. $P_{ET_{CO_2}}$'s and temperatures are from the means of the values obtained at the halothane concentrations preventing and permitting movement in response to stimulation. All values are means ± SEM. Note that the percentage change in MAC was obtained from the percentage changes for individual animals, and hence the mean value differs slightly from that given by dividing the average MAC value after injection by the average value before injection.

was tested in an additional group of 12 rats. The treatment of these animals was identical to that of the rat in the first experiment except that the investigators were not blind to the dose of naloxone given.

THIRD EXPERIMENT

Nine awake unmedicated rats were restrained in individual clear plastic cylinders from which their tails protruded. They were given pure oxygen to breathe for 2 min or more, after which naloxone, 100 mg/kg, was given intravenously. Each rat was observed for convulsions for the ensuing 2–5 min.

Statistical evaluations included analysis of variance and t tests for paired and unpaired data. We accepted as significant $P < .05$.

Results

FIRST AND SECOND EXPERIMENTS

End-tidal carbon dioxide partial pressure values and rectal temperatures were comparable for all groups both before and after injection of saline solution or naloxone (table 1). Although it appeared that relative to the saline control a small dose-related increase in MAC was associated with the lowest three doses of naloxone, analysis of variance did not indicate that the changes were significant. A t test for unpaired data comparing the change in MAC obtained with saline solution with that obtained with naloxone, 50 mg/kg, also was not significant. Finally, t tests for paired data for the changes in MAC achieved with individual dose groups revealed no significant change except for the small (7.1 per cent) decrease in MAC in the control group.

Anesthetic requirement was also estimated from the inspired halothane concentrations associated with movement and lack of movement in response to tail clamping (data not shown). The results were similar to those obtained using end-tidal halothane concentrations (MAC). However, the use of inspired concentrations increased variance. In addition, as might be expected with the decreasing anesthetic uptake associated with an increase in the duration of anesthesia, there was a tendency at every dose level of naloxone to see a greater decrease in anesthetic requirement using inspired concentrations rather than end-tidal values to determine anesthetic requirement. Analysis of variance again did not reveal any significant difference among the treatments (including control) in the first experiment.

THIRD EXPERIMENT

Seven of the nine rats given naloxone, 100 mg/kg, evidenced tonic, clonic seizures, which often occurred repetitively and then subsided after 5 min. All animals survived this dose. In the first experiment, two of the rats given 50 mg/kg also convulsed. Surprisingly, none of the rats convulsed when given 250 mg/kg.

Discussion

Although we did not find that naloxone in any dose caused a statistically significant shift in the anes-

thetic dose–response curve (*i.e.*, MAC), our results are not in conflict with those found by Finck *et al.*[1] The difference between MAC's with saline solution and naloxone, 10 mg/kg, was 8.1 per cent (*i.e.*, minus 7.1 per cent with saline solution and +1.0 per cent with 10 mg/kg naloxone—see table 1). Given the steepness of the anesthetic dose–response curve, this difference would be sufficient to explain the 35 to 69 per cent increase in rats responding in the study of Finck *et al.*

The doses of naloxone used in our experiment far exceed those necessary to antagonize the action of opiates or compete with enkephalins at their receptors in rat brain.[5] The analgesia produced by intracerebral injection of beta-endorphin (a substance closely related to the enkephalins) in mice is fully reversed by naloxone.[3]

Finally, in awake rats the lethal intravenous dose for 50 per cent of animals (LD_{50}) is 109 mg/kg.[6] Others have found that this dose in unanesthetized rats is associated with an analeptic effect evidenced by excitation, hyperactivity, tremors and tonic–clonic convulsions (unpublished data from Endo Laboratories), a finding confirmed by the results of our third experiment. Finck *et al.* found a small decrease in anesthestic depth and an alteration in the electroencephalogram in some but not all animals given naloxone. An analeptic effect might explain both of these phenomena.

We conclude that any antianesthetic effect exerted by naloxone probably is the result of its analeptic properties. Our data do not indicate that release of enkephalins or other morphine-like factors in the central nervous system by anesthetics plays an important role in the causation of general anesthesia by inhaled agents.

The crystalline naloxone hydrochloride (Narcan®) was donated by Endo Laboratories; halothane (Fluothane®) was a gift of Ayerst Laboratories.

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