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NALOXONE DECREASES THE DURATION OF PRIMARY APNEA WITH NEONATAL ASPHYXIA

Victor Chernick,[25] Deborah L. Madansky and Edward E. Lawson [24]

Department of Pediatrics

University of Manitoba and Children's Hospital

Winnipeg, Canada

and

Department of Pediatrics

Harvard University and Children's Hospital Medical Centre

Boston, Massachusetts, U.S.A.

SUMMARY

Naloxone, a specific opiate antagonist with no agonist properties, in doses of 0.4 and 4.0 mg/kg was found to markedly reduce the duration of primary apnea in asphyxiated newborn rabbits. There was no effect on the duration of the hyperpneic phase (time to primary apnea) or on survival time (time to last gasp). It is suggested that endogenous opiate-like peptides are released during asphyxia and are the major factor in the suppression of medullary inspiratory neuronal discharge during primary apnea.

SPECULATION

Apnea is a serious problem in the asphyxiated newborn infant, the pre-term infant and has been implicated in the sudden infant death syndrome. It is tempting to speculate that endogenous opiate-like peptides prolong primary apnea in these conditions but further studies are required. It is also possible that naloxone should be used in the resuscitation of the asphyxiated neonate whether or not the mother has received exogenous opiates; this hypothesis could be tested in appropriate animal models.
Endorphins and enkephalins are recently discovered polypeptides with opiate-like activity found in the pituitary gland and in various areas of vertebrate brain, including the medulla, in association with opiate receptors (3,7,8,17). They have been implicated as neurotransmitters involved in the control of pain, emotional behavior and narcotic addiction. We postulated that since exogenous opiates modify respiration and the ventilatory response to hypercapnia and hypoxemia (19), the endogenous opiate-like compounds might modify the respiratory response to neonatal asphyxia. We now report that naloxone, a specific opiate antagonist known to block opiate receptor sites (15), markedly decreases the duration of primary apnea produced by asphyxia in the newborn rabbit.

MATERIALS AND METHODS

Newborn rabbits, 3 to 5 days of age and weighing 41-108 g were prepared as described in detail by Lawson and Thach (12). Briefly each was anesthetized with ether, a tracheal cannula inserted and the animal allowed to awaken from the anesthesia for approximately 20 minutes. Body temperature was maintained between 37-39°C by a heating lamp. Twelve rabbit pups received 1 ml saline I.P. and eight pups from the same litters as the control pups received 1 ml (0.4 mg) naloxone I.P. or approximately 4 mg/kg. Five minutes were allowed for absorption. The tracheal cannula was then occluded at end-expiration and tracheal pressure monitored with a Statham pressure transducer (PM 6) and recorded on a Beckman 411 polygraph. Occlusion produced progressive asphyxia during which the animal made increasing respiratory efforts until primary apnea commenced (Fig. 1). Following the initial gasp after primary apnea, the cannula was reopened and the animal resuscitated with a few positive pressure inflations if necessary. Each animal underwent four tracheal occlusions, with three to five minutes allowed between occlusions for recovery. Recordings were analyzed for time to primary apnea, duration of primary apnea and tracheal pressure (P_T) generated at 5, 10, 20, 30 and 40 seconds after occlusion as well as the tracheal pressure of the first gasp after primary apnea. In addition, the average frequency of respiratory efforts prior to primary apnea was calculated by the following formula: number of breaths/time to primary apnea (sec) X 60.

Following our preliminary report of these experiments (2), Finck and colleagues (5) reported that naloxone (10 mg/kg) altered the depth of inhalational anesthesia in the adult rat. They suggested that anesthetics might release endogenous opiates. Because of our use of ether anesthesia for insertion of a tracheal tube, we were unable to rule out an effect of the anesthesia on the results. We therefore repeated the experiments avoiding the use of exogenous anesthetic or analgesic agents in the following manner.

Newborn rabbits, 3-5 days of age and weighing 53 to 96 g, were injected intraperitoneally with either saline or naloxone (0.04 mg/kg, 0.40 mg/kg or 4.0 mg/kg). Each pup was placed in a small body plethysmograph consisting of plexiglass cylinder with one open end which was closed with a double layer of latex rubber. A cruciate incision in the latex rubber allowed the head to protrude and provided and airtight neck seal without interferring with breathing. Respiration was monitored continuously by a pressure transducer connected through a side port to the body chamber and recorded on an Mingograf recorder. Body temperature, monitored by a thermistor, was maintained at 37-39°C by a heating pad and heating lamps. Nitrogen was administered by head hood until gasping respiration began after the period of primary apnea and the animal resuscitated with oxygen (hypoxia group). Each animal was studied four times allowing 5 minutes recovery between trials. On the fourth trial nitrogen breathing was continued until the stage of terminal apnea (end of gasping respiration).

In a separate series of experiments newborn rabbits were injected I.P. with saline or 4.0 mg/kg naloxone and then given nitrogen plus 7% CO_2 to more closely mimic the effects of asphyxia (asphyxia group). Again four trials were done allowing 5 minutes recovery between trials. On the fourth trial each animal was asphyxiated to the stage of terminal apnea. We measured the time to primary apnea, duration of the primary apnea and the time to the last gasp on each animal in the hypoxia and asphyxia groups.

Statistical analysis was done using analysis of variance and student's t-test for unpaired variates.

RESULTS

A. TRACHEAL OCCLUSION

There was no difference between the four occlusions in the time to primary apnea or the duration of primary apnea for either the saline or naloxone treated pups. Therefore, the data were grouped for comparison. The mean (± SE) data for time to primary apnea, duration of primary apnea, frequency and tracheal pressure (P_T) for the saline and naloxone treated pups are shown in Table 1. Naloxone did not affect the time to primary apnea ($t=1.86; p>.05$). The duration of primary apnea was reduced by 75% ($t=3.72; p<.001$). The average frequency, the P_T at 5, 10, 20, 30 and 40 sec after occlusion and the P_T of the first gasp after primary apnea were not affected by naloxone.

B. HYPOXIA (Table 2)

There was no difference in the time to primary apnea or the time to the last gasp (survival time) between the groups who received saline or varying doses of naloxone (0.04, 0.4 or 4.0 mg/kg). The duration of primary apnea was markedly reduced by 79% and 55% with the higher doses of naloxone, 0.4 mg/kg and 4 mg/kg respectively, but not significantly different from controls at the lower dose of naloxone (0.04 mg/kg).

C. ASPHYXIA (Table 3)

There was a small (19%) but significant decrease in the time to primary apnea in the naloxone (4 mg/kg) injected group. There was no difference in survival time between groups. Again a striking decrease (72%) in the duration of primary apnea was seen in the naloxone treated group.

D. COMPARISON OF ASPHYXIA AND HYPOXIA GROUPS

There was no difference in the time to primary apnea between the control groups subjected to either hypoxia (100% nitrogen) or asphyxia (7% CO_2, 93% nitrogen)(Fig. 2). The time to primary apnea of 39.5 ± 2.5 sec in the hypoxia group and 34.7 ± 1.3 sec in the asphyxia group following the I.P. injection of 4 mg/kg naloxone was also not statistically different. Similarly, there was no significant difference in the duration of primary apnea between the asphyxia and hypoxia control groups and the asphyxia and hypoxia groups following 4 mg/kg naloxone (Fig. 3).

E. COMPARISON OF TRACHEAL OCCLUSION AND PLETHYSMOGRAPH METHODS

The time to primary apnea was significantly longer by approximately 25% in the control group undergoing tracheal occlusion when compared to those control rabbits subjected to asphyxia or hypoxia by the plethysmograph method (Fig. 2). The time to primary apnea in tracheal occlusion rabbits who received naloxone was prolonged by about 57% compared to those in the plethysmograph group who received naloxone. However, there was no difference in the duration of primary apnea between the tracheal occlusion method and plethysmograph method when either control groups or naloxone injected groups were compared (Fig. 3).

DISCUSSION

Naloxone is a potent opiate antagonist with no known agonist properties (6). The serum half-life in the rat is about 30 minutes and there is rapid penetrance into the brain with brain-serum concentration ratios of 2.7 to 4.6 (14). The drug binds to specific opiate receptors in the brain and acts as a competitive inhibitor of both pharmacologic opiates and endogenous opiate-like peptides (15). Opiate receptors are present in the brain during fetal life, increase rapidly after birth while the affinity for opiates remains constant throughout development (16).
Finck and colleagues (5) demonstrated that naloxone antagonized the analgesic effect of cycloproprane, halothane, enflurane and nitrous oxide in adult animals and suggested that inhalational anesthetics caused the release of endogenous opiates. We were concerned that the ether anesthesia used for the tracheal occlusion experiments may have influenced the results and therefore utilized a method of producing asphyxia that did not require anesthesia. The results clearly indicate that ether anesthesia does not influence the duration of primary apnea in either control animals or in those receiving naloxone (Fig. 3). The duration of the hyperpneic phase (time to primary apnea) was prolonged in the groups who underwent tracheal occlusion following ether anesthesia when compared to those in the plethysmographic groups (Fig. 2). However, there was no difference in the time to primary apnea between control and naloxone treated pups in the tracheal occlusion groups. Thus, ether anesthesia per se was not associated with the release of endogenous opiates. The prolonged time to primary apnea in the tracheal occlusion groups is probably related to the more acute onset of asphyxia in the plethysmograph groups since the onset of primary apnea occurs when PaO_2 reaches approximately 10 to 12 Torr (9,12). The demonstration that naloxone does not influence the time to primary apnea in either the tracheal occlusion or plethysmographic experiments indicates that endogenous opiates have no effect on the time of onset of primary apnea. These results are consistent with previous observations that exogenous opiates, morphine and pethidine had no effect on the time to primary apnea in 3 day old rabbits breathing nitrogen (13).

Occlusion pressure has been used as an index of respiratory center "output" (20). In the present study the progressive increase in occlusion pressure following tracheal occlusion, the occlusion pressure of the first gasp following primary apnea and the respiratory frequency during the hyperpneic phase were unaffected by naloxone indicating that endogenous opiates do not modify these parameters.

The time to last gasp (survival time) is known to be prolonged by pentobarbital and meperidine (1), increased carbohydrate stores (10), and greatly reduced by aminophylline which greatly increases cerebral metabolic rate (11). In the present study although the number of observations was small and there was great variability, naloxone did not influence the time to the last gasp (survival time) in the plethysmographic experiments, suggesting that endogenous opiates do not affect survival time in the asphyxiated newborn animal.

The present experiments have demonstrated that administration of naloxone has a striking effect on the duration of primary apnea whether induced by tracheal occlusion, inhalation of nitrogen or 7% CO_2 in N_2. This effect was not seen at a dose of 0.04 mg/kg but was evident at doses of 0.4 and 4.0 mg/kg, some 10 to 100 fold greater than the recommended clinical dose. These results are in contrast to those reported by Stephen and colleagues (18) who placed one day old rabbits in a perspex cylinder, induced primary apnea by flushing the chamber with nitrogen and used a stop watch to time the duration of primary apnea and the gasping phase. Their rabbits were given naloxone in a dose of 0.1 mg/kg or 1.0 mg/kg 30 minutes prior to the experiment and since no difference was seen between the two doses the groups were combined. They did not find a difference between saline injected and naloxone injected rabbits in the duration of primary apnea. In preliminary experiments we used a perspex cylinder flushed with nitrogen as described by Stephen et al. (18) and attempted to time events by direct observation without the benefit of a recording of respiratory activity. We found that the precise duration of primary apnea was extremely difficult to judge since it is preceded by convulsive movements and often interrupted by twitching movements which may or may not have been accompanied by a breath or gasp. This method was therefore abandoned and the plethysmograph method used which allowed continuous recording of respiration as described. With this method the duration of primary apnea in control animals was identical to that found in the tracheal occlusion experiments where tracheal pressure was continuously recorded. Thus, we feel that direct recording of respiratory efforts is important in this type of experiment and that failure to do so might explain the discrepancy between the present results and those reported by Stephen et al. (18).

The onset of primary apnea is related to PaO_2 and has a consistent temporal relation with hypoxic decerebration (12). Heretofore, nothing has been known about what factors govern the duration of primary apnea. Lawson and Thach (12) suggested that since some of the features of gasps were present prior to the onset of primary apnea and since primary apnea was a period of active expiration, that primary apnea resulted from hypoxic depression of medullary inspiratory neurons while medullary expiratory neurones remained active. Somewhat later, expiratory neurones become depressed and the onset of gasping is due to the inherent rhythmicity of medullary inspiratory neurones. They suggested that the existence of a separate gasp center seemed unlikely. If this is so, then our present experiments indicate that hypoxic depression of inspiratory neurones is largely a result of the action of endogenous opiates either acting directly on these neurones or via other neural centers (?pontine). When opiate receptor sites are blocked by naloxone, the duration of primary apnea is extremely brief. This sequence of events is consistent with the demonstration in adult cats that methionine-enkaphalin markedly reduces peak discharge frequency of pontobulbar respiratory neurones and this effect is antagonised by naloxone (4).
We postulate the following sequence of events in the ventilatory response to severe hypoxia: regular respiration is inhibited by hypoxia and the time to primary apnea is determined by either cerebral blood flow (cardiac output) or by differing "sensitivity" to hypoxia. Severe hypoxia is associated with the release of endogenous opiates which temporarily suppresses the onset of gasping respiration. This suppression is antagonized by naloxone. The duration of gasping is determined by cerebral metabolic rate and carbohydrate stores and gasping ceases when there is insufficient energy for repolarization of the responsible neurones.

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24. Present address of Dr. E.E. Lawson is Department of Pediatrics University of North Carolina, Chapel Hill, North Carolina, U.S.A.
25. Request for reprints should be addressed to Dr. V. Chernick, Children's Hospital, 685 Bannatyne Avenue, Winnipeg, Canada, R3E 0W1.

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Table 1. Mean (± S.E.) data for tracheal occlusion experiments for saline and naloxone treated pups

	Tracheal pressure (P_T) at various time intervals following the onset of tracheal occlusion	
	Saline	Naloxone (4.0 mg/kg)
Time to primary apnea (sec)	51.2 ± 2.2 (47)[1]	58.3 ± 3.3 (31)
Duration of primary apnea (sec)	50.0 ± 9.9 (47)	12.6 ± 1.7 (31)[2]
Frequency (breaths/min)	70.3 ± 3.8 (46)	71.7 ± 4.6 (31)
P_T (cm H_2O) 5 sec	27.0 ± 1.8 (46)	29.4 ± 1.5 (31)
10 sec	31.3 ± 2.0 (47)	34.1 ± 1.7 (31)
20 sec	36.2 ± 1.9 (46)	39.4 ± 1.7 (31)
30 sec	36.3 ± 2.2 (44)	38.6 ± 2.1 (30)
40 sec	35.9 ± 3.1 (32)	36.1 ± 2.0 (25)
First gasp	49.3 ± 2.3 (46)	50.9 ± 2.5 (31)

[1]Numbers in parentheses refer to number of observations.

[2]Significantly different from controls.

Table 2. Mean (± S.E.) data for pups in whom apnea was induced using 100% nitrogen

	Saline	Naloxone		
		0.04 mg/kg	0.4 mg/kg	4 mg/kg
Time to primary apnea (sec)	39.7 ± 1.1(32)[1]	42.1 ± 1.6(24)	39.8 ± 1.4(16)	39.5 ± 2.5(24)
Duration of primary apnea (sec)	56.0 ± 11.7(32)	73.4 ± 17.1(24)	11.6 ± 1.9(16)[2]	25.2 ± 4.9(24)[2]
Time to last gasp (sec)	705.2 ± 140.0(8)	510.6 ± 115.4(6)	1013.8 ± 170.7(4)	682.0 ± 96.2(4)

[1] Numbers in parentheses refer to number of observations.

[2] Significantly different from control.

Table 3. Mean (± S.E.) data for pups in whom apnea was induced with 7% CO_2 balance N_2

	Saline	Naloxone (4 mg/kg)
Time to primary apnea (sec)	42.7 ± 2.4 (24)[1]	34.7 ± 1.3 (24)[2]
Duration of primary apnea (sec)	63.1 ± 16.6 (24)	17.7 ± 5.3 (24)[2]
Time to last gasp (sec)	1117.4 ± 157.3 (6)	1108.9 ± 113.5 (6)

[1] Numbers in parentheses refer to number of observations.

[2] Significantly different from control.

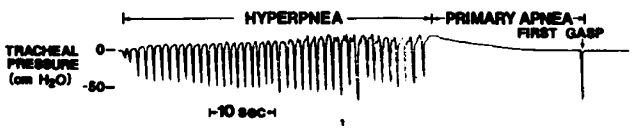

Fig. 1 Tracing illustrating respiratory response to tracheal occlusion in a newborn rabbit pup. Tracheal occlusion done at first arrow is followed by a "hyperpneic" phase, primary apnea and a gasp. Tracheal pressure calibration and time as noted on the figure.

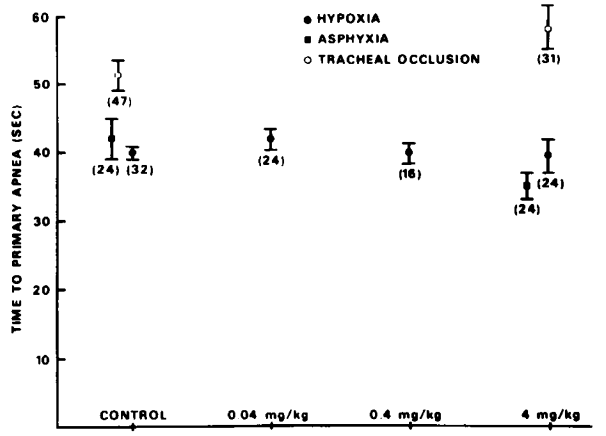

Fig. 2 Comparison of time to primary apnea in newborn rabbits by either the tracheal occlusion method (○) or plethysmograph method. In the latter instance either nitrogen (hypoxia) (●) or 7% CO_2 balance N_2 (asphyxia) (■) was administered to the pup. Values shown represent mean ± 1 S.E. Shown here are data from control animals (saline injected) and that following I.P. injection of various doses of naloxone.

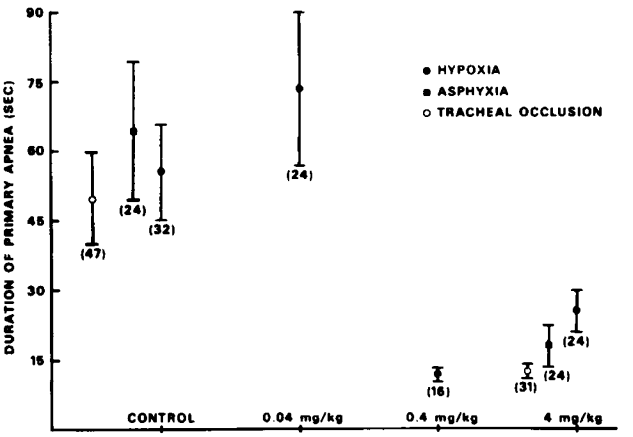

Fig. 3 Legend identical to Fig. 2 except comparison here of duration of primary apnea in saline and naloxone treated pups by either the tracheal occlusion or plethysmographic method.

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