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Anesthesia and Stimulation of Pituitary β-Endorphin Release in Rats (41870)

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Abstract. The effects of several anesthetic drugs and artificial respiration on the release of pituitary β-endorphin-like immunoreactivity (β-END-LI) were examined in rats. Plasma β-END-LI responses to halothane and pentobarbital were similar in magnitude and duration, being maximal (2- to 3-fold) by 10 min and returning to control values by 30 min after induction. Urethane anesthesia was associated with an 8-fold increase in plasma β-END-LI throughout the 30-min treatment period. In comparison to anesthesia alone, anesthesia plus intubation with artificial respiration (standard parameters) was associated with considerably greater elevations in plasma β-END-LI (up to 30-fold). Further, intubation and artificial respiration appear to have contributed separately, and in an additive fashion, to the overall β-END-LI responses observed. As compared to halothane anesthesia alone, intubation evoked a 4-fold increase in circulating β-END-LI, whereas intubation plus ventilation was associated with a 12-fold increase. Treatment with morphine (1 or 5 mg/kg), but not pancuronium (0.3 mg/kg), attenuated the plasma β-END-LI response to mechanical ventilation, suggesting that a subconscious phenomenon, perhaps related to pain, was partially responsible for the profound release of pituitary β-END-LI associated with artificial respiration. Chromatographic analysis of the molecular forms of β-END-LI released into plasma revealed that both β-END- and β-lipotropin (β-LPH)-sized peptides were secreted under the present experimental conditions. Since the analgesic form of β-END (β-END_{1-31}) is cosecreted with β-LPH from the pars distalis, increases in the fraction of plasma β-END-LI corresponding to β-END in size were probably due to the release of opiate active β-END_{1-31}.

Various theories have been proposed describing the analgesic mechanism of anesthetic drugs. Several groups of investigators have suggested that anesthetic analgesia may be mediated through the release of endogenous opiate peptides (1–3). Evidence supporting this hypothesis includes findings that the opiate receptor antagonist, naloxone, partially reversed the analgesia (1, 2, 4, 5) and attenuated the circulatory (6, 7) and hypnotic (6) effects of inhalation anesthetics in laboratory animals. Also, Berkowitz (8) reported a cross-tolerance between nitrous oxide and morphine in rats. In man, nitrous oxide decreased the perception of pain (subjective reports) (3, 9) and reduced the amplitude of cerebral evoked potentials to painful tooth pulp stimulation (9). Importantly, both these responses were partially reversed by naloxone (3, 9), again suggesting a role for endogenous opiate peptides in anesthetic analgesia. By contrast, results of several comparable studies do not support this conclusion. For example, Harper *et al.* (10) observed that naloxone had little influence on the anesthetic requirement for halothane in rats. Also, Way *et al.* (11) failed to observe an increase in cerebral spinal fluid β-endorphin (β-END) following thiopental–nitrous oxide–halothane anesthesia in humans. Although these findings suggest that the effects of anesthetics may not be mediated through the release of endogenous opiate peptides within the central nervous system (CNS), the possible release of pituitary β-END into blood and its contribution to the development of anesthesia remain to be determined. Accordingly, we have investigated the effects of halothane, pentobarbital, and urethane on the secretion of pituitary β-END in rats. The present findings indicate that all three agents increase plasma levels of β-END-like immunoreactivity (β-END-LI); however, these responses are modest in comparison to the release of pituitary β-END-LI evoked by anesthesia plus artificial respiration. Chromatographic analysis suggests that the molecular form of β-END released under the present condition is, indeed, the analgesic form β-END_{1-31}.

Materials and Methods. *Animals and treatments.* Male Sprague–Dawley rats (Taconic Farms, Inc., Germantown, Pa.) weighing 300 to 500 g were housed at 22°C with lights on

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from 0600 to 1800 hr having free access to standard rat chow and tap water. Sodium pentobarbital (50 mg/kg, Sigma Chemical Co., St. Louis, Mo.), urethane (1.5 g/kg, Sigma), or morphine (1 and 5 mg/kg, Mallinckrodt Chemical Works, St. Louis, Mo.) were injected intraperitoneally in a volume of 0.4 ml; control animals received injections of 0.9% NaCl vehicle. Pancuronium bromide (0.3 mg/kg, Organon, Inc., West Orange, N.J.) was injected via a tail artery cannula 8 to 10 min after pentobarbital administration; a maintenance dose of 0.1 mg/kg was given 14 min after the first injection of pancuronium. Control animals received corresponding vehicle injections. Halothane was administered in room air at a flow rate of 7.5 liters/min via a vaporizer (Flotec MK2, Cyprane Ltd., Keighley, England). Halothane induction was carried out in a plastic chamber having an atmosphere of 4% halothane; control animals were handled similarly but were not exposed to halothane. Anesthetized rats were maintained on 1.5% halothane administered via a nose cone. Mechanical ventilation (Rodent Respirator, Harvard Apparatus, South Natick, Mass.) was performed via an endotracheal tube using a stroke volume (5.5 cm^3) and frequency (60 cycles/min) determined to maintain blood gases (pO_2, pCO_2) and pH within the normal ranges. Colonic temperatures were monitored (Yellow Springs Instrument Co., Yellow Springs, Ohio) and maintained between 36 and 38°C by a heated water pad (Hamilton Industries, Cincinnati, Ohio). Additional details for treatments within individual experiments are presented under Results.

Blood collection, radioimmunoassay, and gel filtration chromatography. Serial blood samples (0.8 to 1.0 ml) were collected via tail artery cannulae (PE-90, Clay Adams) into plastic syringes containing 40 μl of 10% EDTA and 5 mg% bacitracin; cannulations were completed within 10 min after exposure to anesthetic. The cells and fluid volume removed were returned (in 0.9% NaCl) immediately following each collection. Decapitation blood samples (approximately 6 ml) were collected into plastic tubes containing 600 μl of 10% EDTA and 5 mg% bacitracin and placed on ice. Plasmas were prepared by centrifugation and stored at −70°C until assayed. It was determined that within the same anesthetic treatment group, the two methods for blood collection yielded comparable plasma hormone values, indicating that cannulation and blood sampling, per se, did not affect circulating levels of β-END-LI.

Plasma levels of β-END-LI were measured by radioimmunoassay using a rabbit antiserum (C-55) raised against camel $\beta\text{-END}_{1\text{-}31}$. As previously described by Mueller (12), this antibody recognizes the C-terminal portion of $\beta\text{-END}_{1\text{-}31}$ and reacts equally with shorter and α-*N*-acetylated β-endorphins, as well as β-lipotropin (β-LPH). The antibody does not recognize α-endorphin, the enkephalins, or other peptides structurally related to the N-terminal sequence of $\beta\text{-END}_{1\text{-}31}$. Similarly, non-opiate hormones of the hypothalamus, pituitary, and gastrointestinal tract are not recognized by this assay. Because more than one molecular form of β-END-LI is detected, data are expressed in picograms per milliliter based on the camel $\beta\text{-END}_{1\text{-}31}$ standard used (Peninsula Laboratories, Inc., Belmont, Calif.).

To determine the relative contribution of β-END- and β-LPH-sized material to total circulating β-END-LI under the various conditions reported here, treatment group pools of plasma (5.0 to 10.0 ml each) were chromatographed at 4°C on a Sephadex G-50 (superfine, Pharmacia, Inc., Piscataway, N.J.) column (2.5 × 80 cm) using 0.1 *N* acetic acid containing 0.05% BSA, 0.02% sodium azide, and 5 mg% bacitracin as the mobile phase. Aliquots of each 7.0-ml fraction were lyophilized, resuspended in assay buffer, and immunoassayed for β-END-LI. Recovery of applied β-END-LI averaged 87 ± 7% for all the runs ($N = 7$). Purified preparations of human β-LPH (gifts of D. Orth and A. Parlow) and rat β-LPH (prepared in house), as well as synthetic $\beta\text{-END}_{1\text{-}31}$, were used to characterize the column.

Statistical analysis. Data were analyzed statistically using an analysis of variance and Duncan's multiple range test; differences were considered significant at the level of $P < 0.05$.

Results. Shown in Fig. 1 are the time course effects of pentobarbital, urethane, and halothane on plasma β-END-LI levels in spontaneously breathing and mechanically ventilated rats. When given alone, pentobarbital

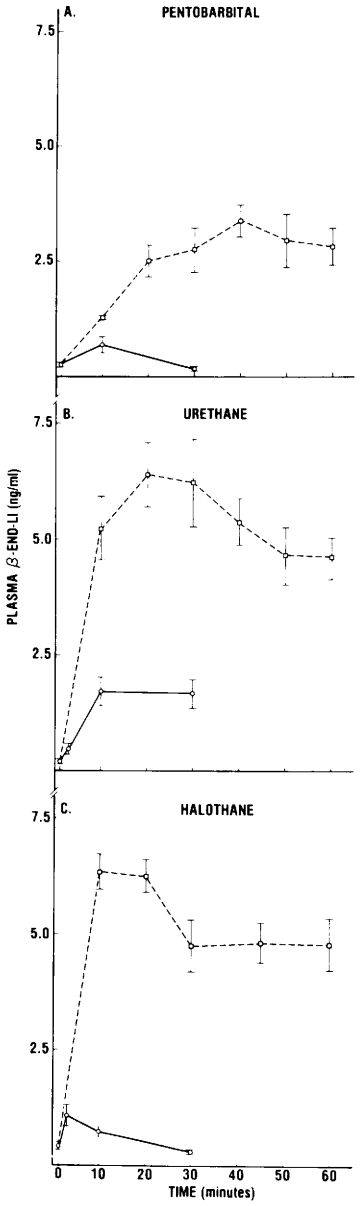

FIG. 1. The time course effects of pentobarbital (50 mg/kg), urethane (1 mg/kg), and halothane (induction with 4%, maintenance with 1.5%) on plasma levels of β-END-LI in spontaneously breathing (O —— O) and mechanically ventilated (O --- O) rats. Serial blood samples were collected in the ventilated groups; decapitation samples in the anesthetized spontaneously breathing and unanesthetized control groups. Mean plasma β-END-LI values for the unanesthetized control groups of the pentobarbital, urethane, and halothane experiments were 253 ± 29, 209 ± 38, and 434 ± 80 pg/ml, respectively. Values are group means ± SEM (N = 6–10). All values are significantly different ($P < 0.05$) from 0 time control values with the exception of the 30-min means in spontaneously breathing rats anesthetized with either pentobarbital or halothane.

and halothane evoked rapid but small increases in circulating β-END-LI as compared to 0 time control values ($P < 0.05$). Plasma β-END-LI responses to both anesthetics were similar in magnitude (2- to 3-fold) and duration (maximal by 10 min; return to control values by 30 min following induction). Urethane anesthesia resulted in a more prolonged (30 min) 8-fold increase in plasma β-END-LI in spontaneously breathing animals. Though significant, the increases in plasma β-END-LI evoked by pentobarbital, halothane, and urethane in spontaneously breathing animals were modest as compared to the rises observed in anesthetized animals receiving mechanical ventilation. Anesthesia plus intubation with mechanical ventilation was associated with dramatic 15- to more than 30-fold elevations in plasma levels of β-END-LI over normal control values.

Although tail artery cannulation and serial blood sampling did not produce any noticeable physical or hormonal responses (data not shown), endotracheal intubation characteristically resulted in a brief physical response (generalized muscle contraction with irregular breathing and gagging) in spite of a surgical plane of anesthesia. To examine the possibility that the intubation procedure might contribute separately to marked release of β-END-LI in artificially respirated animals, the experiment presented in Fig. 2 was conducted. Under halothane anesthesia, intubation resulted in significant elevations in plasma β-END-LI (10 to 30 min) over values measured in rats receiving just anesthetic; a maximal 4-fold response was elicited 10 min following endotracheal intubation. Mechanical ventilation in combination with intubation further increased circulating levels of β-END-LI to a value which was 11-fold greater than that observed in animals under anesthesia alone.

As painful stimuli are potent stimulators of pituitary β-END release in laboratory animals (13–15), it is possible that some effect related to pain caused the dramatic plasma β-END-LI increases associated with intubation and mechanical ventilation (Fig. 1). Accordingly,

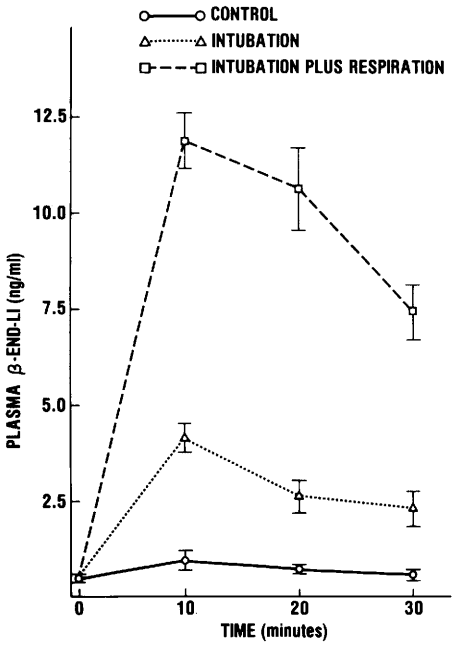

FIG. 2. Effects of endotracheal intubation and intubation plus mechanical ventilation on plasma levels of β-END-LI in rats anesthetized with halothane. Following induction groups of animals were intubated or intubated and ventilated; all anesthetized animals were rapidly cannulated for blood collection (completed within 5 min of induction). The 0 time control value (493 ± 107 pg/ml) represents plasma β-END-LI in normal rats sacrificed by decapitation; the 10-, 20-, and 30-min control values represent plasma β-END-LI in rats treated with halothane alone. Data plotted are group means ± SEM (N = 6 or 7). Plasma β-END-LI values in intubated rats are significantly greater ($P < 0.05$) than values in animals treated with halothane alone. Intubation plus respiration significantly elevated ($P < 0.05$) plasma β-END-LI above values observed in intubated animals.

we examined the sensitivity of the plasma β-END-LI response to pretreatment with morphine. Under pentobarbital anesthesia, artificially respirated animals had plasma β-END-LI concentrations which averaged 7-fold higher than those of spontaneously breathing animals by 10 to 30 min after anesthetic administration (Fig. 3). Morphine (1 mg/kg, ip), administered concurrently with pentobarbital, significantly attenuated the plasma β-END-LI response to artificial respiration at 30 min but not at earlier time points (10 and 20 min). A higher dose of morphine (5 mg/kg, ip) given 10 min prior to pentobarbital almost completely prevented the stimulated release of β-END-LI at all time points (Table I). By contrast, neuromuscular blockade by pancuronium (0.3 mg/kg, iv) was without effect on the time-related increases in plasma β-END-LI associated with mechanical ventilation (data not shown).

Gel filtration chromatographic analysis revealed that anesthesia along or together with mechanical ventilation resulted in elevated levels of β-END-LI (Figs. 1–3) resembling both β-END_{1-31} and β-LPH standards in molecular size (Table II). Some variation in the ratio of

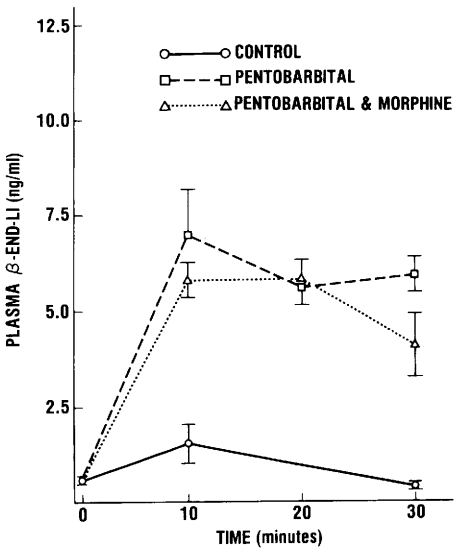

FIG. 3. Effects of morphine treatment on plasma β-END-LI in anesthetized and mechanically ventilated rats. Morphine (1 mg/kg, ip) was administered following induction of pentobarbital (50 mg/kg) anesthesia and just prior to intubation. The 0-min control value (595 ± 10 pg/ml) represents plasma β-END-LI in untreated rats; the 10-, 20-, and 30-min control samples were collected from animals anesthetized but not ventilated (○ —— ○). The groups designated pentobarbital (□ --- □) and pentobarbital morphine (△ · · · △) were mechanically ventilated. Serial blood samples were collected in the ventilated groups; decapitation samples in the spontaneously breathing groups. Values are group means ± SEM (N = 5 or 6). Circulating β-END-LI levels in both groups of respirated animals were significantly greater ($P < 0.05$) than those observed in control animals. Morphine significantly attenuated the plasma β-END-LI response to mechanical ventilation at the 30-min time point.

TABLE I. EFFECTS OF MORPHINE (5 mg/kg) PRETREATMENT ON PLASMA LEVELS OF β-END-LI IN MECHANICALLY VENTILATED RATS

Treatment	β-END-LI (pg/ml)
Control	180 ± 18
Pentobarbital	
10 min	914 ± 131[a]
20 min	983 ± 162[a]
30 min	1275 ± 229[a]
Pentobarbital plus morphine:	
10 min	381 ± 45[a,b]
20 min	276 ± 37[b]
30 min	187 ± 30[b]

Note. Control animals received vehicle injections; all animals receiving pentobarbital were mechanically ventilated. Values stated are group means ± SEM ($N = 7$ or 8).

[a] Significantly different from control values, $P < 0.05$.

[b] Significantly different from values of pentobarbital-treated animals at corresponding time, $P < 0.05$.

β-END- to β-LPH-sized immunoreactivity was observed though not quantitated precisely because the chromatographic procedure used required that large volumes of plasma be run in order to fractionate measurable amounts of β-END-LI in an elution profile. Accordingly, no more than two runs could be made with each treatment group plasma pool. It is important to note, however, that β-LPH, a marker for the release of opiate active β-END_{1-31} from the par distalis, was generally increased under conditions examined here (see Discussion).

Discussion. The present findings indicate that pentobarbital, halothane, and urethane all evoke rapid and significant increases in bloodborne β-END-LI in rats. As previously observed with ether anesthesia (15), stimulation of pituitary β-END-LI release by pentobarbital or halothane is of shorter duration (5–20 min) and reduced magnitude (2- to 3-fold) as compared to that caused by a variety of physical stimuli (e.g., physical immobilization) known to increase β-END-LI secretion in this species (13–15). Urethane, a commonly used anesthetic in rodents, was more effective than pentobarbital, halothane, or ether [see Ref. (15)] in stimulating pituitary β-END-LI release. Perhaps a more generalized depression of CNS function, as may have resulted from the carbamate administration (16), produced the enhanced plasma β-END-LI response to urethane. The ability of each anesthetic, however, to stimulate pituitary β-END-LI release indicates that a rise in circulating opiates may

TABLE II. EFFECTS OF HALOTHANE, URETHANE, PENTOBARBITAL, AND MECHANICAL VENTILATION ON THE DIFFERENT MOLECULAR FORMS OF IMMUNOREACTIVE β-END IN RAT BLOOD

Treatment	Total plasma β-END-LI per pool[a] (pg/ml)	% β-END	% β-LPH	β-END/β-LPH ratio
Control	299	83	17	4.9
Halothane				
Spontaneous breathing	682	69	31	2.2
Mechanically ventilated	5402	60	40	1.5
Urethane				
Spontaneous breathing	1289	54	42	1.3
Mechanically ventilated	5481	81	19	4.3
Pentobarbital				
Spontaneous breathing	418	84	16	5.2
Mechanically ventilated	2718	71	24	3.0

[a] Treatment group plasma pools (5–10 ml) were chromatographed on Sephadex G-50 superfine resin. The plasma pools and elution profiles were immunoassayed for β-END-LI and the relative contribution of β-END- and β-LPH-sized immunoreactivity to total plasma β-END-LI are presented as percentages. Where their combined amounts do not equal 100%, a small portion of total plasma β-END-LI (≤5%) eluted in the void volume, suggesting the presence of a high-molecular-weight precursor for β-END. The β-END/β-LPH ratio indicates the β-END-sized material is the predominant form of circulating β-END-LI under most conditions.

be a common phenomenon associated with most, if not all, anesthetics. This relationship between anesthesia and pituitary β-END-LI release points to the possible role(s) for endogenous opiates in anesthetic analgesia. Caution, however, must be taken when considering this possibility [see Ref. (17)]. Although numerous reports indicate that opiate antagonists (naloxone and naltrexone) diminish various *in vivo* effects of a number of anesthetics (1–7, 9, 17–19), comparable experiments have failed to demonstrate such actions for the antagonists in both man (20, 21) and laboratory animals (10, 22–25). That these differences exist raises question as to the importance of endogenous opiates in mediating the anesthesia produced by anesthetic drugs. It is possible, however, that β-END and related opiate peptides influence anesthesia through actions on receptors which do not readily recognize naloxone or naltrexone. If so, these actions would be little affected by the two opiate antagonists. Compelling evidence to support this conclusion is presently lacking but may emerge as the pharmacology of multiple opiate receptors is better elucidated (26). The findings reported here do demonstrate that anesthesia, as produced by a number of agents, is associated with the activation of at least one intrinsic opiate peptide system, the release of pituitary β-END-LI into blood. Whether this occurs as a consequence of the anesthesia or participates in the development of the anesthetic state remains to be determined. Similar to pituitary β-END, release of opiate peptides by neurons within the CNS (27) or from the adrenal medulla (28) may also occur during anesthesia and contribute to the CNS and/or peripheral effects produced by anesthetic drugs.

Reported here are dramatic plasma β-END-LI responses to endotracheal intubation and mechanical ventilation which occur in animals under general anesthesia (Figs. 1–3). The time courses of these hormonal responses suggest mediation via a neuroendocrine reflex mechanism initiated by physical distention and/or trauma of the trachea and upper airways. Interestingly, morphine treatment (1 mg/kg, Fig. 3; 5 mg/kg, Table I) does attenuate this release of pituitary β-END-LI. This suggests the possibility that a morphine sensitive, yet subconscious, phenomenon is involved in the sequence of events leading to the greatly stimulated β-END-LI release. Similar observations have also been made in man. DuBois and coworkers (29) recently reported that pituitary β-END-LI responses occur in anesthetized humans (in response to laparotomy) and these, too, are diminished by administration of an opiate (fentanyl). Unlike the narcotic analgesics, the neuromuscular blocking drug pancuronium (30) (0.3 kg/mg) does not effect the plasma β-END-LI response to mechanical ventilation in rats (data not shown). Therefore, under the present conditions, the release of pituitary β-END-LI is probably not associated with neuromuscular activity in the chest wall or elsewhere in the body.

Gel filtration analysis revealed that the anesthetic drugs investigated here, as well as the procedures of intubation and mechanical ventilation, generally increased circulating β-END-LI resembling both β-END and β-LPH in molecular size. Although β-LPH is not analgesic, it does serve as a marker for β-END-LI secretion from corticotrophs of the pars distalis (31–34). Since opiate active β-END_{1-31} is cosecreted with β-LPH by corticotrophs *in vitro* (32–34), it is reasonable to conclude that increases in β-END_{1-31} also accompany rises in plasma β-LPH *in vivo*. This point may be of importance since only β-END_{1-31} is analgesic (31), whereas the modified forms of β-END_{1-31} (shorter and/or acetylated) released by the melanotrophs of the pars intermedia are not active at known opiate receptors (31–33).

In summary, we have demonstrated that the anesthesia produced by several commonly used anesthetics is associated with the release of pituitary β-END-LI in rats. The amounts released, however, are quite modest as compared to those evoked by intubation or mechanical ventilation. In all cases, however, the data indicate that concentrations of circulating β-END_{1-31}, the analgesic form of β-END, are elevated suggesting the possible involvement of endogenous opiate peptides in anesthesia. The considerable variability in the reported effects of opiate antagonists on anesthesia and anesthetic analgesia [see Ref. (17)], however, indicates that the released pituitary β-END_{1-31} probably does not mediate the pre-

dominant effects of general anesthetics. Possible actions of endogenous opiate peptides mediated through receptors which do not recognize naloxone or naltrexone should be considered in light of the increasing number of pharmacologically distinct receptors to which β-END$_{1-31}$ and related peptides are reported to bind. At present, the physiologic functions of pituitary β-END released in response to anesthetic drugs and mechanical ventilation remain to be determined as do the specific receptors with which this class of peptides may interact.

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