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Do Inhalation General Anesthetic Drugs Induce the Neuronal Release of Endogenous Opioid Peptides?

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Abstract

The antagonism of some effects of inhalation general anesthetic agents by naloxone suggests that there may be an opioid component to anesthetic action. There is evidence that this opioid action component is due to neuronal release of endogenous opioid peptides. The strongest evidence is provided by studies that monitor changes in the concentration of opioid peptides in the perfused brain following inhalation of the anesthetic. Indirect or circumstantial evidence also comes from studies of anesthetic effects on regional brain levels of opioid peptides, antagonism of selected anesthetic effects by antisera to opioid

peptides and anesthetic-induced changes radioligand binding to opioid receptors. It is likely that some inhalation general anesthetics (e.g., nitrous oxide) can induce neuronal release of opioid peptides and that this may contribute to certain components of general anesthesia (e.g., analgesia). More definitive studies utilizing in vivo microdialysis or autoradiography in selected areas of the brain during induction and successive states of general anesthesia have yet to be conducted.

Introduction

The concept of general anesthesia has been realized for some 150 years and, despite its monumental impact in medicine, exactly how general anesthesia is produced pharmacologically remains uncertain. Interest in the mechanism of anesthesia remains high, though, and there has been no shortage of hypothesized mechanisms. Foremost among these are the "lipid theory" and the "protein theory".

Earlier studies suggested that general anesthetics might produce their effects by acting on the neuronal membrane. Meyer and Overton independently described the correlation between lipid solubility of inhaled anesthetic agents and the amount of drug required for anesthesia (Miller et al., 1972). According to this so-called "lipid theory," it was the number of anesthetic molecules, irrespective of which anesthetic agent, dissolved in the phospholipid bilayer of the neuronal membrane that was actually responsible for anesthetic state (Kaufman, 1977). It was postulated that dissolution of anesthetic molecules in the neuronal membrane expanded the volume of the hydrophobic region, producing a structural perturbation of ion channels and preventing the sodium influx necessary for synaptic release of neurotransmitter (Cantor, 1997).

The newer opposing view, the purported "protein theory," holds that anesthetics directly interact with hydrophobic pockets on specific membrane proteins to produce the anesthetic state. Franks and Lieb have amassed an impressive body of evidence that general anesthetics may act stereoselectively on ligand-gated ion channels that facilitate postsynaptic inhibitory channel fluxes (Franks and Lieb, 1994, Franks and Lieb, 1998). Studies that correlated anesthetic potency with prolongation of GABA-mediated inhibition of neuronal excitability implicate the $GABA_A$ receptor as a possible protein target of anesthetic drug action (Zimmerman et al., 1994).

There are multiple components to the anesthetic state induced by general anesthetic drugs and these may vary from one anesthetic drug to another (Kissin, 1993). It is not unreasonable that different drug actions may underlie different components of general anesthesia. Since insensitivity to pain is one component of general anesthesia, it has been suggested that perhaps some general anesthetic drugs work through activation of endogenous opioid systems to evoke analgesia. The prima facia evidence implicating an opioid basis for any physiological or pharmacological event is blockade by an opioid receptor antagonist.

An opioid component to general anesthesia?

Continuous perfusion of dogs with 20 µg/ml naloxone through the fourth cerebral ventricle—but not the lateral or third cerebral ventricles—reversed the hypotension, bradycardia, depressed baroreceptor response, and EEG synchronization induced by inhalation of halothane (Arndt and Freye, 1979). The antagonism was competitive as it was overcome by doubling the concentration of inspired halothane and it was reversible as it dissipated with discontinuation of the naloxone perfusion. Intravenously

administered naloxone also attenuated in a dose-related manner the hypotensive effect observed in halothane-anesthetized dogs (Artru et al., 1980).

Intravenous (i.v.) treatment of rats with naloxone also reportedly antagonized the antinociceptive effects of cyclopropane, halothane and enflurane following 30 min exposure to the anesthetic (Finck et al., 1977). In additional experiments, electroencephalographic readings in cyclopropane- and halothane-anesthesized rats showed conversion to patterns that were more characteristic of lighter planes of general anesthesia following naloxone administration (Finck et al., 1977).

Other studies have reported equivocal results in the ability of narcotic antagonists to shorten the duration of anesthesia and restore the righting reflex. Naloxone failed to alter the duration of anesthesia induced by halothane, diethylether, ketamine pentobarbital, Althesin (a mixture of alphaxalone and alphadolone) or hyperbaric nitrous oxide as determined by recovery of righting reflex (Bennett, 1978, Smith et al., 1978, Lawrence and Livingston, 1981). This was confirmed by another study in which naloxone failed to alter the MAC values for halothane, enflurane and isoflurane (Levine et al., 1986). However, comparable doses of naloxone markedly reduced the antinociceptive effect of ketamine and nitrous oxide but not halothane, diethylether or xylazine (Smith et al., 1978, Lawrence and Livingston, 1981). In another study, naloxone and naltrexone asymmetrically shortened the sleeping times induced by ketamine, halothane, or pentobarbital; high-dose naltrexone was less effective than lower doses in producing this analeptic effect suggesting this action was unrelated to blockade of opioid receptors (Kraynack and Gintautas, 1982).

It seems most likely that the naloxone antagonism of some inhalation anesthetics is due to stimulation by the anesthetic drug of a neuronal release of endogenous opioid peptides that then activate opioid receptors. In a series of intriguing experiments Berkowitz et al., 1976, Berkowitz et al., 1979 reported that the antinociceptive effect of nitrous oxide (N_2O) was attenuated in rats and mice pretreated with naloxone. Morphine-tolerant animals were cross-tolerant to N_2O, yet N_2O-tolerant animals remained sensitive to morphine-induced antinociception (Berkowitz et al., 1977, Berkowitz et al., 1979). This unilateral cross-tolerance between N_2O and morphine suggested that tolerance to N_2O resulted from depletion of an opioid mediator rather than change in sensitivity of opioid receptors or signaling pathways (Berkowitz et al., 1979), hence the idea that general anesthetics might induce neuronal release of opioid peptides.

Further evidence that N_2O, in particular, might stimulate neuronal release of endogenous opioid peptide is indirectly shown by studies utilizing rabbit antisera against opioid peptides. The antinociceptive effect of N_2O in the rat hot plate test was antagonized dose-dependently by an antiserum against β-endorphin but not another against ME (Hara et al., 1994). Similarly N_2O-induced antinociception in the mouse acetic acid-induced abdominal constriction test was antagonized by intracerebroventricular (i.c.v.) or intrathecal (i.t.) pretreatment with antisera against various dynorphin fragments but not antisera against ME or β-endorphin (Branda et al., 2000, Cahill et al., 2000). The suggested neuronal release of dynorphin in the mouse was supported by the finding that N_2O-induced antinociception was selectively and significantly enhanced by i.c.v. pretreatment with phosphoramidon, an inhibitor of endopeptidase 24.11, which has been implicated in dynorphin degradation (Branda et al., 2000).

More recently, it was found that the methylnaloxone-sensitive, early-phase hypotension produced in rats by isoflurane was attenuated by pretreatment with rabbit antisera against methionine–enkephalin but not β-endorphin (Ellenberger et al., 2003). Such reports of functional antagonism of anesthetic

effects by antisera against endogenous opioid peptides is further evidence that at least some effects caused by anesthetic drugs involve a stimulated release of opioid peptides.

Effects of general anesthetics on plasma levels of endogenous opioid peptides

Exposure to halothane produced a 2–3-fold increase in plasma β-endorphin-like immunoreactivity that peaked at 10 min and subsided by 30 min following induction (Maiewski et al., 1984). Treatment with pentobarbital and urethane intravenously produced 2–3-fold and 10-fold increases in plasma β-endorphin-like immunoreactivity, respectively. Halothane also increased plasma levels of β-endorphin but not met–enkephalin or dynorphin in ponies (Luna and Taylor, 1995). In a clinical study, isoflurane proved to be more effective than halothane in increasing plasma β-endorphin concentrations in children aged 1–6 years (Garcia-Sanchez et al., 1993).

Thus, while there appears to be consistent evidence that inhalation and injectable anesthetics can increase plasma levels of β-endorphin, such studies fail to demonstrate whether the agents in question directly or indirectly cause such changes. The influence of anesthetics on circulatory levels of opioid peptides may reflect an effect far downstream from the site of action of the anesthetic drug. These studies also failed to correlate the change in plasma opioid peptide concentration to any specific physiological change.

Effects of general anesthetics on concentrations of endogenous opioid peptides in the brain

Another line of evidence supporting the hypothesis that general anesthetics can stimulate the neuronal release of endogenous opioid peptides is that many inhalation anesthetics are capable of altering brain tissue concentrations of opioid peptides. However, the findings of such studies are heavily influenced by the anesthetic concentration and the time of exposure leading to a variety of interpretations. The level of peptides is only a rough approximation of neuronal function. Increased tissue levels of peptides may represent an increase in the neuronal release of peptides; however, it is plausible that the stimulated release of peptides might occur in the absence of any change in the tissue concentration of the peptide. On the other hand, decreased tissue levels of peptides may indicate excessive neuronal release of the peptides without adequate replenishment of peptide concentrations. Despite these caveats, various investigations have reported both increases and decreases in whole brain or regional brain concentrations of multiple opioid peptides following anesthetic exposure.

Compared to room air-exposed rats, animals exposed to 75% N_2O in $O_2$for 60 min had significant (12–18%) increases in the concentrations of ME-like immunoreactivity in the brainstem, spinal cord, hypothalamus and corpus striatum but not in the cerebral cortex or diencephalon (Quock et al., 1986), although a similar study showed no effect of N_2O on ME levels (Morris and Livingston, 1984). In rats exposed to N_2O in $O_2$for 60 min, there was a concentration-related increase in levels of β-endorphin in the medial basal hypothalamus and the periaqueductal gray, which are sites along the neuroaxis functionally involved with analgesia (Zuniga et al., 1987a). Chronic exposure (8 h/day over one estrous cycle) of female rats to N_2O resulted in increased levels of ME in the brainstem and increased levels of β-EP in the pituitary (Kugel et al., 1991). Hypothalamic concentrations of both ME and preproenkephalin mRNA were elevated in rats exposed to 60% N_2O, compared to rats exposed to room air (Agarwal et al.,

1996). Levels of preproenkephalin mRNA were progressively greater in proportion to the length of exposure. The increase might be considered compensatory replenishment of opioid peptide following neuronal release.

Reported effects of fluorinated hydrocarbons on brain opioid peptides reinforced suspicions of a more global interaction between general anesthetics and endogenous opioid systems; however, effects appear to be site-specific and time-dependent. A 2-h exposure of rats to 1.5% halothane in O_2 significantly reduced levels of ME in the pituitary gland and several brain regions but levels generally recover approximately 4 h later (Agarwal et al., 1994). Exposure to halothane also significantly decreased β-endorphin in the olfactory bulb, thalamus and midbrain but significantly increased β-endorphin in the pituitary gland and spinal cord. In contrast, exposure to 0.5% methoxyflurane in O_2 lowered ME content in the olfactory bulb, thalamus and hippocampus (Karuri et al., 1998). Methoxyflurane caused less drastic reductions in ME but markedly depleted ME levels in the rat olfactory bulb, thalamus and hippocampus. Methoxyflurane also decreased β-endorphin in the olfactory bulb, thalamus and brainstem and had a time-dependent biphasic effect on β-endorphin in the pituitary gland (Karuri et al., 1998).

A 1-hr exposure of rabbits to halothane increased ME levels in the hypothalamus, hippocampus and mesencephalon, increased LE content in the hippocampus and reduced LE levels in the hypothalamus (Chmielnicki et al., 1995). Some of these changes persisted up to 60 min following the end of halothane exposure. Isoflurane increased levels of LE in the rabbit hypothalamus, hippocampus and mesencephalon (Chmielnicki et al., 1997). There were also decreases in ME and LE content in selected segments of the spinal cord. Short (5 min) exposure to enflurane increased levels of ME and LE in hippocampus and mesencephalon but depleted ME in the hypothalamus and striatum (Kmieciak-Kolada et al., 1998). Exposures of up to 1 h resulted in increased ME content in the hypothalamus and hippocampus and also LE levels in the hypothalamus and mesencephalon, but LE levels were decreased in the striatum and hippocampus.

Such findings of altered regional brain levels of opioid peptides are indicative of some direct or indirect interaction. Changes are peptide-specific as ME and LE do not appear to change in parallel fashion nor does β-endorphin. Levels in different brain regions are differentially affected by general anesthetics indicating a site-specificity of anesthetic action in the brain. More importantly, the duration of exposure to an anesthetic has tremendous impact on the direction of the change. Short-term exposure is more likely to increase regional levels of an opioid peptide until the rate of depletion exceeds the rate of repletion when tissue levels of the peptide decline. Following termination of anesthesia, levels of the affected peptide may exceed resting levels depending on the rate of replenishment.

Effects of general anesthetics on release of opioid peptides

In the face of reports of anesthetic-induced changes in brain concentrations of opioid peptides—and the attendant differences in interpretation of such findings—other investigators have sought to detect changes in the levels of opioid peptides released either into the plasma or the cerebrospinal fluid (CSF).

The most enlightening data comes from studies that directly monitored the release of endogenous opioid peptides in the brain. In ventricularly–cisternally-perfused rats under basal urethane anesthesia, a 60-min exposure to 75% N_2O in oxygen (O_2) increased methionine–enkephalin (ME)-like immunoreactivity in 8-min fractions of artificial cerebrospinal fluid (CSF) perfusate (Quock et al., 1985).

In a similar study, samples of CSF were drawn from the third cerebral ventricle of dogs and fractionated to improve selectivity of the radioimmunoassay (RIA) procedure. Samples drawn during exposure to N_2O showed that ME was dramatically increased by 28–400 times over control and ME–Arg^6–Phe^{7}increased 1.5–8.3 times over control (Finck et al., 1985). On the other hand, N_2O appeared not to alter levels of dynorphin A, dynorphin B or β-endorphin in the third ventricular CSF.

In an elegantly designed in vitro experiment, rat basal hypothalamic cells were mechanically dissociated, affixed to cytodex beads and placed in a continuous flow superfusion column (Zuniga et al., 1987b). The effluents collected every 5 min during superfusion with 60% and 80% N_2O had significantly higher levels of immunoreactive β-endorphin than did effluents collected during superfusion with saline or 30% N_2O. These results suggest that N_2O has a stimulatory effect on central pro-opiomelanocortin neurons and evokes the release of β-endorphin. In a clinical study, human subjects were administered thiopental (2–5 mg/kg) plus 70% N_2O followed by halothane; this produced no change in levels of β-endorphin and leucine–enkephalin immunoreactivity in the cerebrospinal fluid (Way et al., 1984).

Effects of general anesthetics on opioid radioligand binding

There has been a single report of opioid receptor binding following in vivo exposure to an inhalation anesthetic. Ngai and Finck (1988) showed that 18 h of N_2O produced a 20% reduction in B_{max} which would be consistent with the release of endogenous opioid peptides by N_2O and subsequent down regulation. In vitro binding experiments, both saturation binding experiments in the presence or absence of anesthetic (Tejwani et al., 1991, Inoki et al., 1983, Ori et al., 1989, Campbell et al., 1995) and inhibition experiments where anesthetics inhibit the binding of a single concentration of opioid radioligand (Ahmed and Byrne, 1980, Tejwani et al., 1991), provide evidence that inhalation anesthetics may directly affect radioligand binding of opioid receptors. Several reports demonstrate that anesthetics inhibit opioid radioligand binding by reducing the apparent affinity of the receptor (Campbell et al., 1995, Inoki et al., 1983, Tejwani et al., 1991), although there has been one report where no effect was found (Lawrence and Livingston, 1981) and most studies report no effect under certain tissue regions, different conditions, or different radioligands (Ori et al., 1989, Campbell et al., 1995, Inoki et al., 1983). It is unclear whether the reported anesthetic-induced inhibition of radioligand binding is due to competitive inhibition at the opioid binding site, noncompetitive inhibition, or a direct alteration of either receptor protein or surrounding membrane by the anesthetic drug.

Although affinity is most often affected, there have been two reports where receptor concentration (B_{max}) has been altered in vitro by presence of anesthetic (Ori et al., 1989, Inoki et al., 1983). These changes in opioid binding in vitro suggest that although release of endogenous opioid peptides may be the prime mechanism by which volatile anesthetics interact with the opioid system, there may be a direct membrane effect or an effect on opioid receptors as well.

Conclusion

Do general anesthetics induce the neuronal release of endogenous opioid peptides? The answer appears to be yes but whether all general anesthetics do so is another question. Does the release of opioid peptides cause general anesthesia? There are undoubtedly multiple mechanisms through which anesthesia is produced and there are multiple components to the anesthetic state that may vary from one anesthetic drug to another. Some but not all of these effects appear to be mediated by endogenous opioid mechanisms. The analgesic effect of certain anesthetics like nitrous oxide is likely to be explained

at least in part of release of opioid peptides. The most direct approach would appear to be in vivo microdialysis, in which a dialysis probe is inserted into the brain to collect dialysate in the conscious state, during induction of anesthesia and through several stages and planes of anesthesia. Quantification of the opioid peptide content of these samples will provide more definitive evidence of whether general anesthetic agents can release endogenous opioid peptides. Another interesting approach would be in vivo autoradiography to localize brain regions where the release of endogenous opioid peptides might competitively displace a radioligand. To date there have been no such studies.

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