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Pontine Cholinergic Mechanisms Modulate the Cortical Electroencephalographic Spindles of Halothane Anesthesia

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Background: **Halothane anesthesia causes spindles in the electroencephalogram (EEG), but the cellular and molecular mechanisms generating these spindles remain incompletely understood. The current study tested the hypothesis that halothane-induced EEG spindles are regulated, in part, by pontine cholinergic mechanisms.**

Methods: **Adult male cats were implanted with EEG electrodes and trained to sleep in the laboratory. Approximately 1 month after surgery, animals were anesthetized with halothane and a microdialysis probe was stereotaxically placed in the medial pontine reticular formation (mPRF). Simultaneous measurements were made of mPRF acetylcholine release and number of cortical EEG spindles during halothane anesthesia and subsequent wakefulness. In additional experiments, carbachol (88 mM) was microinjected into the mPRF before halothane anesthesia to determine whether enhanced cholinergic neurotransmission in the mPRF would block the ability of halothane to induce cortical EEG spindles.**

Results: **During wakefulness, mPRF acetylcholine release averaged 0.43 pmol/10 min of dialysis. Halothane at 1 minimum alveolar concentration decreased acetylcholine release (0.25 pmol/10 min) while significantly increasing the number of cortical EEG spindles. Cortical EEG spindles caused by 1 minimum alveolar concentration halothane were not significantly different in waveform, amplitude, or number from the EEG spindles of nonrapid eye movement sleep. Microinjection of carbachol into the mPRF before halothane administration caused a significant reduction in number of halothane-induced EEG spindles.**

Conclusions: **Laterodorsal and pedunculopontine tegmental neurons, which provide cholinergic input to the mPRF, play a causal role in generating the EEG spindles of halothane anesthesia. (Key words: Measurement techniques: microdialysis. Monitoring: electroencephalogram burst suppression. Neurotransmission: cholinergic. Potency, anesthetic: minimum alveolar concentration.)**

THE physiologic and behavioral responses to painful stimuli during anesthesia are characterized by a high degree of individual variability. About 30 yr ago, a seminal series of investigations showed that, in spite of the variable response to pain, a useful standard of anesthetic potency was the minimum alveolar concentration (MAC) of anesthetic necessary to prevent movement in response to a painful stimulus. The concept of MAC[1] thus provided a standard behavioral response for comparing different inhalational anesthetics.[2] The positive correlation of MAC with lipid solubility of volatile anesthetics[3] also facilitates ongoing efforts to elucidate the neuronal mechanisms causing anesthesia.[4,5]

As reviewed recently,[6] there is an extensive literature on the use of electroencephalographic (EEG) monitoring for assessing anesthetic depth. Less is known, however, about the central nervous system mechanisms generating changes in the cortical EEG at various anesthetic concentrations. For the volatile agent halothane, anesthetic concentration was shown to be correlated with the production of 10–14 Hz activity in the human EEG.[7] Increased 12–15 Hz EEG activity, described as similar to barbiturate spindles, also was noted in cat after administration of halothane.[8] This relationship between halothane concentration and EEG spindles suggests that understanding the cellular mechanisms generating halothane-induced EEG changes may also contribute to efforts seeking to elucidate a neuronal basis underlying altered behavior during anesthesia.

During both natural sleep and anesthesia, cholinergic and cholinoceptive pontine neurons alter EEG and behavioral arousal. During natural sleep, for example, cholinergic neurotransmission in the pons plays a key

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role in generating the EEG.[9–12] The medial pontine reticular formation (mPRF) contains neurons that are known to be cholinoceptive, and microinjecting cholinergic agonists into the mPRF reliably produces a desynchronized EEG, similar to the EEG of natural rapid eye movement (REM) sleep (reviewed in reference 13). Acetylcholine release within the mPRF is enhanced during natural REM sleep[14] and during the cholinergically induced REM sleeplike state.[15]

Data also emphasize the importance of cholinergic neurotransmission for the generation of anesthetically induced states. During isoflurane anesthesia in rat, increasing brain acetylcholine levels by intracerebroventricular administration of physostigmine significantly increased MAC.[16] During halothane anesthesia in cat, acetylcholine levels in the pontine reticular formation were decreased.[11] These data inspired the current study designed to test the hypothesis that halothane-induced EEG spindles are generated by cholinergic mechanisms localizable to a specific region of the pontine brain stem. The results suggest that cholinergic neurons in the laterodorsal and pedunculopontine tegmental (LDT/PPT) nuclei, known to modulate the EEG spindles of non-REM sleep in cat,[17] also cause the EEG spindles characteristic of halothane anesthesia.

Methods and Materials

Surgical Preparation

All experiments were conducted in accordance with guidelines established by the National Institutes of Health.‡ As described in detail previously,[13,18] adult male cats (N = 8) were implanted during halothane anesthesia with standard, indwelling electrodes for recording the EEG, electrooculographic, electromyographic, and ponto-geniculo-occipital waves from the lateral geniculate bodies of the thalamus. These variables enabled an objective measurement of sleep and wakefulness according to well-developed criteria.[19] A craniotomy was created to provide painless access to the brain stem for subsequent insertion of dialysis probes and/or microinjection cannulas. After recovery from surgery, the animals were trained to sleep in the laboratory. The experiments were begun approximately 1 month after electrode implantation.

Experimental Procedure

Before each experiment, the animals were anesthetized by mask induction with halothane, nitrous oxide, and oxygen. Tracheal intubation was accomplished after laryngoscopy and lidocaine spray was applied to the vocal cords. The lungs were ventilated to achieve an end-tidal carbon dioxide concentration of approximately 30 mmHg. Respiratory gas and anesthetic vapor concentrations were measured using a Raman spectrometer. End-tidal halothane concentration was adjusted to 1.2%, previously determined to be the 1 MAC value for cats.[20] Using techniques described in detail elsewhere,[12,15] a microdialysis probe was aimed stereotaxically for the mPRF. The initial coordinates were posterior = 3.0, lateral = 1.5, height = −5.0, theta = 30° posterior, according to the atlas of Berman.[21] For each experiment, continuous polygraphic recordings were obtained by connecting the EEG, electrooculographic, ponto-geniculo-occipital, and electromyographic electrodes to a Grass Model 7 Polygraph *via* a shielded cable.

Initial mPRF dialysis samples reflecting acetylcholine release due to injury induced by inserting the microdialysis probe were discarded. Once the chromatograms revealed a stable level of acetylcholine release, dialysis samples were obtained every 10 min during anesthesia at 1 MAC halothane and during emergence from anesthesia. The animals' tracheas were extubated during emergence when they were able to breathe spontaneously and maintain airway patency. After extubation, particular attention was given to confirming that the transitional state ended and that each animal was able to maintain prolonged intervals of wakefulness defined by behavioral and electrographic data. In no case were dialysis samples obtained during the transitional state classified as coming from a fully awake animal. Once normal arousal was present, dialysis samples continued to be collected from the mPRF for longer than 1 hr. At the end of this procedure, the dialysis probe was withdrawn, the craniotomy tube was closed, and the animals were returned to their home cages. After at least 1 week, procedures were repeated by placing the dialysis probe in a different location within the pontine reticular formation. It was thus possible to obtain multiple acetylcholine dialysis samples from the same animal during each experiment and to perform several experiments with each animal.

‡ Guide for the Care and Use of Laboratory Animals: Publication #86-23. Washington, DC, Institute of Laboratory Animal Resources, Department of Health and Human Services, National Institutes of Health, 1985.

Microdialysis and High-performance Liquid Chromatography

The microdialysis probe (CMA/10, Carnegie Medcine, Stockholm, Sweden) had a 2-mm polycarbonate tip and a 20-kD molecular mass cutoff. As described in detail previously,[12] the dialysis probe was perfused at 3.0 μl/min with Ringers solution and mPRF dialysis samples were collected every 10 min. The modified Ringers solution comprised: 147 mM NaCl; 4.0 mM KCl; 2.4 mM $CaCl_2$; 10 μM neostigmine; *p*H = 6.0. The 30-μl dialysis samples were injected into a high-performance liquid chromatography system (BAS LC-4B) that separated acetylcholine and choline. An immobilized enzyme reactor column generated H_2O_2 and chromatograms proportional to acetylcholine and choline.[22] Acetylcholine release was quantified by integrating the area under the chromatographic peak relative to known concentrations of acetylcholine and choline.[12]

Pontine Carbachol Administration

As reviewed in detail elsewhere,[10] administration of cholinomimetics into the medial pontine reticular formation of intact, unanesthetized cat causes a REM sleeplike state,[13,23] and significant increases in mPRF release of acetylcholine.[24] This increase in mPRF acetylcholine has been shown to arise from cholinergic LDT/PPT neurons[15] and to parallel the increased mPRF release of acetylcholine observed during natural REM sleep.[14] Rapid eye movement sleep and the cholinergically induced REM sleeplike state are characterized by increased discharge of LDT/PPT neurons[25,26] and by a complete absence of EEG spindles.[9] This made it possible to determine whether microinjecting 88 mM carbachol (4.0 μg/0.25 μl) into the mPRF before halothane induction would significantly decrease the number of halothane-induced EEG spindles. For these studies, EEG spindle frequency (spindles/min) was quantified during halothane anesthesia preceded by mPRF carbachol administration and compared to EEG spindle frequency during halothane alone. Thirty-minute samples of spindle activity were quantified for each of four concentrations of administered halothane (0.6–0.7%, 0.8–1.2%, 1.8%, and 2.4%). These data also made it possible to test the hypothesis of a dose-dependent relationship between number of EEG spindles and percentage of halothane administered.

Data Analyses

Acetylcholine levels in the pontine brain stem were expressed as pmol/10 min dialysis. Statistically significant alterations in acetylcholine caused by halothane anesthesia were quantified with analysis of variance and Student's *t* test. Frequency of cortical EEG spindles was measured during halothane anesthesia and compared to cortical spindle activity during recordings of naturally occurring nonrapid eye movement (NREM) sleep not associated with anesthesia. Two-way analysis of variance, *t* test, and Sheffe's F test were used to evaluate the effect of halothane dose, and mPRF carbachol administration on EEG spindle frequency. Dialysis probe placement was localized histologically within the mPRF for each brain by examining cresyl violet-stained, serial sections of formalin-perfused brain stem.

Results

Halothane Decreased Medial Pontine Reticular Formation Acetylcholine

Detailed histologic analysis confirmed that all dialysis probe sites were within a region of the mPRF referred to in Berman's[21] atlas as the gigantocellular tegmental field. Figure 1A shows representative chromatograms

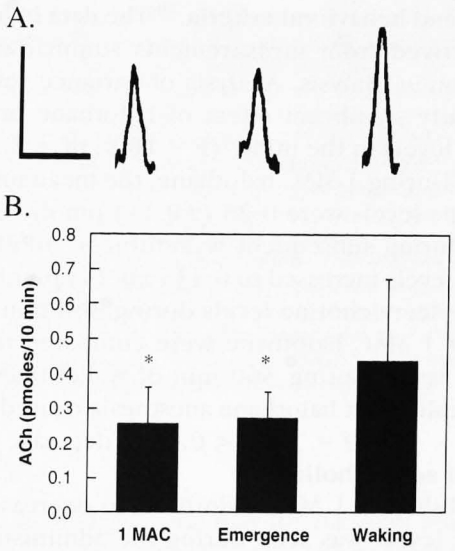

Fig. 1. Individual chromatograms (*A*) and mean (+SD) mPRF acetylcholine levels (*B*). Halothane anesthesia significantly depressed acetylcholine levels in the medial pontine reticular formation. Calibration for chromatogram (*A*) is shown at left; horizontal bar ≈110 s; vertical bar = 0.05 nA. Histograms (*B*) illustrate acetylcholine levels measured in four cats during 1 MAC halothane anesthesia (N = 36 dialysis samples), emergence (N = 13), and wakefulness (N = 36). *$P < 0.05$ compared to acetylcholine levels during wakefulness.

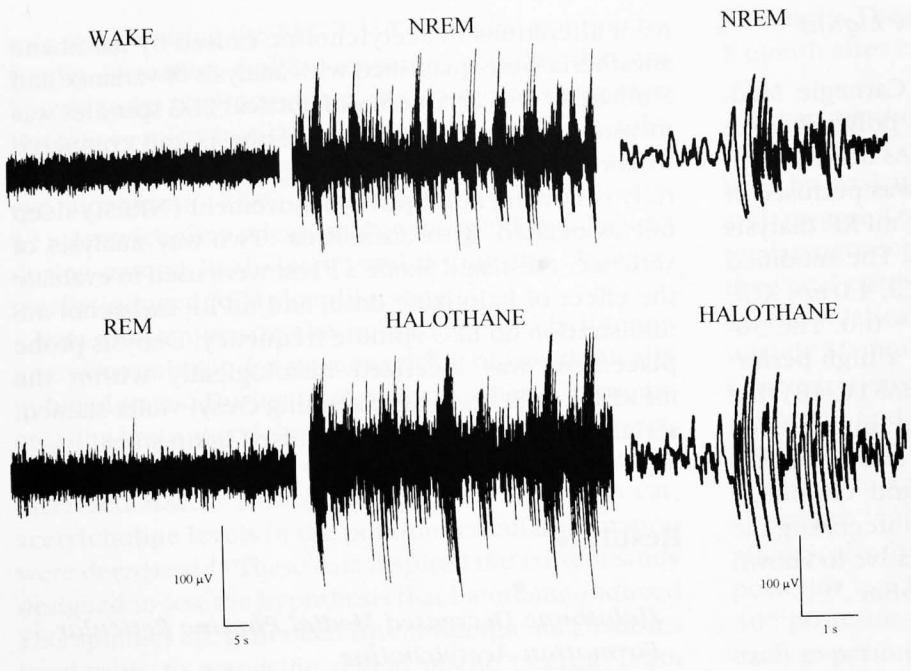

Fig. 2. Cortical electroencephalogram recorded during wakefulness, nonrapid eye movement sleep, rapid eye movement sleep, and halothane anesthesia. The low amplitude, high frequency electroencephalogram of wakefulness (WAKE) is similar to the EEG recorded during rapid eye movement (REM) sleep.[9] Non-REM sleep (NREM) is characterized by high amplitude (>100 μV) synchronized waves with frequencies of 8–14 Hz (called spindles). The lower tracing in the center shows that electroencephalogram spindles during halothane anesthesia are similar to electroencephalogram spindles of NREM sleep. The right-most traces show a single spindle with an expanded time-scale during NREM sleep (*top*) and during halothane anesthesia (*bottom*).

of mPRF acetylcholine during administration of 1 MAC halothane (left), emergence from halothane anesthesia (middle), and wakefulness (right). For all of the current results, the fully aroused waking state always was unambiguous and was based on electroencephalographic and behavioral criteria.[19] The data in figure 1B were derived from measurements summarizing 850 min of brain dialysis. Analysis of variance revealed a statistically significant effect of halothane on acetylcholine levels in the mPRF ($F = 10.9$; df = 2, 84; $P < 0.001$). During 1 MAC halothane, the mean mPRF acetylcholine levels were 0.25 (±0.11) pmol/10 min dialysis. During subsequent wakefulness, mPRF acetylcholine levels increased to 0.43 (±0.24) pmol/10 min dialysis. Acetylcholine levels during 360 min of anesthesia at 1 MAC halothane were compared to acetylcholine levels during 360 min of wakefulness, and *t* test revealed that halothane anesthesia caused a significant ($t = 4.2$; df = 70; $P < 0.001$) decrease in mPRF levels of acetylcholine.

The ability of 1 MAC halothane to decrease acetylcholine levels was seen during the administration of every halothane anesthetic. When halothane administration was discontinued, the first behavioral (movement) and EEG (loss of cortical spindles) signs indicating emergence from anesthesia were observed at end-tidal halothane concentrations ranging from 0.6% to 0.2% halothane. When end-tidal halothane concentrations were <0.1%, the animals regained waking consciousness. The acetylcholine levels during the period of emergence from halothane anesthesia averaged 0.27 (±0.07) pmol/10 min dialysis. Acetylcholine levels during emergence (fig. 1B) were significantly less than acetylcholine levels measured during wakefulness ($t = 2.4$; df = 47; $P < 0.05$). There was no statistically significant difference in mPRF acetylcholine levels comparing 1 MAC halothane to emergence from anesthesia. Arterial blood pressure measurements showed that halothane-induced decreases in systemic blood pressure were not significantly correlated with mPRF acetylcholine levels.

Halothane, Isoflurane, Enflurane, and Electroencephalogram Spindles

Figure 2 depicts cortical EEG recordings that are characteristic of wakefulness, NREM sleep, REM sleep, and halothane anesthesia. The phenomenological similarity in the cortical EEG recording between NREM sleep and 1 MAC halothane also is apparent in figure 2. Quantification of EEG spindles showed that the mean (±SD) spindle frequency during sample intervals comprising 44 min of NREM sleep (10.2 ± 2.6 spindles/min) was not significantly different from spindle frequency during 44 min of halothane anesthesia (8.7 ± 2.8 spindles/min).

Figure 3 shows representative recordings of cortical EEG obtained during anesthesia with halothane, isoflurane, and enflurane. Note that during 1 MAC halothane

anesthesia, EEG spindles were superimposed on a background of low-voltage, high-frequency activity. This pattern is similar to the occurrence of spindles during natural NREM sleep (fig. 2, NREM). During 1 MAC anesthesia with isoflurane and enflurane (fig. 3), spindles arise from a nearly isoelectric background. Electroencephalographic spindles never occurred during wakefulness (fig. 3, bottom).

Decreased acetylcholine levels in the pontine reticular formation caused by halothane, also were observed while administering 1 MAC concentrations of the halogenated ethers, isoflurane and enflurane (fig. 4). During anesthesia with 1 MAC halothane, isoflurane, and enflurane, mPRF acetylcholine levels averaged 0.22 (±0.06) pmol/10 min, 0.18 (±0.14) pmol/10 min, and 0.23 (±0.10) pmol/10 min, respectively. During wakefulness, acetylcholine levels increased to 0.43 (±0.03) pmol/10 min.

To further characterize the relationship between EEG spindle frequency and halothane concentration, four different concentrations of halothane were administered while quantifying EEG spindle frequency. Analysis of variance performed on these data (fig. 5, solid squares) revealed a statistically significant main effect of halothane dose on number of EEG spindles per min. The greatest number of spindles occurred at end-tidal halothane concentrations of 0.6–0.7% halothane according to both *t* test and Scheffe's *post hoc* comparison. With 2.4% halothane there was EEG burst suppression reflected by a significant decrease, compared to 1.8% halothane, in number of EEG spindles.

Pontine Carbachol Administration Decreased Halothane Spindles

As a further test of the hypothesis that pontine cholinergic mechanisms causally contribute to halothane-induced EEG spindle generation, carbachol was microinjected into the mPRF before halothane administration. The results (fig. 5, solid circles) show that mPRF microinjection of carbachol caused EEG desynchrony and a statistically significant decrease in the number of EEG spindles associated with halothane concentrations less than 1 MAC (0.6–0.7%), at 1 MAC (1.2% halothane), and at 1.8% halothane. When end-tidal halothane was at 2.4%, the EEG began to reveal intervals of EEG burst suppression and there was no significant difference in spindle frequency between halothane alone and halothane plus pontine carbachol.

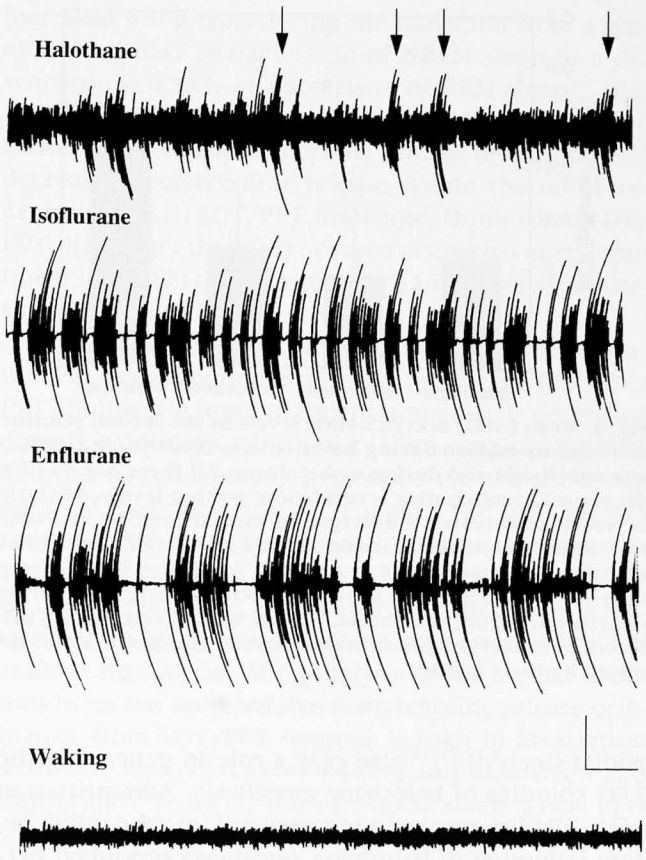

Fig. 3. Cortical electroencephalogram (EEG) recorded during 1 MAC anesthesia induced by three inhalational agents. Each EEG trace represents 60 s of recording time. The top trace (halothane) shows EEG spindle activity superimposed on a tonic background of low voltage, fast EEG activity. Arrows mark some of the halothane-induced EEG spindles recorded at sweep speed of 2.5 mm/s. The isoflurane trace shows an EEG comprising multiple spindle bursts superimposed on an isoelectric background. The enflurane trace shows a distinctly different pattern of EEG activity characterized by more single spikes and fewer clusters of spindles. The bottom tracing illustrates the desynchronized EEG typical of wakefulness. All EEG recordings were obtained from the same animal and all recordings were from the same implanted electrode configuration. At lower right, vertical calibration bar indicates 50 μV amplitude; horizontal bar indicates time of 5 s.

Discussion

The current data show that inhalational anesthetics decreased acetylcholine levels in the pontine reticular formation, and that halothane caused EEG spindles similar in appearance and frequency to the EEG spindles observed during NREM sleep. The results provide neurochemical, EEG, and behavioral data suggesting, for the first time, that cholinergic neurons in the LDT/PPT involved in generating the EEG spindles of natural

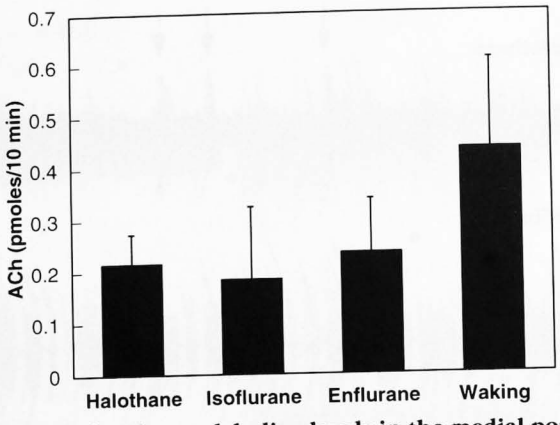

Fig. 4. Mean (±SD) acetylcholine levels in the medial pontine reticular formation during halothane, isoflurane, and enflurane anesthesia and during wakefulness. All three anesthetics decreased acetylcholine levels below waking levels. Analysis of variance revealed a decrease in medial pontine reticular formation acetylcholine levels due to a statistically significant anesthesia main-effect ($F = 4.5$; df = 3, 18; $P < 0.02$). These data are consistent with previous reports noting cholinergic depression during anesthesia.[16] There were no significant differences in acetylcholine levels between anesthetic states induced by these agents.

NREM sleep[9,12,27,28] also play a role in generating the EEG spindles of halothane anesthesia. Administration of the cholinergic agonist carbachol into the mPRF before induction of halothane anesthesia produced EEG desynchrony and significantly decreased the ability of halothane to cause EEG spindles. The results are discussed later with regard to: (1) possible mechanisms through which halothane alters acetylcholine levels in the mPRF, and (2) recent evidence showing that LDT/PPT cholinergic neurons contribute to the regulation of both pontine acetylcholine levels and thalamocortical mechanisms generating cortical EEG spindles.

Electroencephalogram as a Correlate of Arousal State

Although various phases of anesthesia have been recognized for some time,[29] the complexity of classifying states of anesthesia based on any single physiologic measure is well appreciated.[10,30] This difficulty emphasizes the importance of the concept of MAC as a tool for ensuring adequate anesthetic depth,[2] even while providing little insight into the neural mechanisms responsible for the discrete, nonlinear progression of states composing the spectrum of anesthesia. Similar to anesthesia, naturally occurring states of sleep also have distinct phases. The REM phase of sleep is synonymously referred to as desynchronized sleep,[9] active sleep,[31] and paradoxical sleep.[32] All three of these terms refer to a state during which the EEG is actively desynchronized (see low-voltage, high-frequency REM EEG in fig. 2). Jouvet's terminology points out the paradox that during this dreaming phase of sleep the EEG is similar to the desynchronized EEG of wakefulness.

Most of sleep is composed of the NREM phase, also referred to as quiet sleep, slow wave sleep, or synchronized sleep. During this phase of sleep, the EEG is characterized by high-voltage, synchronized waveforms.[33] The phenomenological descriptions of these EEG waves refer to them as spindles because these EEG recordings have a horizontal shape that progresses from broad to narrow, similar to a thread-bearing spindle in a fabric mill (figs. 2 and 3). It is noteworthy that EEG spindles that occur during the loss of wakefulness associated with natural NREM sleep also are observed

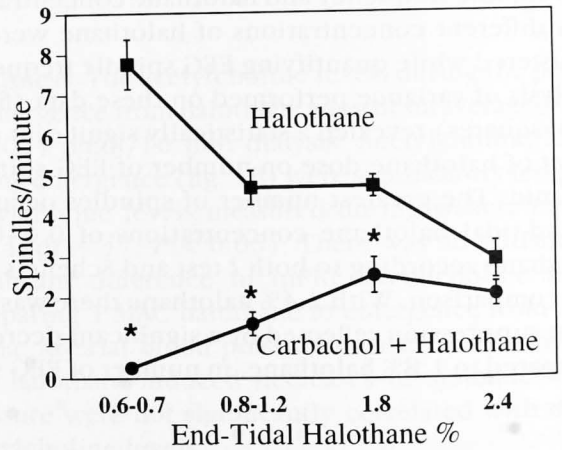

Fig. 5. Mean (±SEM) electroencephalogram (EEG) spindles caused by increasing doses of halothane. These data were obtained from six experiments on three cats. Solid squares (halothane) indicate frequency of EEG spindles as a function of end-tidal halothane concentration. Solid circles show the effect on EEG spindle frequency of microinjecting carbachol into the medial pontine reticular formation before halothane administration (carbachol + halothane). Two-way analysis of variance revealed a statistically significant effect of halothane concentration on EEG spindle frequency ($F = 5.36$; d.f. = 3,120; $P < 0.001$); a statistically significant effect of medial pontine reticular formation carbachol on EEG spindle frequency ($F = 196.9$; d.f. = 1,239; $P < 0.0001$); and a statistically significant interaction between halothane and mPRF carbachol on EEG spindles ($F = 33.36$; d.f. = 3,239; $P < 0.0001$). Asterisks indicate statistically significant differences ($p < 0.05$) in EEG spindle frequency comparing halothane alone and carbachol pretreatment of the medial pontine reticular formation before halothane administration. Note that at all end-tidal halothane concentrations, except for 2.4% halothane, medial pontine reticular formation carbachol significantly decreased the ability of halothane to produce EEG spindles.

during the loss of wakefulness caused by certain anesthetic agents. The data presented in this study suggest that the EEG spindles caused by halothane are the same as those observed during natural NREM sleep, and that cholinergic neurons in the LDT/PPT are involved in generating the EEG spindles of both NREM sleep and anesthesia. This working hypothesis is experimentally testable, and confirmation or refutation of this hypothesis will advance our understanding of neuronal mechanisms generating states of anesthesia.

Halothane Differentially Alters Acetylcholine Levels throughout the Brain

Limited information exists concerning the relationship between levels of neurotransmitters in the central nervous system and inhalational anesthetics.[16,34] The cellular and molecular mechanisms by which inhalational anesthetics alter acetylcholine levels are not presently known. Mulder and Schoffelmeer[35] have noted that some opioid agonists produce presynaptic inhibition and decrease acetylcholine levels. Previous studies from our laboratory have shown that systemic opioid administration causes decreased acetylcholine release within the mPRF.[12] The decreased acetylcholine in the mPRF caused by halothane, isoflurane, and enflurane (figs. 1 and 4) reflects transmitter release from cholinergic LDT/PPT neurons terminating in the mPRF.[15]

Changes in acetylcholine turnover and acetylcholine release are known to vary as a function of brain region studied.[34] For example, in the interpeduncular nucleus, acetylcholine release has been shown to increase when rats were exposed to 3% halothane anesthesia.[36] In the striatum, however, additional studies in rat noted a decreased interstitial concentration of acetylcholine during exposure to halothane.[37] These varied cholinergic responses within different brain regions during anesthetic exposure negate the possibility that halothane uniformly alters acetylcholine release throughout the brain.

Cholinergic Modulation of Halothane Spindle Generation

Cellular studies recently have advanced our understanding of the mechanisms involved in EEG spindle generation (reviewed in reference 28). For example, extracellular recordings from single LDT/PPT neurons revealed a diminished discharge during NREM sleep relative to firing rates recorded during wakefulness or REM sleep.[25] These same LDT/PPT neurons also exhibit increased firing rates during the transition from a synchronized EEG characteristic of NREM sleep to a desynchronized EEG characteristic of REM sleep[26] (fig. 2). Because electrical stimulation of the LDT/PPT causes enhanced acetylcholine release in the mPRF,[15] decreased acetylcholine release within the mPRF reflects decreased LDT/PPT discharge. Diminished LDT/PPT discharge, therefore, causes decreased acetylcholine release, EEG synchronization, and the onset of cortical EEG spindles.

The current finding of halothane-induced reductions in mPRF acetylcholine levels (fig. 1) suggests that LDT/PPT cholinergic input to the thalamus also is decreased during halothane anesthesia. Cholinergic LDT/PPT neurons simultaneously project to the thalamus and mPRF of the brain stem.[38,39] The dual LDT/PPT projection to mPRF and thalamus is fortuitous because the small size of the nucleus reticularis, and the proximity of adjacent thalamic nuclei currently precludes selective dialysis of nucleus reticularis, even in large-brained animals such as cat. Microdialysis of the medial thalamus in rat has revealed that acetylcholine release originating from LDT/PPT neurons is high in association with EEG desynchrony of waking and REM sleep and significantly reduced during NREM sleep when EEG spindles are present.[40] The finding of enhanced thalamic acetylcholine release during the EEG desynchrony of REM sleep is consistent with increased acetylcholine release in cat mPRF during the EEG activation of REM sleep.[12,14,24] Similarly, the diminished acetylcholine release in rat thalamus[40] during the EEG synchrony of NREM sleep is consistent with the current finding (fig. 1) of decreased mPRF acetylcholine in association with halothane-induced EEG synchrony. The ability of cholinergic agonists microinjected into the mPRF to cause EEG desynchrony[13,23] and to cause a decrease in halothane-induced EEG spindles (fig. 5), supports our conclusion that cholinergic LDT/PPT neurons play a causal role in generating the EEG spindles of halothane anesthesia.

A schematic summary of cholinergic mechanisms modulating EEG spindle generation is shown in figure 6. Two lines of evidence are key to figure 6, and to our interpretation of the current findings. First is the finding noted earlier that LDT/PPT neurons provide simultaneous cholinergic input to both the pontine reticular formation and to the thalamus.[38,39] Thus, decreased levels of acetylcholine in the pons (fig. 1) provide an indirect index of decreased cholinergic input to the thalamus. Within the thalamus, the centromedian and

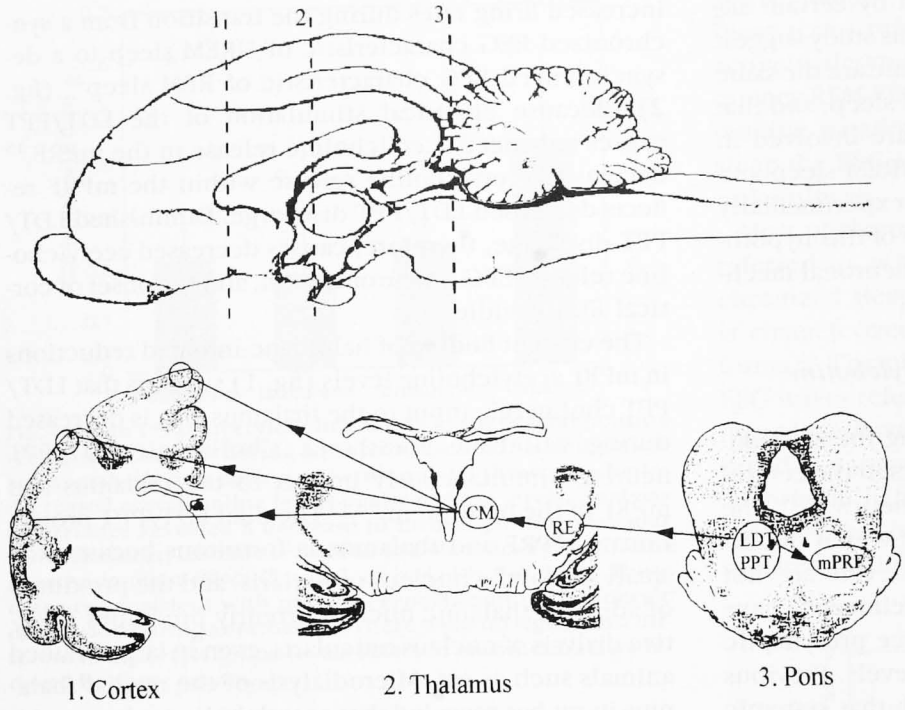

Fig. 6. Schematic summary of the brain regions regulating pontine acetylcholine levels and cholinergic modulation of the cortical electroencephalogram (EEG). The top image is a lateral view of cat brain with broken lines (1, 2, 3) identifying the origin of coronal sections illustrated below. EEG spindles are measured in the cortex (1), which electrophysiologic studies have shown are regulated by cells in the thalamic (2) nucleus reticularis (RE) and centromedian (CM) nucleus.[17,28] The pontine section (3) shows the mPRF region from which the acetylcholine dialysis samples were obtained (see fig. 1) and into which carbachol was administered (see fig. 5). Pontine cholinergic neurons in the laterodorsal and pedunculopontine tegmental (LDT/PPT) nucleus have been shown by anatomic studies[38,39] to project to both the thalamus and the mPRF, where they regulate acetylcholine release.[15] The lines connecting the cortex, thalamus, and pons schematically indicate pathways and neurons through which halothane induces cortical EEG spindles.

nucleus reticularis have been shown to generate cortical EEG spindles.[27] Additional studies[28] revealed that any reduction in thalamic acetylcholine causes the removal of cholinergic inhibition within the nucleus reticularis, thus enabling the nucleus reticularis/centromedian circuit to generate spindles in the cortical EEG.

A second line of evidence supporting the schematic shown in figure 6 is provided by the current carbachol microinjection experiments. Pontine administration of the cholinergic agonist carbachol significantly decreased the ability of systemically administered halothane to produce EEG spindles (fig. 5). Microinjection of carbachol into the mPRF has been known for more than 10 years to cause EEG desynchrony and elimination of all EEG spindles.[23] Carbachol administration into the mPRF has been shown to cause increased acetylcholine release[24] from cholinergic neurons in the LDT/PPT.[15] Thus, the current finding that mPRF microinjection of carbachol caused a significant decrease in halothane-induced EEG spindles provides a new line of evidence supporting the view that the EEG spindles of halothane anesthesia are caused, in part, by cholinergic neurons in the LDT/PPT.

Limitations and Conclusions

One limitation of the current study is the inability to provide a cellular-level explanation for the phenomenological differences in the cortical EEG caused by different inhalational anesthetics (fig. 3). Whereas spindles during halothane anesthesia arise from a low-voltage, high-frequency background, EEG spindles during isoflurane and enflurane anesthesia appear from a nearly isoelectric background (fig. 3). Although all three agents can cause spindles and decreased pontine acetylcholine levels, isoflurane and enflurane have the additional effect of suppressing the background, low-voltage, high-frequency EEG activity. It is not possible from the present data to make inferences regarding the neuronal mechanisms underlying these EEG differences.

Since mask inductions with halothane are commonly accompanied by movement, and since such movement would produce brain damage from the stereotaxically positioned dialysis probe, it was not possible to obtain measures of mPRF acetylcholine levels before administering halothane. The results, however, reveal no significant differences between postanesthesia measures of acetylcholine levels (waking, figs. 1 and 4) compared to our previous measures of waking acetylcholine levels obtained without prior anesthesia.[12,15,41] The lack of a significant difference in waking mPRF acetylcholine levels, with and without prior anesthesia, suggests that the electroencephalographic and behavioral measures of the current study accurately defined and differen-

tiated wakefulness from obtunded levels of arousal known to immediately follow recovery from anesthesia.

No state of consciousness is regulated by any single brain region.[10] In addition to identifying membrane-bound proteins as important sites of anesthetic action,[4,5] the anatomically distributed neuronal networks by which general anesthesia causes insensibility to pain, immobility, and loss of consciousness are known to range from spinal to cerebral levels.[42] Data have been presented showing that MAC in rat is independent of the forebrain,[43] and the importance of subcortical brain structures in generating states of general anesthesia has been emphasized.[44] The current results obtained from the pontine brain stem are consistent with the view that subcortical brain structures play a key role in generating states of general anesthesia.

No single neurotransmitter system generates any state of consciousness.[10,30] The current results, however, are in agreement with a large body of evidence showing that the cholinergic and cholinoceptive neurons in the pontine brain stem play a major role regulating EEG arousal[9,10] and anesthetic requirement.[16] Topographic EEG monitoring shows great promise for assessing depth of anesthesia,[6] but it is not yet possible to determine with 100% reliability from EEG data whether a patient administered general anesthesia is completely unconscious during surgery.[45] Because EEG desynchronization is known to be positively correlated with electrical[9] and metabolic[46] activation of the cortex, understanding the cellular and molecular mechanisms generating the EEG ultimately may help avoid the clinically important problem of patient awareness during anesthesia.[47]

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