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Research Report

The ventrolateral preoptic nucleus is not required for isoflurane general anesthesia☆

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ABSTRACT

Neurons of the ventrolateral preoptic nucleus (VLPO) promote sleep and VLPO loss produces insomnia. Previous studies show that general anesthetics including isoflurane activate VLPO neurons, and may contribute to their sedative effects. However, it is not clear to what extent the activation of VLPO neurons contributes to general anesthesia. We tested whether destruction of the VLPO neurons would affect the onset, depth, or recovery from isoflurane's general anesthetic effects. The VLPO was ablated in 25 rats by bilateral local injection of orexin-saporin, and polysomnography was performed to measure baseline sleep loss and responses to isoflurane anesthesia at 1% and 2%. Eight rats received sham (saline) injections. We measured isoflurane effects on time to loss of righting reflex, onset of continuous slow wave activity, and burst suppression; burst-suppression ratio; and time to recovery of righting reflex and desynchronized EEG. VLPO neuron cell loss was quantified by *post hoc* histology. Loss of VLPO neurons as well as lesion size were associated with cumulative sleep loss ($r=0.77$ and $r=0.62$, respectively), and cumulative sleep loss was the strongest predictor of high sensitivity to anesthesia, expressed as decreased time to loss of righting reflex (–0.59), increased burst-suppression ratio ($r=0.52$) , and increased emergence time ($r=0.54$); an interaction-effect of isoflurane dose was observed (burst-suppression ratio: $p<0.001$). We conclude that the sleep loss caused by ablation of VLPO neurons sensitizes animals to the general anesthetic effects of isoflurane, but that the sedation produced by VLPO neurons themselves is not required for isoflurane anesthesia.

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1. Introduction

General anesthesia is a drug-induced, reversible condition with five components: hypnosis (loss of consciousness), amnesia, analgesia, immobility (no movement in response to pain stimuli), and hemodynamic stability with control of the stress response (Evers and Crowder, 2006). About 2–5% of the population is placed under general anesthesia per year, but a detailed understanding of the neural basis for this condition is still lacking. Anesthesia and natural sleep share some molecular mechanisms. First, most commonly used general anesthetics potentiate gamma-amino-butyric acid (GABA)-induced chloride currents (Franks, 2008) and GABA is the principle neurotransmitter used by sleep-promoting neurons in the ventrolateral preoptic nucleus (VLPO). Sleep requires the inhibition of multiple pathways in the arousal system and available evidence suggests that the VLPO is a major source of sleep-active neurons that provide GABAergic, inhibitory input to many elements of the arousal system (Sherin et al., 1996), including the tuberomammillary nucleus (TMN), locus coeruleus, and orexinergic neurons (Saper et al., 2010). In addition, many compounds that enhance GABAergic transmission promote sleep at low doses (Kales et al., 1968; Kay et al., 1972), but induce anesthesia at high doses (Eikermann et al., 2009).

It has recently been shown that GABA-potentiating general anesthetic drugs engage hypothalamic sleep-promoting neurons in the VLPO (Lu et al., 2008; Nelson et al., 2002). Drugs as diverse as isoflurane, pentobarbital, and chloral hydrate at general anesthetic doses activate VLPO neurons, as judged by expression of the immediate early gene cFos (Lu et al., 2008). Although activation of VLPO neurons would presumably potentiate the drug effects (by causing more GABA release onto arousal neurons), whether this activity plays an important role in the effects of these drugs at general anesthetic doses remained unclear. If it did, rats with VLPO lesions may exhibit lighter anesthesia at controlled end tidal levels of isoflurane. Furthermore, ablation of the VLPO may be expected to prolong the time to onset of anesthesia and shorten time to recovery. On the other hand, if VLPO neurons are not required for isoflurane general anesthesia, anesthetic effects may be potentiated by accumulated sleepiness that occurs in VLPO lesioned animals (Lu et al., 2000). Therefore, we tested whether ablation of VLPO neurons would cause increased or decreased sensitivity to isoflurane anesthesia by measuring the effects of isoflurane in terms of clinical and electroencephalographic metrics of onset latency, depth, and recovery from anaesthesia in animals with VLPO lesions vs. sham lesions.

2. Results

2.1. *Lesion extent*

As intended, the degree of cell loss in the VLPO varied in these experiments from none (n=3) to nearly complete (n=3), with the majority of cases (n=19) having partial lesions with varying degrees of involvement of surrounding structures including the median preoptic nucleus and the nucleus of the diagonal band (Fig. 1). Rats with saline injections had no damage (n=8).

2.2. *The effects of lesions on natural sleep*

Both the number of VLPO neurons lost as well as lesion size were associated with sleep loss (r=0.77 and r=0.62, respectively). However, multiple regression analysis revealed that loss of VLPO neurons and not lesion size predicted the cumulative sleep loss, indicating that loss of VLPO neurons rather than an unspecific brain damage represents the biologically relevant mechanism of sleep deprivation observed in our study. The VLPO lesions produced a significant increase in wakefulness (to 55.3% ±1.5, n=25, ranging from 47.9 to 70.4%) compared to sham lesioned animals (46.6% ±0.9, n=8, ranging from 43.4 to 51.1%).

2.2. *The effects of VLPO lesions on general anesthesia*

The transition from the awake to the anesthetized state was marked by typical EEG waveforms progressing from desynchronized to elevated slow wave activity to synchronized bursting alternating with silence (burst suppression pattern) (Fig. 2). At steady-state 2% isoflurane, the burst suppression ratio (BSR) in VLPO-lesioned rats was 0.75±0.02 n=25, indicating electrographic silence 75% of the time (Figs. 3B, D). The BSR at 1% isoflurane averaged 0.25±0.03; n=25 (Figs. 3A, C). For comparison, the BSRs in sham-lesioned rats were 0.66±0.12 and 0.10±0.04 in 2 and 1% isoflurane, respectively (n=6). The depth of anesthesia as estimated by the burst suppression ratio was correlated with the degree of VLPO lesion as taken by cell counts (p=0.0001; linear mixed model, see Statistics. The greater the cell loss in VLPO, the greater percentage of time the EEG was in the isoelectric state, indicating a deeper level of anesthesia. This effect was dose-dependent; an interaction-effect between isoflurane dose and number of remaining VLPO neurons was detected for burst-suppression ratio (p=0.0001), indicating that the effects of VLPO lesion on EEG signs of depth of isoflurane-induced unconsciousness were manifested only at shallow levels of anesthesia (1% isoflurane; Fig. 3). The correlations between VLPO neuron number and latency to loss of righting reflex (LORR; r=0.377; p=.063; Fig. 4) and to the first silent second (r=0.381; p=.060) were not significant but were trending in the direction of VLPO neuron loss contributing to anaesthesia induction. There was no correlation between VLPO lesions and the onset time of slow wave activity (r=0.77, p=0.715).

We measured emergence time from a steady-state level of 1% isoflurane. The number of remaining VLPO neurons was associated with time to emergence as measured by recovery of righting reflex (r=−0.488; p=0.013).

Overall, these correlations were not consistent with the concept that VLPO neurons play a role in induction of general anesthesia with isoflurane, but rather suggested the opposite. We thus explored the idea that the observed changes were related to sleep loss induced by VLPO lesions. We found that the amount of sleep loss was correlated with BSR (again, only at 1% isoflurane, r=0.571; p=0.003) (Fig. 5), time to LORR and RORR (r=−0.585, p=0.002; r=0.587, p=0.002 respectively) (Fig. 6). Finally, since LORR and RORR include motor