Review Article

Consciousness loss during epileptogenesis: implication for VLPO-PnO circuits

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Abstract: There is a growing concern about consciousness loss during epileptic seizures. Understanding neural mechanisms could lead to a better comprehension of cerebral circuit function in the control of consciousness loss in intractable epilepsy. We propose that ventrolateral preoptic area (VLPO)-PnO (nucleus pontis oralis) circuits may serve a major role in the loss of consciousness in drug-refractory epilepsy. Future behavioural and neuroimaging studies are clearly needed to understand the functional connectivity between the VLPO and PnO during loss of consciousness in drug-refractory epilepsy, to greatly prevent unconsciousness in this disorder and improve the quality of life in patients with intractable epilepsy.

Keywords: Consciousness loss, drug-refractory epilepsy, ventrolateral preoptic area, nucleus pontis oralis

Introduction

It is widely perceived that seizures may produce reversible loss of consciousness [1-3]. There is a growing concern about loss of consciousness during epileptic seizures [4-9]. Some patients with epileptic seizures have experienced frequent episodes of consciousness loss, with eyes staring, often during swallowing or chewing. Many studies have found that exposure to frequent seizures can result in widespread neuronal degeneration and cognitive impairment [10-13]. Studying neural circuits of sleep-wake regulation can enhance our understanding of the pathophysiology of loss of consciousness during epileptic seizures, and provide optimal therapies for loss of consciousness during epileptic seizures.

Sleep-wake regulation in the VLPO-PnO circuits

The rapidly growing field of neural circuits provides a wealth of biological information on sleep-wake regulation. Utilizing the Cre-dependent AAV encoding hrGFP as a tracer in the projections of A2AR neurons in nucleus accumbens (NAc) mediating sleep-wake regulation, Zhang et al. found that there existed the NAc-preoptic area-pontine reticular formation pathway [14].

It is known that the ventrolateral preoptic area (VLPO) is an important sleep-promoting nucleus [15, 16]. Wang et al. and colleagues previously evaluated the effects of morphine on sleep-wake profiles after administration of a mu receptor antagonist D-Phe-Cys-Thr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP) or kappa opioid receptor antagonist nor-BIN to the VLPO using electroencephalogram (EEG) and electromyogram recordings in freely moving rats, and found that morphine could inhibit the activity of sleep-promoting neurons in the VLPO by affecting mu receptors and induced arousal in a dose-dependent manner [17]. Some data reported that GABA agonist Magnolol (a major bioactive constituent of the bark of Magnolia officinalis) and GABA transporter-1 inhibitor NO-711 increased c-Fos expression in the VLPO whereas they
decreased c-Fos expression in the wake-promoting nuclei (e.g., lateral hypothalamus) [15, 18, 19].

The hypocretin (orexin), a hypothalamus-specific peptide with neuroexcitatory activity, is involved in the regulation of waking and sleep states [20, 21]. The oral part of the pontine reticular formation (nucleus pontis oralis, PnO) has been shown to have an important somnogenic role and receives hypocretinergic input [22-25]. It is reported that wakefulness occurs following injections of hypocretin-1 (orexin A) into the PnO [26]. Watson and colleagues previously showed that wakefulness is either increased or decreased, respectively, by PnO administration of nipecotic acid (a GABA uptake inhibitor that increases extracellular GABA levels) or 3-mercaptopropionic acid (a GABA synthesis inhibitor that decreases extracellular GABA levels; 3-MPA), and indicated that PnO administration of hypocretin-1 increased PnO GABA levels and increases wakefulness [27]. A study by Watson et al. provided evidence that mu-opioid receptor agonist morphine disrupted sleep and obtunded wakefulness by decreasing GABAergic transmission in the brain regions regulating arousal, including the pontine reticular nucleus, oral part [22].

Our research data about VLPO-PnO circuits and melanocortinergic signaling

The ventrolateral preoptic area (VLPO) and the oral part of the pontine reticular formation (PnO) selectively express various receptors or

Figure 1. MC4R-GFP-positive neurons in the VLPO-PnO circuits. Injection of PRV-614 into the left kidney resulted in retrograde infection of neurons in the VLPO (A), NPO (B and C) by the sympathetic pathway. PRV-614/MC4R-GFP dual-labeled neurons were detected in the VLPO (A3) and NPO (C3). PRV-614/TH dual-labeled neurons were detected in the PPTg (B3). (A1, B1 and C1) show PRV-614-labeled cells; (A2 and C2) show MC4R-GFP-positive cells; (B2) shows TH-positive cells; and (A3, B3 and C3) show overlaid images of (A1, B1, and C1) plus (A2, B2, and C2), respectively. MC4R, melanocortin-4 receptor; PnO, pontine reticular formation; TH, tyrosine hydroxylase; VLPO, ventrolateral preoptic area. Arrows indicate double-labeled neurons. Scale bars, 50 μm. Some photomicrographs were taken from [28, 29, 42, 43].
peptides, the property that can define their phenotypes. Recent studies have indicated that the PnO may be involved in rapid eye movement (REM) sleep dysfunction in epilepsy by melanocortinergic signaling [28, 29]. We examined the molecular basis of this selectivity in the VLPO and PnO by microinjections of pseudorabies virus vector PRV-614 expressing red fluorescent protein (RFP) into the kidney of transgenic mouse containing MC4R gene promoter using expressing green fluorescent protein (GFP) as the reporter [28-49]. 5 days following injection of the PRV-614, immunohistochemical assays of GFP expression from these constructs were done to determine whether the GFP reporter co-localizes with PRV-614-immunoreactivity in the VLPO and PnO. The results show that PRV-614/MC4R-GFP double-labeled neurons were detected in the VLPO and PnO (Figure 1), suggesting that there may exist the VLPO-PnO circuits and the key elements in the MC4R gene promoter may be involved in regulating the cell-type specific expression the VLPO and PnO (Figure 2).

**The occurrence of seizures, sleep disturbances and loss of consciousness**

The intimate relationship between the occurrence of seizures and loss of consciousness has long been recognized [31, 50-53]. Wyllie et al. reported a 9-year-old girl case with loss of consciousness and seizures [54]. It is known that seizures are a devastating neurological disorder associated with sleep disturbance [55, 56]. Our understanding of the mechanisms for sleep disturbances and loss of consciousness during the occurrence of seizures is changing rapidly.

The roles of GABA transporter subtype 1 (GAT1) inhibitors for the treatment of epileptic seizure activity have been extensively reported [57, 58]. Xu et al. observed sleep behaviors of mice treated with a selective GAT1 inhibitor NO-711, and found that NO-711 caused a marked enhancement of EEG activity during rapid eye movement (REM) sleep and wakefulness, indicating that NO-711 may be useful in sleep-wake regulation for the treatment of epilepsy [15]. Magnolol exerting anti-epileptic effects via the GABA receptor has been studied [18, 19]. A study of Chen et al. showed that magnolol increased the number of state transitions from wakefulness to NREM sleep and increased c-Fos expression in the neurons of VLPO, a sleep center in the anterior hypothalamus [18]. These results suggest that the occurrence of seizures is tightly linked to sleep disturbances.

It is generally accepted that generalized seizures are commonly associated with ictal alterations in consciousness [59-62], whereas simple-partial seizures do not disrupt conscious-
ness. Generalized seizures produce distinct, active and highly organized patterns on the electroencephalogram (EEG), the most common of which is synchronous or irregular slow spike-wave discharges [1, 53]. Yang et al. used the prospective responsiveness in epilepsy scale (RES) to investigate impaired consciousness in 52 patients with epilepsy during continuous video-EEG monitoring, and found that RES impairment was greatest during and after tonic-clonic seizures whereas less in partial seizures, suggesting that RES impairment was related to EEG changes during seizures [62]. Blumenfeld and Taylor have indicated that generalized seizures result in abnormal increased activity in brain networks (e.g., fronto-parietal association cortex and related subcortical structures) to impede the normal communications of arousal and cognition [1]. A study of Farzampour et al. has pointed that transient loss of consciousness accompanied by focal temporal lobe seizures is a complex life-threatening phenomenon [63].

Conclusion

Though the precise structures involved in loss of consciousness in epileptic seizures are far from complete, remarkable progress has been made in elucidating the neurobiological, neurophysiological, and neuropharmacological mechanisms underlying the unconsciousness due to seizures [64-66]. Understanding these neural mechanisms could lead to a better comprehension of cerebral circuit function in the control of loss of consciousness in intractable epilepsy. We propose that VLPO-PnO circuits may serve a major role in the loss of consciousness in drug-refractory epilepsy. Future behavioural and neuroimaging studies are clearly needed to understand the functional connectivity between the VLPO and PnO during loss of consciousness in drug-refractory epilepsy, to greatly prevent unconsciousness in this disorder and improve the quality of life in patients with intractable epilepsy.

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Disclosure of conflict of interest

None.

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