主論文の要旨

Dual orexin and MCH neuron-ablated mice display severe sleep attacks and cataplexy

（オレキシン神経とMCH神経を両方脱落させたマウスは重篤な睡眠発作と脱力発作を呈する）

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【Introduction】

The lateral hypothalamus (LH) has long been known to be involved in the regulation of sleep/wakefulness, feeding behavior and metabolism. Orexin/hypocretin and melanin-concentrating hormone (MCH)-producing neurons are distributed within the LH. Orexin and MCH neurons project throughout the brain and are implicated in feeding and sleep/wakefulness. Absence of orexin signal in mice and human display narcolepsy. Narcolepsy is a chronic sleep disorder caused by the specific degeneration of orexin neurons by the immune system. Narcolepsy patients have characteristic symptoms including excessive daytime sleepiness, hallucinations and cataplexy, a sudden loss of muscle tone triggered by positive emotions such as laughter. On the other hand, optogenetic activation of MCH neurons increases the total time in rapid eye movement (REM) sleep and reduces non-REM (NREM) sleep in mice. Ablation of MCH neurons promotes wakefulness and decreases time in NREM sleep but has no effect on REM sleep. These results suggest that MCH neurons are likely involved in the regulation of both NREM and REM sleep. Nevertheless, it is still unclear how interactions between orexin and MCH neurons contribute to sleep/wake regulation.

【Methods】

To understand the functional communication between orexin and MCH neurons in sleep/wakefulness regulation, I generated tiple-transgenic orexin-tTA (Tg/-); MCH-tTA (Tg/-); TetO diphtheria toxin A fragment (DTA) (Tg/-) (OXMC) mice in which both orexin and MCH neurons were simultaneously ablated. DTA induces cell death by inactivating eukaryotic elongation factor 2 through ADP ribosylation, thereby blocking protein synthesis. DTA expression can be controlled by the presence or absence of doxycycline (DOX)-containing chow since the Tet-off system is used to control the timing of DTA expression.

【Results】

Sleep and wakefulness was assessed in three groups of mice, OXMC DOX(-), OXMC DOX(+) and orexin-tTA (Tg/-); TetO DTA (Tg/-) (OX) DOX(-) mice by EEG and EMG recording. Spectral analyses of the EEG and EMG during wakefulness, NREM and REM sleep were indistinguishable across the three groups under the DOX(+) condition. After DOX removal, there was no difference in EEG spectrum and EMG integral of OXMC DOX(-) mice in all vigilance state. These results indicate that neither orexin nor MCH neuron ablation affected the EEG power spectrum.

Ablation of orexin neurons is known to induce narcolepsy-like symptoms, such as fragmentation of sleep and wakefulness, sleep onset REM sleep, and cataplexy-like behavioral arrests. Since orexin neurons were ablated in both OX DOX(-) and OXMC

DOX(-) mice, these mice showed cataplexy-like behavioral arrests, but OXMC DOX(+) mice did not.

The total wakefulness time significantly increased in OXMC DOX(-) mice at 4 weeks after DOX removal in the both light and dark phases as compared with OX DOX(-) mice. Conversely, OXMC DOX(-) mice exhibited a significant reduction in total REM and NREM sleep and the number of bouts in both light and dark phases at 4 weeks DOX(-) when compared with OX DOX(-). The total time in cataplexy and mean cataplexy bout duration was greater in OXMC DOX(-) mice as compared with OX DOX(-) mice, indicating that the loss of MCH neurons exacerbated cataplexy symptomatology. The differences in sleep/wakefulness phenotype between these two strains suggest that MCH neurons are part of a circuit that normally suppresses cataplexy (Figure 1).

Interestingly, OXMC DOX(-) mice frequently showed behavioral arrest episodes that were similar to cataplexy but which occurred after a sustained period of wakefulness. The EEG showed high amplitude spectral power in the δ and θ bandwidths during the behavioral arrests. These episodes were defined as a sudden cessation of motor activity characterized by decreased EMG and relatively high-power ratios of δ and θ in the EEG, preceded by at least 40 s of wakefulness and followed by a return to wakefulness. Consequently, I called the state during these arrests ‘delta/theta sleep’ (DT sleep, Figure 2A and 2B).

To further understand whether DT sleep is related to sleep or cataplexy, behavioral assessment, tactile stimulation, was performed on OXMC DOX(-) mice during immobile stages. The probability of wakefulness was 33% after tactile stimulation during cataplexy compared to 100% in NREM and DT sleep (Figure 2C). These results suggest that DT sleep differs from cataplexy and may be more similar to NREM sleep.

The position of mice in the home cage during each stage was identified. The cage was divided into 4 areas, with a nest located in one of them. NREM and REM sleep occurred with higher probability when mice were in the nest area. However, both DT sleep and cataplexy occurred in the nest area at almost a chance level (Figure 2D). These results suggest that DT sleep occurs with a similar timing to cataplexy and, like cataplexy, may be a spontaneous, uncontrolled state.

Pharmacological assessments were also performed. OXMC DOX(-) mice were exposed to chocolate to increase cataplexy, administered clomipramine to suppress cataplexy, or modafinil to promote wakefulness. Chocolate administration to OXMC DOX(-) mice significantly increased wakefulness and cataplexy, it decreased REM and NREM sleep but it did not affect DT sleep (Figure 3). Clomipramine administration significantly decreased time in wakefulness and REM sleep, increased total time of NREM sleep, inhibited cataplexy, but did not affect DT sleep time (Figure 4). Modafinil administration increased the total time of wakefulness, inhibited NREM and REM sleep, but did not affect cataplexy

(Figure 5). Among the results above, they suggest that DT sleep is a novel brain state different from other states.

Since both δ and θ power in the EEG were high during DT sleep, I evaluated the EEG during sleep/wake and wake/sleep transitions to compare EEG spectral characteristics. I found that the EEG spectrum in the transition from NREM to REM sleep was similar to DT sleep. Spectral power in the δ, θ, α and β bands of the EEG was indistinguishable between DT sleep and the NREM to REM sleep transition (Figure 6).

【Conclusion】

To understand functional interactions between orexin and MCH neurons in the regulation of sleep and wakefulness, I generated dual orexin and MCH neuron-ablated mice and compared the resultant sleep abnormalities to those of orexin neuron-ablated mice. Double-ablated mice exhibited pronounced cataplexy and the total time in cataplexy and mean cataplexy bout duration were significantly increased, suggesting that MCH neurons normally have a suppressive role on cataplexy. Double-ablated mice also had exaggerated sleep abnormalities compared to singly-ablated or intact mice; specifically, increased time in wakefulness and decreased time in NREM and REM sleep. Double-ablated mice also exhibited a novel state that I called DT sleep, defined as an episode of sudden behavioral arrest of brief (~15 s) duration preceded by at least 40 s of wake and characterized by high δ and θ power in the EEG. Behavioral, electrophysiological and pharmacological assessments discriminated DT sleep from NREM, REM and cataplexy.