論　文　概　要

論 文 題 目　Mechanism analysis of Japanese sake yeast induced non-rapid eye movement sleep in mice
（マウスにおける清酒酵母によるノンレム睡眠誘導メカニズムの解析）

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Purpose

To investigate the effects of oral administration of Japanese sake yeast on sleep/wake behavior and its molecular and neural mechanisms of action in mice.

Material and method

The effects of oral administration of test samples on the spontaneous locomotor activity and sleep/wake behavior were examined in adult male C57BL6/N mice. The locomotor activity in individual mice was assessed using a passive infrared sensor. For the evaluations of effects on the sleep/wake behavior, mice were implanted with electroencephalogram (EEG) and electromyogram (EMG) electrodes for polysomnographic recording. After recovery, animals were habituated and vehicle or the test samples were orally administered to mice for 15 min before the onset of dark period. EEG and EMG were then analyzed and scored as wakefulness, rapid eye movement (REM) and non-REM (NREM) sleep, by using SLEEPSIGN software, according to the standard criteria. To clarify the molecular mechanism of sake yeast induced NREM sleep, the effects of intraperitoneal pretreatment of adenosine receptor antagonists were examined after oral administration of sake yeast. To clarify the neural mechanism, the effects of sake yeast on neuronal activity was investigated using immunohistochemical staining for c-Fos, a marker of neuronal activation.

Results

Oral administration of Japanese sake yeast (100, 200, and 300 mg/kg) decreased the locomotor activity by 18%, 46%, and 59% and increased the amount of NREM sleep by 1.5, 2.3, and 2.4-fold, compared with vehicle-administered group, respectively, in a dose-dependent manner for 4 hours after the oral administration. However, Japanese sake yeast did not change the amount of REM sleep, the electroencephalogram power density during NREM sleep, or show any adverse effects such as rebound of insomnia during 24 hours post-administration and on the next day. An intraperitoneal pretreatment with an adenosine A_{2A} receptor ($A_{2A}R$)-selective antagonist, ZM241385 (15 mg/kg), reduced the amount of NREM sleep of sake yeast-administered mice to the basal level, without changing basal amount of sleep. In contrast, an A_1 receptor (A_1R)-selective antagonist, 8-cyclopentyltheophylline (10 mg/kg), did not affect the sleep promoting effect of Japanese sake yeast. The effects of sake yeast on neuronal activity were examined using immunohistochemical staining for c-Fos, a marker of neuronal activation, 4 hours post-administration at the onset of the dark period. The activities of the nucleus accumbens (NAc) and tuberomammillary nucleus (TMN), a wake-promoting neuron downstream to the NAc, in response to $A_{2A}R$-induced NREM sleep were investigated. Oral administration of sake yeast significantly increased the number of c-Fos positive neurons in the NAc shell by 1.7-fold ($p < 0.05$) but did not change the expression in the core. In contrast, sake yeast significantly decreased c-Fos expression in the TMN by 57% ($p < 0.01$). These

results indicate that oral administration of sake yeast activates the NAc shell and suppresses the TMN.

Discussion

The current study provides pharmacological and neural evidence of the sleep-promoting effect of Japanese sake yeast via $A_{2A}R$ in mice. Sake yeast-induced NREM sleep was very similar to physiological optimal NREM sleep, as judged by the mean episode duration and EEG power spectral analysis. The NREM sleep-promoting effect of sake yeast was reduced to the basal level by pretreatment with an $A_{2A}R$ antagonist but was not affected by A_1R antagonist, indicating that sake yeast promotes NREM sleep via activation of $A_{2A}R$. In addition, oral administration of sake yeast increased the number of c-Fos positive cells in the NAc and decreased in the TMN, suggesting that sake yeast activates NAc and suppresses TMN neuronal activity. In rodent studies, intracerebroventricular infusion of $A_{2A}R$-selective agonist, CGS21680, activates the shell of the NAc, inhibits the TMN, and induces NREM sleep. Furthermore, the wake-promoting effect of caffeine was abolished by $A_{2A}R$ gene knockdown in the shell regions of the NAc. The present study's results correspond with these previous findings, and sake yeast appears to have an opposite mechanism of action than caffeine in sleep/wake regulation. Nevertheless, from the pharmacological analysis, it cannot be excluded the possibility that peripheral $A_{2A}R$ is involved in the NREM sleep-promoting effect of sake yeast. To clarify the involvement of $A_{2A}R$ in the brain, the effects of sake yeast in the brain specific $A_{2A}R$ knockout mice need to be tested.

Conclusion

This study demonstrated that Japanese sake yeast promoted NREM sleep via activation of $A_{2A}R$. Japanese sake yeast is a potential candidate for a supplement improving the quality of sleep.