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論文の要旨
Abstract of thesis

Introduction: Insomnia is one of the most common sleep problems with an estimated prevalence of 10-15% in the general population and 30-60% in the older population. The adenosine A_{2A} receptor ($A_{2A}R$) agonist CGS 21680 induces sleep when infused into the brain of rodents. However, it is commonly believed that administration of an $A_{2A}R$ agonist has limited clinical potential for treating sleep disorders, because of its cardiovascular side effects, including hypotension and tachycardia. Moreover, all currently existing $A_{2A}R$ agonists are not suitable for treating the central nervous system due to the lack of brain permeability, as it is widely accepted that the basic adenosine scaffold must be maintained in an $A_{2A}R$ agonist. Selective physiologic $A_{2A}R$ responses may, however, be evoked by a positive allosteric modulator, because its action, in contrast an orthosteric ligand, is limited to when and where the endogenous ligands are released. Allosteric modulation may be an alternative strategy for the treatment of insomnia, because the elevation of extracellular adenosine levels in the brain is positively associated

with sleep. The main objective of this study was to prove this hypothesis.

Materials and methods: The author established $A_{2A}R$-expressing Chinese hamster ovary cells to measure cAMP produced upon $A_{2A}R$ activation by using a fluorescence resonance energy transfer immunoassay and subsequently, screened 1194 small-molecule compounds for allosteric effects at $A_{2A}R$ in the cell-culture bioassay. The author determined sleep-inducing effects of the compound measured by using SLEEPSIGN, a sleep recording systems, effects of compound on blood pressure measured by using an electrosphygmomanometer, and heart rhythm monitored by using an electrocardiography, and body temperature by using body core temperature sensors, iButton.

Results: The author identified a positive allosteric modulator for $A_{2A}R$ ($A_{2A}R$ PAM-1) in cell-culture system. When the author examined the sleep inducing activity of $A_{2A}R$ PAM-1 by monitoring the electroencephalogram, the author found that the intraperitoneal (i.p.) administration of $A_{2A}R$ PAM-1 dose dependently (30-75 mg/kg) increased the total amount of slow wave sleep (SWS). The SWS-inducing effect of $A_{2A}R$ PAM-1 was suppressed by $A_{2A}R$ antagonist ZM 241385 (15 mg/kg, i.p.) and abolished in $A_{2A}R$ knockout mice. Moreover, direct infusion of $A_{2A}R$ PAM-1 (200 nmol/ml) into the brain of mice during the night strongly induced SWS. In contrast to $A_{2A}R$ agonist CGS 21680, blood pressure measured by using an electrosphygmomanometer, heart rhythm monitored by using electrocardiography, and body temperature measured by using temperature sensors iButton was not affected after i.p. administration of $A_{2A}R$ PAM-1.

Discussion: The author suggestd that enhancing $A_{2A}R$ signaling by intraperitoneal administration of $A_{2A}R$ PAM-1 induces SWS without cardiovascular effects in mice. Therefore, $A_{2A}R$-modulating compounds may provide safe options for the treatment of insomnia and poor- quality sleep. The observation in animals that adenosine levels are elevated during prolonged wakefulness may explain why an allosteric modulator could effectively enhance the sleep-inducing effect of endogenous adenosine in the brain. On the other hand, adenosine was absent or its concentration was too low in the cardiovascular system under physiologic conditions to affect blood pressure and heart function after administration of an allosteric modulator of $A_{2A}R$. Small lipophilic monocarboxylates like $A_{2A}R$ PAM-1 likely pass through the blood-brain-barrier by passive diffusion or via a monocarboxylate transport system. Therefore, allosteric modulation of $A_{2A}Rs$ has the potential to cause pharmacologic effects in the central nervous system after systemic administration, resulting in good quality sleep. However, the author's study did not elucidate where and how the $A_{2A}R$ PAM-1 binds to the receptor and exert its allosteric effects. Moreover,

the author's work did not address the possible sleep-inducing effects of the $A_{2A}R$ PAM-1 in the human use. Many obstacles remain to be overcome in generating a novel drug for the treatment of insomnia in humans.

Conclusions: Small molecules like $A_{2A}R$ PAM-1 may help people with sleep problems to fall asleep.

審査の要旨
Abstract of assessment result

【批評 Review】

The findings of this study indicate that enhancing $A_{2A}R$ signaling promotes slow-wave sleep without affecting cardiovascular functions and body temperature. Therefore, small molecules that allosterically modulate $A_{2A}R$s could help people with sleep problems to fall asleep and thus also be a potential treatment for psychiatric disorders. The strong points of this work could be summarized as like below: This study elucidated at the first time that positive allosteric modulation of adenosine $A_{2A}R$ promotes slow-wave sleep in a dose-dependent manner in mice. Inducing slow-wave sleep via adenosine $A_{2A}R$ positive allosteric modulator does not cause hypotension or affect blood pressure and heart rate/rhythm. On the other hand, the weak points of this work could be counted as below: This study did not investigate how and where the $A_{2A}R$ PAM-1 binds at the receptor to exert its allosteric effect. Therefore, an important next step will be to examine the allosteric interactions of $A_{2A}R$ PAM-1 and the receptor using binding assays and crystal structure analysis.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 17 December, 2018. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded a Doctor of Philosophy in Human Biology.