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学位論文内容要旨

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CIRCADIAN PHASE-ADJUSTMENT BY DAILY INJECTIONS OF HISTAMINE RECEPTOR ANTAGONIST, KETOTIFEN

Ahmad Alsawaf

Summary

Studies have shown that histaminergic neuronal activity in the brain involves maintenance of arousal, whereas blocking of histamine H_1 receptors (H_1R) either pharmacologically or genetically, enhances sleep. Drowsiness following generic (i.e., over-the-counter) anti-allergic drug administration, which may contain classic H_1R antagonists, may be due to transport of anti-histamines to the brain. In fact, modern H_1R antagonists such as cetirizine, fexofenadine, and mizolastine, which were designed to be less permeable to the blood-brain barrier, produce little or no drowsiness. Conversely, one of the first generation H_1R antagonists, diphenhydramine, is currently sold as a sleeping pill (Drewell® and Neoday®) in Japan. In addition, doxepin (Sinequan®), which is a first generation H_1R antagonist known to induce sleep, has been approved by the FDA to treat insomnia in the USA since 2010. One concern of using these classic H_1R antagonists to treat insomnia is their non-specific actions, including anti-cholinergic actions. An overdose of classic H_1R antagonists produces cocaine-like arousal-inducing actions, resulting in a narrow effective dose-window (i.e., sharp bell-shape dose-response curve) for sleep induction. Because drowsiness is an undesirable property for the general use of antihistamines, the newer and more specific H_1R antagonists have been designed to reduce brain permeability. Therefore, developing brain-permeable specific H_1R antagonists with fewer side effects is still imperative for advancing insomnia treatment. However, development of new drugs requires significant time and effort. Moreover, use of newly patented drugs is costly. Therefore, we have suggested an alternative strategy (i.e., drug re-positioning) to treat insomnia using ketotifen, which is categorized as an early phase second generation H_1R antagonist, likely to be more specific than the first generation antihistamines, while maintaining its permeability to the brain.

In our previous rat model studies, ketotifen represented a typical S-shape dose-response curve for non-REM sleep induction. Also, repeated administration of ketotifen (3 mg/kg, i.p.) for 5 days at the late active phase (ZT21) consistently facilitated non-REM sleep at the end of the active phase (Unno et al, *Eur J Pharmacol* 2012). To further characterize the effects of

ketotifen on circadian clock movements, the present study examined free-running locomotor activity rhythms (Ahmad et al, *Sleep and Biological Rhythms* 2016), core body temperature (Tb) rhythms, and clock gene (*mPer2*) expression rhythms in rats following the repeated injections of ketotifen (3 mg/kg, i.p. at ZT21). The results are described as follows.

(i) Locomotor activities of Sprague-Dawley rats were monitored using infrared sensors under 12:12 h light-dark cycles. Daily intraperitoneal injections of ketotifen (3 mg/kg, i.p.) or vehicle (saline) were examined for 5 days 3-h before light onset time (ZT21) or 3-h before dark onset time (ZT9). Following the final injection day, room light was switched to constant dim red light to monitor free-running (i.e., endogenous circadian) rhythms. The eye-fitting activity onset time on the free-running rhythms demonstrated significant advancement of circadian phase of locomotor activity rhythms when ketotifen was injected at ZT21. Since non-REM sleep was enhanced by ZT21 ketotifen injections, we speculated that enhanced sleep might cause circadian phase-shifts in this experimental paradigm.

(ii) Rats Tb were monitored by thermistor-based coin-shape data loggers, which were intraperitoneally implanted one week before recordings. The same ketotifen injections (3 mg/kg, i.p., for 5 days) were examined as above for locomotor activity recordings. The results demonstrated that ketotifen did not decrease or increase Tb levels immediately after the injections. However, during the injection days, circadian Tb rhythms were slightly amplified. These results suggest that circadian phase-shifts by daily ketotifen injections were not directly caused via Tb modulations.

(iii) To conclude the effects of daily ketotifen injections on the circadian clock, we also evaluated *mPer2* transcriptional rhythms in the hypothalamic suprachiasmatic nucleus (SCN) and hippocampus using a real-time RT-PCR. After 5 day injections of ketotifen (3 mg/kg, i.p., at ZT21), rats were sacrificed and their brain tissues were sampled at ZT0, ZT3, ZT6, ZT9 or ZT12 to estimate *mPer2* transcriptional levels. The results demonstrated that peak *mPer2* transcription in the SCN was located at ZT6 in the ketotifen injected group and at ZT9 in the saline injected control. In addition, peak *mPer2* transcription in the hippocampus was located at ZT9 in the ketotifen injected group and at ZT12 in the saline injected control. Thus, these results indicate that daily ketotifen injections advance *mPer2* transcriptional rhythms ubiquitously in the brain, including the central SCN clock. Hence, we suggest ketotifen-induced sleep at the end of active phase may advance the critical clock movements, resulting in an advancement of behavioral rhythms even after drug injection period.

Although further experimental and clinical trials will be needed to conclude the possible benefits of ketotifen to treat insomnia, the present study provides a strong evidence that daily ketotifen administrations at the end of active phase will be effective to treat circadian rhythm sleep disorders, such including the delayed sleep phase disorder.

【博士学位論文審査結果の要旨】

アハマド アルサワフ君は、睡眠改善薬としてヒトへの応用が期待されているH_1ヒスタミン受容体アンタゴニスト（ケトチフェン）の作用を、ラットを用いた動物実験により詳細に検討し、以下の新しい知見を得た。つまり、ラットの活動期後半である明期開始3時間前（ZT21時）に、暗赤色光下でケトチフェンを腹腔内投与する実験を 5 日間連続して行い、その直後に環境の明暗サイクルを恒常暗赤色条件とすることで、歩行活動の自由継続リズムを観察した。その結果、このケトチフェンの連日投与により、活動開始時刻が有意に前進することを見出した。以前の報告により、ZT21時でのケトチフェン投与が、ラットのノンレム睡眠を増加させることがわかっているので、この効果は、「睡眠誘発性概日位相変位」と捉えることが可能である。時間生物学の領域においては、これまでストレスや強制運動が、位相前進を引き起こすことはよく知られており、つまり、「行動誘発性位相変位」というコンセプトは広く定着していたが、定時に繰り返される睡眠が、体内時計の位相を変化させることを実験的に証明したのは、本研究が初めてである。アハマド アルサワフ君は、この睡眠誘発性概日位相変位のメカニズムをさらに詳しく解析し、ケトチフェンが、ラットの深部体温には大きな影響を及ぼさないことや、あるいは、体内時計中枢である視床下部視交叉上核の時計遺伝子（*Per2*）転写リズムの位相前進作用を伴うことを明らかにしている。これらの結果は、筆頭著者として国際原著論文 *Sleep & Biological Rhythms* に発表されたほか、和文レビュー誌と第23回日本時間生物学会において発表されている。なお、iThenticate を用いて審査対象論文の剽窃チェックを行ったところ、過去の出版物との重複率は1%以下であり、オリジナル原稿として認められることを確認した。また、平成29年2月3日に開催された公聴会での発表や質疑応答も十分な内容であった。これらを総合的に判断し、博士学位論文審査を合格とすることとした。